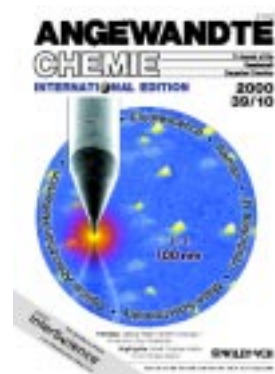
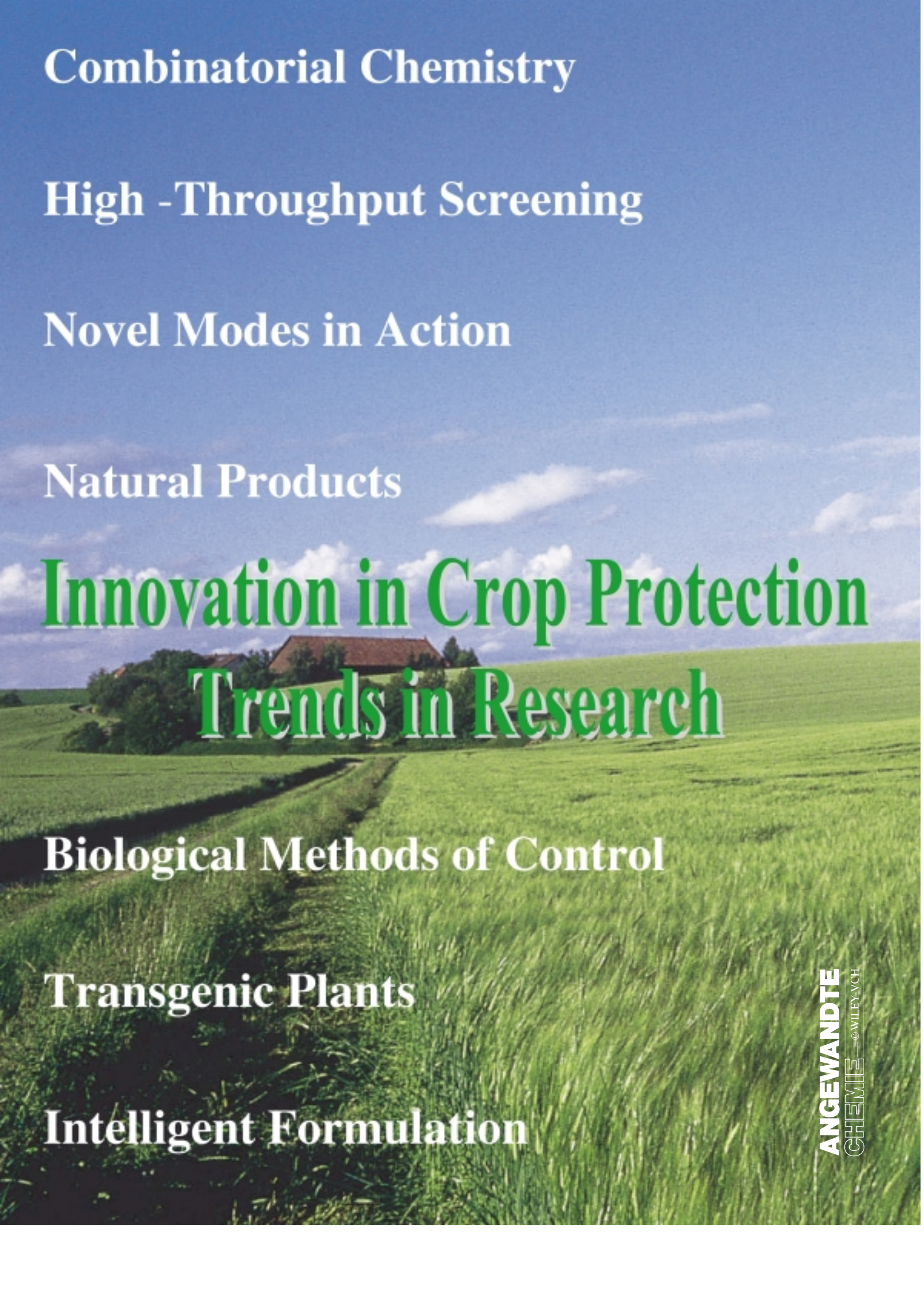


Cover Picture

Renato Zenobi and Volker Deckert

The cover picture shows an etched and metallized optical fiber tip for chemical analysis on the nanometer scale using near-field optical microscopy and spectroscopy. The background shows an image of nanospheres embedded in a polymer matrix that were visualized via fluorescence. Further details are given in the review by R. Zenobi and V. Deckert on pages 1746–1756.





Combinatorial Chemistry

High -Throughput Screening

Novel Modes in Action

Natural Products

Innovation in Crop Protection
Trends in Research

Biological Methods of Control

Transgenic Plants

Intelligent Formulation

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Innovation in Crop Protection: Trends in Research

Jörg Stetter* and Folker Lieb

In the absence of the remarkable levels of growth in the yields of important crops, neither the rapid increase in living standards in industrialized countries nor the adequate standard of nutrition for the greater part of the world's population would have been possible. Alongside high-yielding varieties, improved agricultural techniques, and rapid mechanization, the chemical industry has also contributed

substantially to progress in agriculture since roughly the middle of the nineteenth century. From the chemists "kitchens" came two "magic weapons": artificial fertilisers and chemical agents for crop protection. Today both have become indispensable to modern yield- and quality-orientated agriculture. This review spans the development of the crop-protection industry from its earliest beginnings to the

present day and attempts to portray how the research-based crop-protection industry is prepared for current and future challenges. Considerable space is thus dedicated to the discussion of trends in research.

Keywords: active substance research
• chemical crop protection • pesticides
• transgenic plants

1. Introduction

In "Gulliver's Travels", author Jonathan Swift says of King Brobdingnag: "...and he gave it for his opinion that whoever could make two ears of corn, or two blades of grass to grow upon a spot of ground where only one grew before, would deserve better of mankind and do more essential service to his country than the whole race of politicians put together".^[1] By breeding high-yielding cereal grain varieties, dedicated scientists such as the Nobel peace prize laureate of 1970, Norman E. Borlaug, have achieved considerably more than that, yet their names are virtually unknown to the general public.^[2, 3]

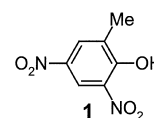
The first practicable crop-protection agents, such as the legendary Bordeaux mixture^[4] (Figure 1), were chance discoveries, with the user reaching, in a sense blindly, into the chemicals cupboard; they represented the first modest weapons in the battle against diseases of potatoes and vines in the latter part of the nineteenth century. In the beginning the agents were generally inorganic in nature, often unselective, active only at high application rates, and frequently toxic. Arsenic-based insecticides remained the mainstay of insect control well into the twentieth century. Thus, in 1941, approximately 30000 tonnes of arsenic trioxide were still used in the USA alone for the production of arsenic-based insecticides.^[5, 6]

Insecticides

As₂O₃

Cu(OAc)₂ · 3 Cu(AsO₂)₂
Paris green

Pb₃(AsO₄)₂



Antinonin (1892)

Herbicides

FeSO₄

H₂SO₄

NaClO₃

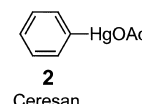
CuSO₄

Fungicides

[Cu(OH)₂]_x · CaSO₄
Bordeaux mixture
(1885)

S_x

HgCl₂



Ceresan

Figure 1. Historical agents of crop protection, 1st generation (1800–1930).^[5]

Organised industrial research with the aim of finding less toxic, selective organochemical active ingredients for crop-protection applications first started in the 1930s. Substances which were simple to synthesize but which were nonetheless already effective, such as TMTD^[7], E 605^[8, 9], DDT^[10–12] or 2,4-D,^[13] are synonymous with the first breakthroughs achieved (Figure 2). With these substances the foundations were laid for a flourishing crop-protection industry with an estimated market volume of around 30 billion \$US annually.^[14]

Initially, there was an immense need to protect the increased yields, which were accruing from the rapidly developing agricultural industry as a result of improved crop

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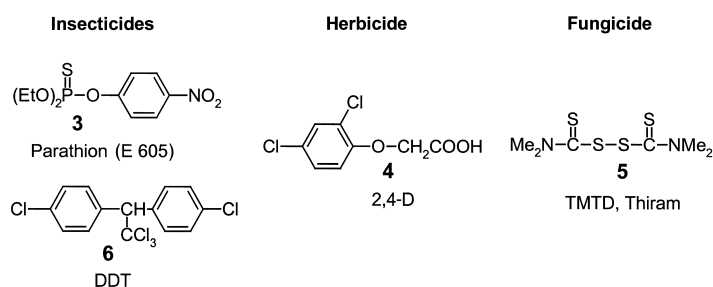


Figure 2. Historical agents of crop protection, 2nd generation (1930–1950).

varieties, artificial fertilisers, and mechanization, from the effects of pests and pathogens. User acceptance of crop-protective agents was extraordinarily high, which is attributable to the improved quality of life they brought to the farming community, as well as to the reduced economic risks. The use of crop-protective agents was an investment which yielded considerable returns. Chemistry in harmony with agriculture, an ordered world, an ideal partnership, or so it appeared for a long time. However, where there is light, there must inevitably also be shadows!

1.1. The Problem of Acceptance

With publication of Rachel Carson's book "Silent Spring",^[15] if not beforehand, the relationship between agriculture and chemistry became, at the very least, tainted in the eyes of the general public. The

spectrum reaches from a lack of acceptance to outright rejection, coupled with demands that the use of chemical agents in agriculture be banned. A discussion of the reasons for this is beyond the scope of this review. Two sources for the lack of acceptance should, however, be named at this juncture: first, the all pervasive "chemophobia" in contemporary culture. Irrationally, chemophobia is limited to synthetic chemicals. Ignoring their properties, the natural "environmental" chemicals, which dominate by orders of magnitude in terms of their numbers and amounts, are regarded as gentle, benign, and almost as though willed by God. This view is naive since nature makes use of an inexhaustible arsenal of chemicals, which to a large part are nothing less than chemical weapons for defence against hostile organisms.^[16, 17] Even those cultivated plants which primarily serve for the nutrition of man and animals contain many secondary metabolites of proven toxicity (Table 1).^[18–26]

The second reason for the lack of acceptance lies in widespread ignorance over the conditions for production in modern agriculture. Who in our modern industrialized society still faces the battle for yield and quality with which the farmer is continually confronted? With regard to the achievements of agriculture, yield and quality are either taken for

Table 1. A very healthy meal.^[a]

Foodstuff	substance	action
carrots	carotatoxin	nerve poison ^[18]
pepper	piperine	mutagen ^[19]
parsley, celery	psoralen	mutagen, carcinogen ^[20]
raddish, onion, broccoli	thiols, disulfides ^[21]	inhibitors of thyroxin synthesis in the thyroid gland ^[22]
mushrooms	agaritine	promoter of ulcers and cancer in the stomach ^[23]
apples	phlorizin	cause of glycosuria ^[24]
mustard, horseraddish	allylisothiocyanate	cause of damage to chromosomes ^[25]
raspberries	coumarin	liver toxin, ^[26] inhibitor of blood clotting

[a] If the same safety requirements were demanded for "natural substances" or food additives as are applied to synthetic substances, the consumption of foodstuffs would have to be banned!

Jörg Stetter, born in 1945, studied chemistry in Aachen and Munich, gaining a PhD from the Institute for Organic Chemistry at the Ludwig-Maximilians University in 1973 under the supervision of Prof. Gompper. In 1974 he joined the staff of Bayer AG as the head of a crop-protection research laboratory at Wuppertal-Elberfeld. By way of various positions—section head in the Central Research Division in Leverkusen, section head of crop-protection research in Wuppertal and Monheim, and the head of process development in the Crop-Protection Division—he became head of a subject field in 1989 and then, from 1997 onwards, departmental head in the Central Research Division in Leverkusen. Since 1993 he has held an honorary professorship in technical chemistry at the Bergische Universität Wuppertal. He is a committee member of the Liebig Vereinigung für Organische Chemie and a member of the board of governors of the GDCh accreditation agency for studies in chemistry.



J. Stetter



F. Lieb

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granted or even seen as excessive in the present day. The consumer finds cheap, high quality foodstuffs just around the corner in the supermarket at the same time as reading about surpluses which have to be destroyed to maintain price stability. That there is little understanding for the necessity of crop-protection agents should, therefore, come as no surprise.

1.2. The Situation Today

The transformation of agrochemicals to a high-tech industry in the last few years has been almost unnoticed by the general public. In terms of scientific rigour and demand for high quality results, today's research and development in the crop-protection sector can no longer be distinguished from pharmaceutical research and development. Comparable average product-development time spans of 8–12 years speak clearly for themselves. Crop-protection chemicals today are amongst the most thoroughly investigated substances with which man and the environment come into contact. This is a fact which cannot seriously be called into question. Worldwide, there is an impressive, although dwindling, arsenal of active ingredients from which to draw, and more and more is known about them. This is because even older active ingredients are regularly tested according to the latest safety criteria in the course of re-registration procedures.

1.2.1. The Transparent Active Ingredient

The days when all that was known about a pesticide was the chemical structure and the observed beneficial effects are long gone. For example, when DDT was first introduced for crop protection, many of its properties were unknown; it was, so to speak, a “black box” (Figure 3).^[27] Today the box is transparent, packed full of the latest scientific know-how in various disciplines and data generated by selected experts. “Transparency” of the active ingredient means elucidation of all relevant properties of a substance. Determination of the environmental fate of the substance and its effects in an ecosystem provides an interesting challenge for scientists from a wide variety of disciplines. How is the substance taken up and transported? How efficacious is it against the target organism and what is its mode of action? How is it broken down by plants, by animals, in water, in the soil, and in the air?

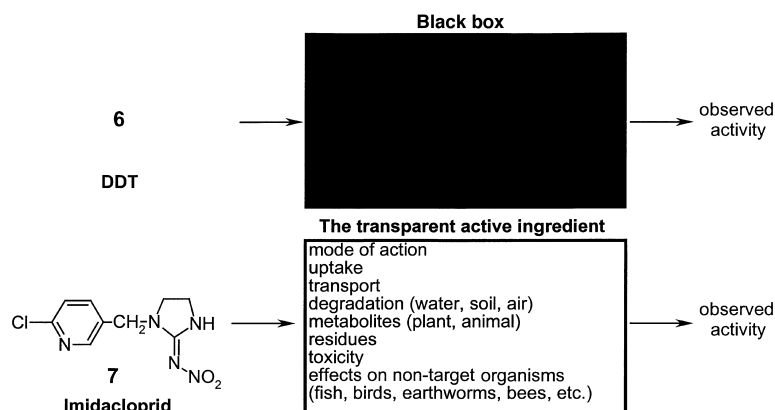


Figure 3. The transparent active ingredient.

Which metabolites are formed? Which residues remain? Which side-effects occur in so called “non-target organisms”? What is the toxicological profile of the substance? An army of dedicated scientists investigate these and many more questions, using the most modern methods of analytical, physicochemical, toxicological, ecological, and biological sciences, including genetically based biotechnological techniques.

1.2.2. Pressure of Costs and Market Perspective

Fascinating as all this is, the costs for the research and development of an active ingredient and suitable forms of application (formulation) have skyrocketed as a result. Today they lie in the order of 250 to 300 million DM, dimensions not far removed from the costs incurred in the development of a pharmaceutical product. However, unlike the pharmaceuticals sector, in which a single market segment such as anti-infectives has a turnover of 40 billion DM (equivalent to some 80% of the total value of the market for crop-protective agents), the segments of the agrochemicals market are substantially smaller, which severely limits the level of research and development expenditure which can be tolerated. Added to this, problems of reduced yield and quality of crops caused by pathogens, weeds, and pest animals can today in principle be more or less satisfactorily solved using existing agents of crop protection. As a rule, the introduction of a new active ingredient leads to displacement competition following the motto “the better is the enemy of the good”. Better can mean many things, for example, cheaper for the user, more favorable toxicological and ecological properties, more easily broken down, less leaching behavior, a breakthrough against resistance, reduced application rates, additional systemic properties, favorable properties for beneficial organisms, or more selective. New active ingredients which open up additional markets are extremely rare.

In the pharmaceuticals field, the perspectives are quite different due to the many hitherto unsolved disease problems. New markets can be established here extremely quickly as soon as an innovative product becomes available (the most recent example is Viagra).

Products are no longer developed specifically for a particular niche pest because of the high developments costs for an active ingredient. All the research-based crop-protection companies concentrate their efforts on the large markets.

The most sought after crop-protection products are those with a large potential market.

No one can sensibly argue with the fact that crops the world over will also need to be protected in order to secure yields in the future. The dynamics of world population growth allow no other choice. Even medium-term predictions demonstrate the need to act (Figure 4).^[28]

2. The Future of Research and Development

The remarks so far have attempted to clarify the framework within which current and future devel-

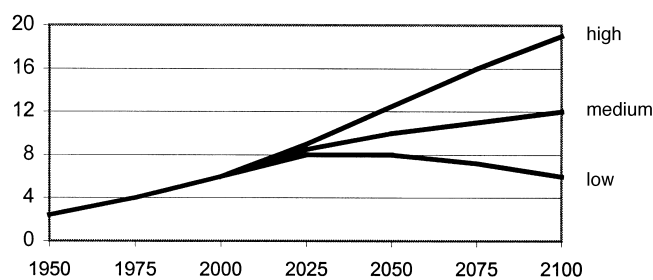


Figure 4. Previous development and predicted growth in world population.

opments in crop protection must operate. Crop-protection companies have adjusted to this with appropriate strategies for research and development. Innovation and efficiency are the magic words. Under the theme innovation and current trends in research and development, various hypotheses can be listed which will be expanded upon and illustrated with examples in the following sections.

Hypotheses for the Future

- Chemical methods will play a dominant role until well into the twenty-first century
- There will be no revolutionary change, established technologies will develop further by a process of evolution
- The process of research and development for new active ingredients will become substantially more efficient, especially through modeling, miniaturization, automation, and electronic data processing in key operations
- There will be fewer, ever safer, “bespoke” active ingredients which will be developed into saleable products
- Improved formulations and application techniques will allow a more targeted use of active ingredients, resulting not only in greater efficiency but also increased safety for the user, the consumer, and the environment
- In the context of integrated pest management, non-chemical methods will increasingly play a supporting role alongside chemical methods
- The introduction of transgenic crops, especially those resistant to herbicides and insect pests, will lead to sometimes substantial shifts in the affected markets
- Predictive and diagnostic methods will be developed much further (the concept of damage thresholds)
- Education and training of users will play an increasingly important part

It should however, be reiterated that ‘chemical methods’ means those that use low molecular weight active ingredients generally, regardless of whether these are of synthetic origin or are natural products, whether they are single defined substances or mixtures, and whether they have a killing or a moderating mode of action. Stated in plain English: the use of pheromones, neem extracts, tobacco juice, and stinging nettle brews is nothing other than chemical pest control.

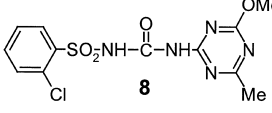
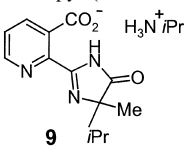
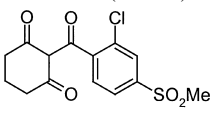
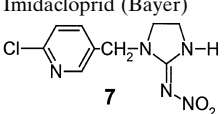
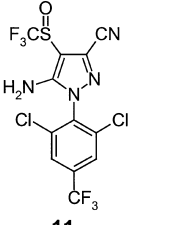
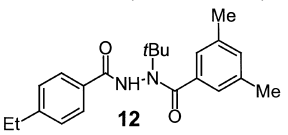
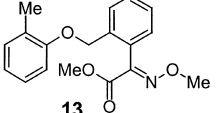
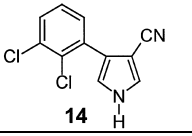
3. The Significance of Synthetic Chemistry for Modern Crop-Protection Research

Synthetic chemistry has not exhausted its ammunition by a long way. As the observation of new patent applications

shows, the flood of new lead structures to be pursued has not yet stilled. Table 2 is a selection of interesting and important classes of structures from the 1980s and 1990s, which have yielded new and valuable solutions to problems. For every class there is an interesting story behind the invention and the inventor. It is remarkable how often luck has played a part.

It is neither to be expected or feared that the stream of valuable new lead structures and classes of active ingredients will dry up sometime in the future. The possibilities for variation offered by modern synthetic chemistry are too

Table 2. Important structural classes from the 1980s and 1990s.

Class of structure	active ingredient	indication	mode of action
sulfonyl ureas ^[29]	Chlorsulfuron (DuPont) 	herbicide	acetolactate synthase (ALS) inhibitor
imidazolinones ^[30]	Imazapyr (American Cyanamid) 	herbicide	acetolactate synthase (ALS) inhibitor
tri-ketones ^[31]	Sulcotrione (Zeneca) 	herbicide	4-hydroxyphenyl-pyruvate dioxygenase inhibitor
nicotinoides ^[32]	Imidacloprid (Bayer) 	insecticide	agonist of the nicotinic acetylcholine receptor (n-AChR)
arylpyr-azoles ^[33]	Fipronil (Rhône Poulenc) 	insecticide	GABA-sensitive chloride-channel (antagonist)
benzoyl-hydra-zine ^[34]	Tebufenozide (Rohm & Haas) 	insecticide	ecdysone agonist
MOAs ^[35]	Kresoxim-methyl (BASF) 	fungicide	inhibitor of cytochrome reductase (in the respiratory chain)
pyr-roles ^[36]	Fenpiclonil (Novartis) 	fungicide	inhibitor of glucose phosphorylation linked to transport; inhibitor of protein kinase PK III

numerous. With the aid of techniques of automated synthesis, it should be possible to probe the expanse of chemical diversity more effectively than was possible in the past.

3.1. Successful Structural Motifs

It is noticeable that there are tried and tested structural motifs which repeatedly occur in successful active ingredients. One of many examples is the pyridine heterocycle. Table 3 shows several active ingredients containing the pyridine building block, none of which have a common mode of action and all of which have different target indications.

Frequently it is not simply the basic chemical units or the templates, in the parlance of the synthetic chemist, but often the presence of very specific substituents in certain selected parts of the molecule, which are decisive in determining the biological activity. In view of the huge, continuously increasing diversity of the chemical building set, it is obvious that many interesting classes of active ingredient await discovery. Just how the tiniest variation in substitution pattern can separate high from negligible activity or even activities in completely different indications, is illustrated by the examples shown in Figure 5. They also show, however, that in the search

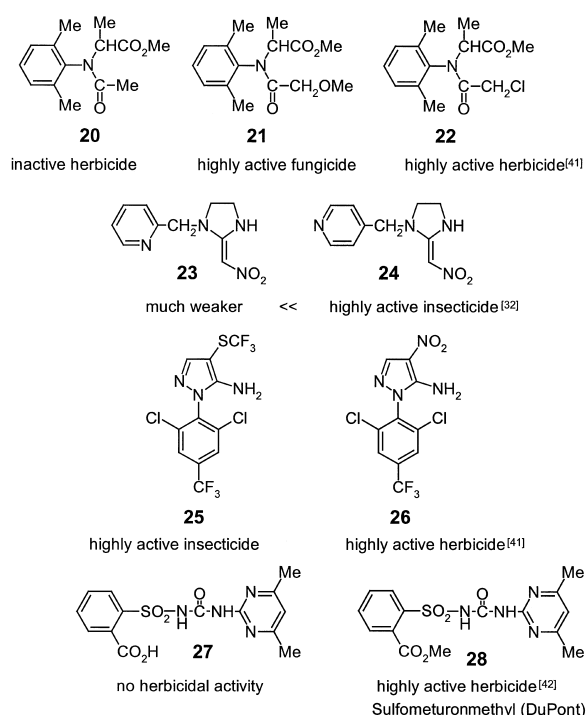
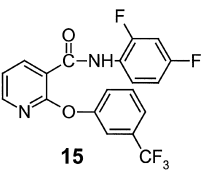
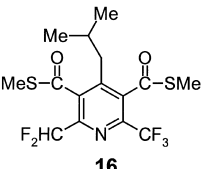
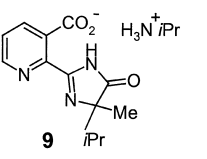
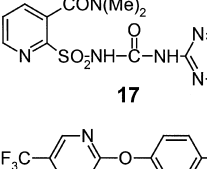
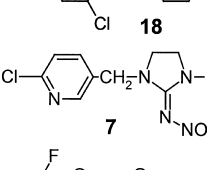
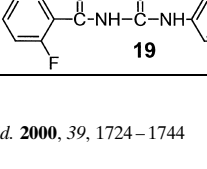


Figure 5. Chemical diversity isn't everything!

Table 3. New commercial products from the 1980s which incorporate a pyridine component.

Product	active ingredient	indication	mode of action
Diffenican ^[37]	 15	herbicide	inhibitor of carotinoid biosynthesis
Dithiopyr ^[38]	 16	herbicide	inhibitor of mitosis
Imazapyr ^[30]	 9	herbicide	acetolactate synthase (ALS) inhibitor
Nicosulfuron ^[29]	 17	herbicide	acetolactate synthase (ALS) inhibitor
Haloxyp ^[39]	 18	herbicide	inhibitor of acetyl-CoA carboxylase
Imidacloprid ^[32]	 7	insecticide	agonist of the nicotinic acetylcholine receptor (n-AChR)>
Chlorfluazuron ^[40]	 19	insecticide	insect growth regulator, inhibitor of chitin biosynthesis

for a lead structure, an absolute prioritization based on chemical diversity can lead in the wrong direction. The real goal must be to find successful structure clusters in the universe of imaginable, low molecular weight, chemical compounds.

3.2. The Obligation to Make an Early Selection

More important in the future than ever before will be the ability for a successful, research based crop-protection company to separate the wheat from the chaff early on and to proceed with the right lead structures and candidate compounds. The key words for such a procedure are more efficient generation and identification of lead structures, and earliest possible and targeted optimization of all parameters having a bearing on development within an integrated concept. Very early knowledge about possible problems regarding toxicity, ecotoxicity and behavior in the environment are essential to-

day. The establishment and assessment of appropriate high performance, meaningful, early test systems is underway everywhere. Only when these yield the necessary certainty that the right choice has been made, can the real development work of field trials throughout the world, process development, wide-ranging experiments on animals, studies on metabolism, and the investigation of residues be justified.

3.3. Everything Revolves around the Lead Structure

The creed of the researcher seeking an active ingredient is that, at the beginning stands the lead structure. The lead structure is a defined chemical substance arising from synthesis or from a pool of natural substances, which demonstrates activity considered worth pursuing in appropriate biological tests. The more relevant a particular test within a screening cascade is to the practical application, the more valid the tested lead structure (Figure 6).

3.3.1. The Screening Cascade

The result from a tried and tested screening system based on whole organisms (insect, plant, or fungus) is of course much closer to real life than an *in vitro* target assay (enzyme, receptor, or ion channel). In the former, the effects of absorption, transport, and metabolism have already been

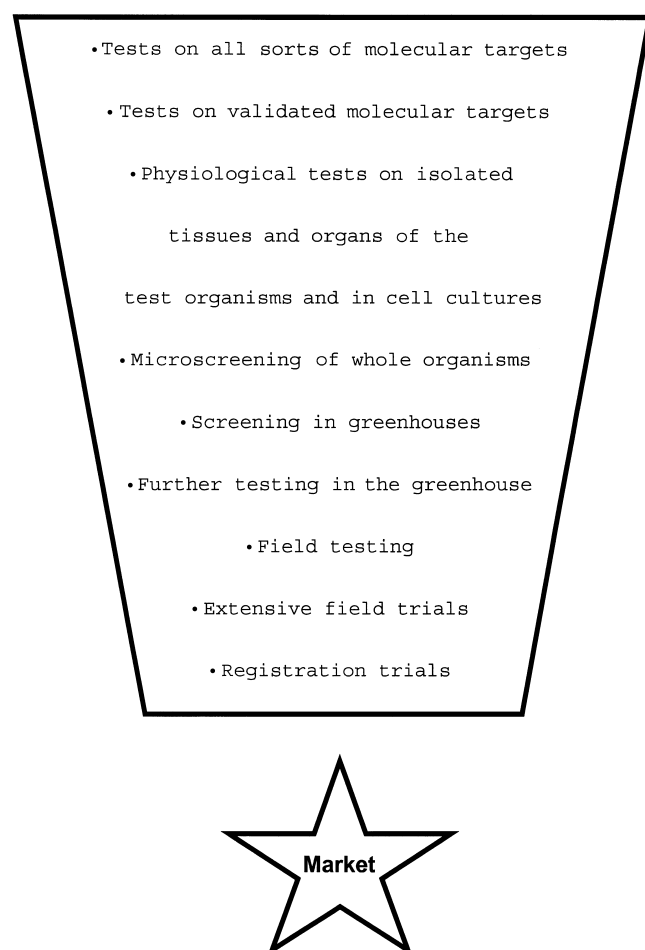


Figure 6. The screening cascade.

taken into account. In principle, all potential targets are also tested, including those hitherto unknown. In target screening, only those substances which interact with a particular target can be found. Target screening can, however, provide valuable, additional information on shifts of activity within a class of substance or on possible toxicological risks.

There is an inverse proportional relationship between the proximity of a test to the real life situation and the number of substances which can be tested with justifiable financial expenditure. One, therefore, tries to use the tests efficiently depending on the number and quality of the test substances available.

Essential as it undeniably is, the lead structure is only the very beginning of the route to a marketable active ingredient. As a rule, in the process of structure–activity optimization, hundreds and often thousands of related substances have to be synthesized in order, if successful, to identify a candidate for product development which can then be taken through to a state of readiness for the market.

A high degree of instinct and intuition is demanded of the pesticide chemist in making the very important strategic decision: Is it at all worthwhile to pursue a lead structure with weak activity? Has the structure perhaps already arrived on a plateau of activity from which no further increase is possible? It then certainly requires the healthy optimism of creative and imaginative chemists and biologists to choose this rather than another lead to follow.

3.3.2. How are Lead Structures Found?

How does one find a lead structure? In answering this central question of active ingredient research, there have been several major changes in paradigm over the last few decades (Table 4).^[43] In the early phase of active ingredient research in the crop-protection industry, the costly, labour-intensive biological screening in greenhouses with a wide range of target

Table 4. At the beginning stands the lead structure.... From random chance to design and back to random chance.

The past (pre 1980)	the vision of the 1980s	the current trends
• costly greenhouse testing • gram quantities of the test substance • virtually no automation	• known biological targets • receptor–inhibitor models (lock & key hypothesis) • de novo design	• all sorts of targets • high-throughput screening (HTS) • automation
↓	↓	↓
• limited testing of natural products • random: chemistry from various sources	• testing limited to designed molecules	• bottleneck: chemical synthesis of test substances • solution: automated synthesis, combinatorial chemistry, molecular diversity
↓	↓	
• bottleneck: greenhouse testing	• deficiencies at the relevant targets • lead structures may not be active <i>in vivo</i>	

organisms was the bottle neck in the process of searching for a lead structure. Due to the high application rates required in those days, several grams of the test compound were needed to conduct a comprehensive primary screening in all indications. All this stood in the way of a broad testing of random substances from a variety of sources (including substances from institutions of higher education and natural substances). Although working in parallel and automation of procedures were known in biological screening in rudimentary form, they were still unheard of in chemical synthesis.

Progress in biochemistry, molecular biology, and electronic data-processing led to the vision of the 1980s; the magic words were rational molecular design and molecular modeling. Based on the well known “lock and key” hypothesis for biological activity and with knowledge about the three-dimensional structure of the biological target, it should be possible after all, using molecular modeling, to design active ingredients with ideal properties of fit as potential effectors.

Many saw the role of the active-ingredient chemist reduced, as it were, to that of “cook’s servant”, who now merely had to make a few bespoke substances. From this collection, in depth biological testing should very quickly be able to select the candidate for development. A nice new world with relatively bad prospects for the synthetic chemist, or so it appeared.

That this could not work was actually already clear to experienced pesticide researchers at the time. First, there was too little of the necessary information about the relevant targets to allow *de novo* design. In addition, the *in vitro* activity at the level of the isolated enzyme or receptor is usually far removed from the *in vivo* activity of a real active ingredient against the plant, insect, or fungus target organism. In short, *de novo* design has so far not succeeded in generating lead structures for the chemical crop-protection industry.

Indisputable however, is the usefulness of molecular modeling as a method for lead structure optimization.^[44] A quantitative structure–activity relationship (QSAR) tool already considered a classic is the Hansch analysis, which allows biological activity to be described in terms of the contribution made by physicochemical properties of individual substituents.^[45] A refinement is comparative molecular field analysis (CoMFA) in which the 3-D field properties of the molecule are linked with biological activity to allow estimates of the biological activity of suggested structures.^[46]

The “active analogue approach” is a widely used method which, by comparison of biologically active substances which are structurally diverse but which share a common mode of action, allows the essential common structural properties to be identified. These so called pharmacophores then serve as a template for the design of a new active ingredient.^[47]

For several years now, the pendulum of lead structure generation has once again swung towards random chance. The ever-less favourable statistical quotient of the chance of finding a hit should henceforth be ‘tricked’ by a kind of bombardment with countless, highly diverse chemical entities. Through progress in genome research, the number of disease-relevant biological targets which are available in the form of an assay has exploded. In high-throughput screening and lately also ultra-high-throughput screening (HTS, UHTS), the bombardment mentioned above can be coped with, since

several 100 000 substances can be tested in an assay in a few days.

An important question for the research-based crop-protection industry in recent years, which has been debated intensively and is also partly controversial is: Can this procedure for finding lead structures derived from pharmaceutical research be transferred to crop-protection research and should one join this shift in paradigm? The answer, at least from the large companies, was initially hesitant, then however, a clear commitment to the additional chances offered by the modern assay formats was made. This, however, did not call into question the classical screening model. One crucial difference between pharmaceutical and pesticide research should not be overlooked: while the former is compelled to rely on biochemical and molecular biological assays for the generation of lead structures, in crop protection one can, so to say, conduct tests directly on the patient. The target organism plants, fungi, and insects are relatively easy and inexpensive to rear in large numbers and are not subject to restrictions comparable with laws to protect higher animals. The test substance is applied to all targets at the same time, including those that are completely unknown. In contrast to pharmaceuticals, the target sites have to be such that an interaction with them neutralizes the target organism; the best reaction of all is when this interaction has a lethal effect. The aim in the end is, after all, to prevent further damage.

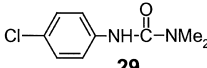
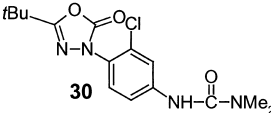
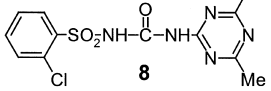
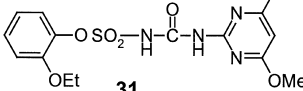
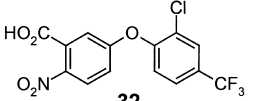
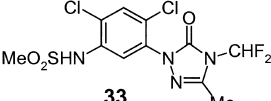
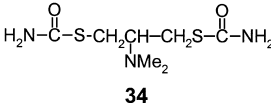
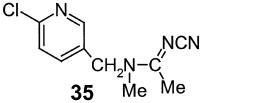
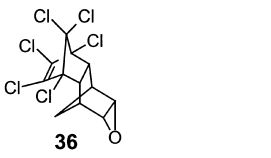
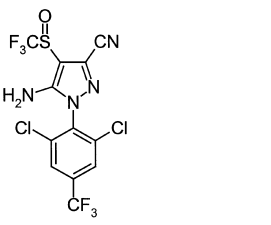
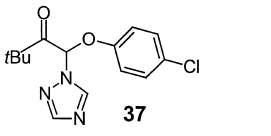
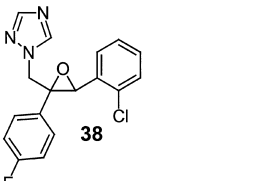
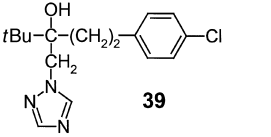
Additionally, the throughput of *in vivo* screenings today is incomparably higher than it was only a few years ago. Besides traditional greenhouse screening on a wide set of weeds and pest organisms, automated medium-throughput screening (MTS) with a few carefully chosen organisms, can be used in advance to select potential lead structures. The amounts of compound required lie in the range of a few milligrams.

For this reason *in vitro* HTS is particularly appropriate in two cases. The first is where analogue chemistry in tried and tested classes of structure brings no perceptible progress. In these cases it is of course tempting to bombard the underlying targets with large, diverse libraries of compounds once again. The hope is to discover completely new classes of substances. Here too, there is a substantial difference to the pharmaceutical industry. Targets in crop protection grow old only very slowly, if indeed at all. While a new mode of action can serve to break down resistance and can be a useful argument for the registration and sale of a new active ingredient, other parameters for the substance such as toxicity, biological selectivity, how easily it can be produced, and application rates play a much more important part. In the end after all, it is the user, the farmer, who decides about its use and for him it is the economical viability which is clearly of paramount concern. Simply stated: mechanisms of action which may already have been known for decades are not “out” just because there are various different products available on the market. Even today, a photosynthesis inhibitor which acted at low application rates, is selective for important crops, has good activity against problem weeds, is cheap to produce, and has good toxicological and environmental properties, would have a very good chance. This is despite the fact that there have been photosynthesis inhibitors for 50 years and more

than 50 different active ingredients from this class have been developed as saleable products.

The azole fungicides which act by inhibition of ergosterol biosynthesis are another of many examples. More than 15 years after the market launch of the first representatives, Epoxiconazole (Opus) from BASF and Tebuconazole (Follicur) from Bayer are two real blockbusters which have come onto the market. Examples for the longevity of mechanisms of action are shown in Table 5.

Table 5. Sites of action in crop protection.^[a]

Site of action	active ingredient	
	early	late
photosynthesis (PSII)	 29 Monuron ^[48] (1951)	 30 Dimefuron ^[49] (1978)
acetolactate synthase (ALS)	 8 Chlorsulfuron ^[29] (1982)	 31 Ethoxysulfuron ^[50] (1996)
protoporphyrinogen oxidase (PPO)	 32 Acifluorfen ^[51] (1979)	 33 Sulfentrazone ^[52] (1995)
nicotinic acetylcholine receptor (n-AChR)	 34 Cartap ^[53] (1965) (antagonist)	 35 Acetamiprid ^[54] (1995) (agonist)
GABA-sensitive chloride-channel	 36 Dieldrin ^[55] (1954)	 11 Fipronil ^[53] (1994)
Ergosterol biosynthesis (C ₁₄ -Demethylation)	 37 Triadimefon ^[56] (1976)	 38 Epoxiconazole ^[57] (1992)
		 39 Tebuconazole ^[58] (1988)

[a] Years of launch are given in parentheses.

The second procedure to be discussed will mean considerable rethinking and probably a change in paradigm. The approach to find potential, hitherto unknown points of attack by the analysis of genomes of pest organisms is a very interesting approach but also filled with risks. In the last few years a great deal of activity has begun here. The large research-based crop-protection companies have all got collaborative projects with new biotechnology companies in the field of genome analysis (Table 6).^[59] It is hoped that by doing

Table 6. Collaborations between genomics companies and crop-protection companies.

Genomics company	crop-protection company	year
Acacia Biosciences	Cyanamid	1998
	DuPont	1998
	Novartis	1998
CuraGen	DuPont	1998
Exelixis Pharmaceuticals	Bayer	1998
Gene Logic	AgrEvo	1998
Lion Bioscience	Bayer	1998
Lynx Therapeutics	BASF	1996
	DuPont	1998
Paradigm Genetics	Bayer	1998

so an important gap will be closed. Experts predict that up to several hundred targets of relevance to crop protection will be found.

There is no doubt that through the use of these targets, screening of large compound libraries will yield hits. That it is an extremely long way from such a hit to an active ingredient with a convincing overall profile in every respect is, however, crystal clear. In a favourable case, the hit identified by target screening will also have detectable activity in the later greenhouse test. Then there is clearly a motivation to undertake chemical optimization work. The decision is, by contrast, particularly difficult when there is no *in vivo* activity. In this case a programme of chemical work only makes sense if there are really viable concepts to overcome this dilemma. Unlike a medicine, a crop-protection agent cannot be applied to the crop orally or intravenously to increase the level of bioavailability to the target organism.

The securing of the expenditure incurred through skilled intellectual property management will be of particular strategic importance. However, the competition doesn't sleep either and, at the very latest, they "jump on the band wagon" when patents to a new class of structure have been published and try to find and develop patent-free active ingredients in the general area of the patent. Experience has shown that no company has so far managed to secure a monopoly over a class of structure and that often it is the late entrants in the game that carry away the big prizes (For example, Opus (Epoxiconazole) from BASF).^[57]

4. Combinatorial Chemistry

Whether they are called MTS, HTS, or UHTS, all these "hungry" test systems require large numbers of compounds. In an analogous situation to pharmaceuticals, it is the

chemical synthesis in crop-protection research which represents the bottleneck in the search for a lead structure. Classical synthesis with an annual output of 200–300 new compounds per laboratory cannot meet the requirements (Figure 7).

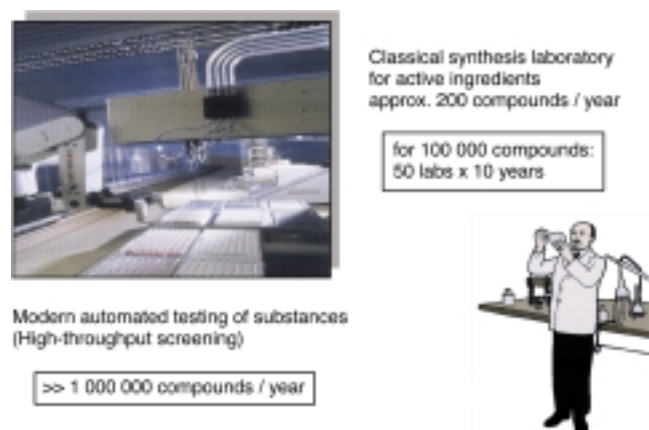


Figure 7. The challenge of high-throughput screening (HTS).

In response to the challenge of HTS, the new technology of combinatorial, automated synthesis was created several years ago. The approach has enjoyed uninterrupted popularity and is still developing at a rapid rate.^[60] The basic idea is to combine small sets of synthetic building blocks to make libraries of modularly constructed molecules in large numbers (Figure 8).

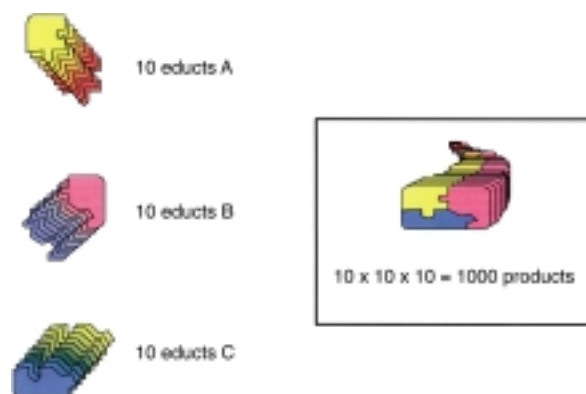


Figure 8. The combinatorial approach.

Trends in Combinatorial Chemistry

The emphasis in combinatorial chemistry has changed dramatically in the last few years. While the first libraries still impress by their immense numbers (often from 100 000 to 1 000 000) of compounds synthesized as mixtures, the trend in the meantime is towards smaller libraries of compounds. Collections of single substances deliver more meaningful test results and because the active molecular structures can more easily be identified, the process of following up hits is speeded up. The smaller number of compounds limits the structural diversity of such a library. Computer-aided analysis of diversity is used in an attempt to optimize this process—more intelligent design of libraries (“smarter” libraries) replaces astronomical numbers. Indispensable for the design of such

libraries is the knowledge of experienced pesticide researchers in making successful active-ingredient templates. At the same time, the realisation is being made that physicochemical parameters should also impact on the design of a library and that these should be investigated in HTS (Figure 9). Therefore: quality libraries instead of mass production!

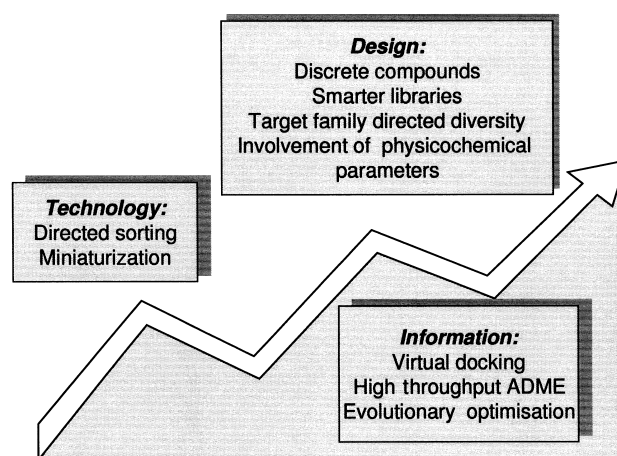


Figure 9. Trends in combinatorial chemistry.

What future has combinatorial chemistry at the threshold of the new millennium? The basic concept that producing chemical diversity can speed up the discovery of substances and reactions will undoubtedly become a reality in many areas of organic chemistry. Combinatorial active-ingredient research shows the way towards further miniaturization, chemical evolution, and the merging of synthetic and analytical chemistry, as well as screening. Analytical techniques are already being combined with methods for detection of activity. The challenge for chemistry will be overcoming the ever-narrower barriers separating parallel-working, automation, and miniaturization. In this context the original solid-phase method is today increasingly becoming augmented by combinatorial synthesis in solution. To summarize: crop-protection research has also embraced the modern or enabling technologies in the process of finding new active ingredients, albeit without doubting the worth of the established approaches.

Understandably, there is no success story to report so far. Only the future will show if the game can really be won with large numbers and whether it is not merely more hits, but ultimately also innovative solutions for the challenges of crop protection, which result.

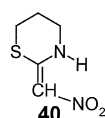
5. Successful Classes of Active Ingredient

Two classes of active ingredient from the recent past, which are particularly good examples for innovation in crop protection, were found by classical routes, either through chemically driven research centred around examples from the patents of competitors, such as the case of the neonicotinoids, or through optimization of a lead structure from natural product screening, such as in the case of the methoxyacrylates (Strobilurin). Both will be briefly presented here.

5.1. Neonicotinoids

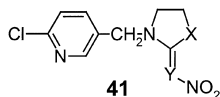
A belief in the possibility to optimize a class of structure, which at the end of the 1970s, on the basis of unsuccessful optimization attempts up to that time, had been deemed as hopeless, was the driving force behind the discovery of a new active ingredient by Bayer, which since then has become the insecticide with the largest turnover in the world.

Arising from a program of chemistry with an entirely different goal, the nitro-substituted ketene aminationals, discovered by Shell in the 1970s were found to act as insecticides and were worked on over a broad front. At the time nithiazin was identified as a development candidate, but it was never launched as a product in the end.^[61] In the early 1980s chemists at Bayer's subsidiary company in Japan started syntheses centered around the nitromethylene lead structure once again. The introduction of a special substituent, the chloronicotiny residue, and its coupling with the new nitro-guanidine building block, brought the breakthrough to a highly potent, light-stable, low-toxicity, widely applicable, and above all systemic group of insecticides, with Imidacloprid **7** as the most interesting candidate for development (Figure 10).^[32] By virtue of its marked systemic properties, **7** is eminently well suited for use in the most environmentally friendly form of application, the seed-coating treatment.



Nithiazine (Shell) ^[61]

Discovery of 2-Chloro-5-methylpyridyl-substituents:



a: X = S, NH
b: Y = CH, N
(CN)

Highly active, light-stable, low toxicity, systemic insecticide

Imidacloprid	NTN 33893
7	Confidor Gaucho (125-250g/ha)

Figure 10. Early in the 1980s, chemists at a Bayer's subsidiary company in Japan began research based on the nitromethylene lead structure once more.

Imidacloprid **7** is also very interesting from the aspect of its mode of action. Figure 11 shows a highly schematic diagram of the cholinergic synapse in the central nervous system. The sites of action of nearly all major insecticidal products are to be found here. While pyrethroids act on the presynaptic sodium channels and carbamates and organophosphates are inhibitors of acetylcholinesterase, **7**, a representative of the neonicotinoids, acts on the nicotinic acetylcholine receptor, like the very small group of so-called nereistoxin analogue products available on the market.^[62]

With complete justification one can say that **7** is the first really widely useable, relevant product which exploits the mode of action described. As a result, insect pests which have developed resistance to insecticides from the established

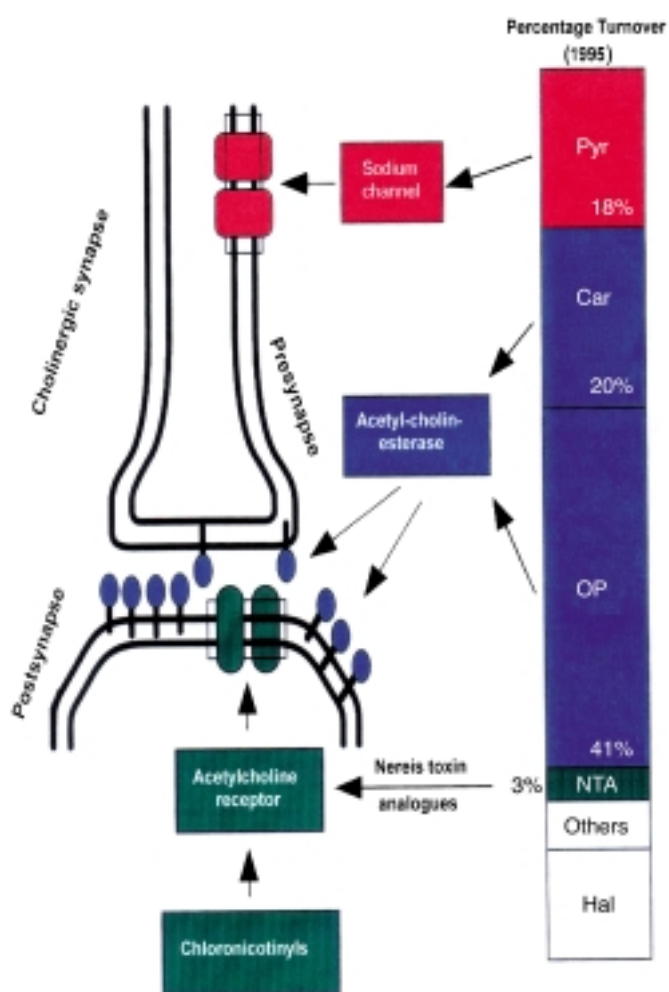


Figure 11. Mode of action of the most important classes of structure. Pyr = pyrethroids, Car = carbamates, OP = organophosphates, NTA = nereis toxin analogues, Hal = halogenated hydrocarbons.

major insecticide classes respond in a completely susceptible manner to **7**. An additional advantage lies in the selective toxicity of **7**. According to the current state of knowledge, this is attributable to the fact that the mammalian acetylcholine receptor is considerably less sensitive to imidacloprid than that of insects.^[63]

The chloronicotiny residue is a particularly important structural element for Imidacloprid and related compounds (Figure 12). At first sight it appears to be a typical chlorinated heterocycle out of the chemist's "synthetic kitchen". All the more surprising therefore, that mother nature seems to have discovered this moiety a very long time ago.^[64] In 1992 a compound, which was named epibatidine, was isolated from the skin of a South American frog. Amazingly this poison from the frog contains the chloronicotiny residue. It acts as a strong analgesic and is also an n-AchR agonist. Thus, there are particularly interesting interrelations between natural substances such as nicotine and epibatidine, which taken together with knowledge about the activity of the Shell nitromethylenes could very much earlier have led to the synthesis of Imidacloprid.

The fact that other companies have not been sleeping is demonstrated by other products which have arisen from

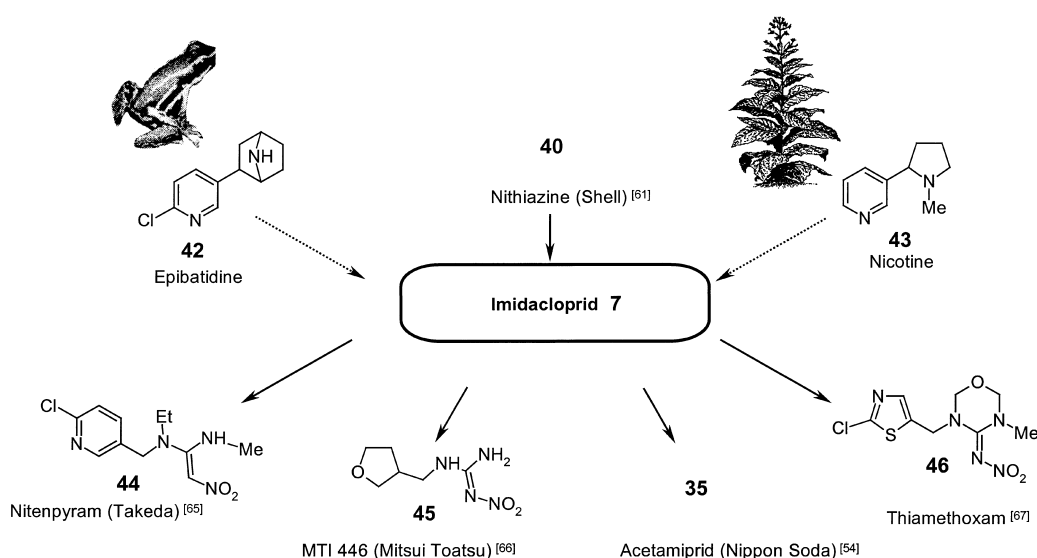


Figure 12. Development of the insecticidal nicotinoids.

analogue research centred around Imidacloprid. Besides the chloronicotinyl residue, the closely related chlorothiazoylmethyl residue and, surprisingly also, the tetrahydrofuranylmethyl residue have proven their worth. The neonicotinoids are on the way to establishing themselves as a significant new class of structure in the insecticides market. The extent of the market share which they will ultimately attain is as yet unknown. One does not, however, have to be a prophet to predict a bright future for such particularly environmentally friendly products as the neonicotinoids.

5.2. Methoxyacrylates (MOAs)

Time and again, both in pharmaceuticals research and in crop-protection research, the screening of pools of natural substances from all over the world and especially of those derived from plants, fungi, marine organisms, and microorganisms has yielded lead structures which were worth pursuing or occasionally even ones which could successfully be developed. As already mentioned, the new possibilities of MTS and HTS screening should open up additional possibilities. For various reasons (availability, costs, stability) the natural substance itself is rarely used in crop protection. However, very recent examples such as Avermectin, a product against spidermites, and Spinosad, which has excellent activity against important lepidopteran species, show that even this is possible from time to time. However, as a rule, improved synthetic analogues of the natural substance are commercialized. The synthetic pyrethroids derived from the natural insecticidal pyrethrum extract are surely the most prominent examples for the particularly successful handling of a natural substance in the past.

Similarly, a new success appears to be developing where a fungicidal lead structure from nature was followed, the synthetic analogues of the strobilurins. More than 30 of these strobilurins and the related oudemansins and myxothiazols have so far been isolated and characterized.^[35]

Seen from a chemical perspective these are derived from β -methoxyacrylic acid (Figure 13). The strobilurins and oudemansins are constituents of several small fungal species, such as *Oudemansiella mucida*, which typically grow on beech trees (Figure 14).

As we know today, the fungicidal mode of action of the strobilurins is attributable to inhibition of mitochondrial electron transfer brought about by blocking a specific cytochrome b binding site.

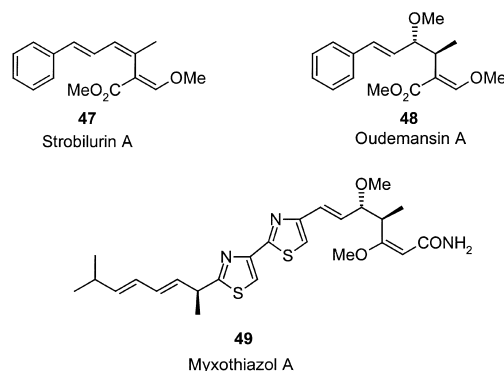


Figure 13. Natural fungicides derived from β -methoxyacrylic acid.



Figure 14. *Oudemansiella mucida*.

Apart from the strobilurins, only the newly introduced active ingredient Famoxate has the same mode of action.^[35] Thus, there is only a very low risk of cross-resistance. The MOAs are remarkably safe, despite a mode of action which, in theory at least, is toxic for warm-blooded animals.^[68] The natural products were never suitable as agricultural fungicides. Apart from inadequate availability, their low light stability, similar to the first Shell neonicotinoids, and too high volatility were the factors responsible.

The process of optimizing the Strobilurin A lead structure proved a slow-moving business for the companies BASF and Zeneca. Steglich and co-workers had already been able to show that making the Z-double bond more rigid through incorporation of a phenyl ring could bring about a breakthrough in terms of the light stability.^[69] Industrial research, initially competitively, between Zeneca and BASF took up this idea. From several cycles of optimization, the landmarks of which are noted in Figure 15, arose the first two marketable

process of innovation and development is today conducted simultaneously across a broad front and over all the scientific disciplines involved.

6. Alternative Methods of Control

6.1. Semiochemicals

So far in this paper the emphasis has been on progress to be expected in the classical field of pest and weed control by means of chemical synthesis. Nature however, has a rich treasure trove of compounds which mediate communication between organisms of the same species and between organisms belonging to different species—the so-called semiochemicals.^[70] The concept of using these substances to control pests, especially insects, is not new and, as an idea, it is fascinating. Research has been conducted in this field throughout the world and with the expenditure of considerable scientific effort for decades. Pheromones, antifeedants, and repellents have been the most frequently chosen objects for this research. In spite of all the efforts, only a very few areas of application have been found to date. In agricultural practice, aggregation and sex pheromones have predominantly been employed in traps to predict pest-populations, for the purposes of mass-trapping, or in the context of a mating-disruption approach.

Without a doubt, in future the use of semiochemicals will increase in the context of integrated crop protection. A few general advantages are worth mentioning: high species specificity with the potential to avoid harm to beneficial species, low toxicity, small amounts of the active substance, low persistence, hardly any problems with environmental residues, and the possibility for a mixed application strategy with reduced insecticide application rates acting as a synergistic and resistance-lowering method of treatment.

The development of semiochemical products requires enormous effort, especially at the point where results from the laboratory have to be transferred into the field. At least the tools to achieve this end will improve markedly through progress, particularly in the fields of molecular biology, behavioral biology, and electrophysiology. The highly volatile nature of semiochemicals will, however, require the development of technically highly sophisticated release systems. An intelligent approach from the standpoint of drawing the insect to the poison in a targeted way rather than bringing the poison to the insect, the so-called “attract and kill strategy” will briefly be discussed here (Figure 16). In the mating-disruption control strategy practised with pheromones until now, the crop which was to be protected was enveloped in a cloud of pheromone emitted from suitable release devices. As a result, male and female insects fail to meet, and copulation is, for the most part, prevented from occurring.

From results so far, the attract and kill strategy, in which the insect is led to the poison, seems more promising. The pheromone and the insecticide are both mixed into a highly viscous formulation. In the fruit orchard the formulation is then applied selectively to relatively few points on the fruit tree, for example (Figure 17).

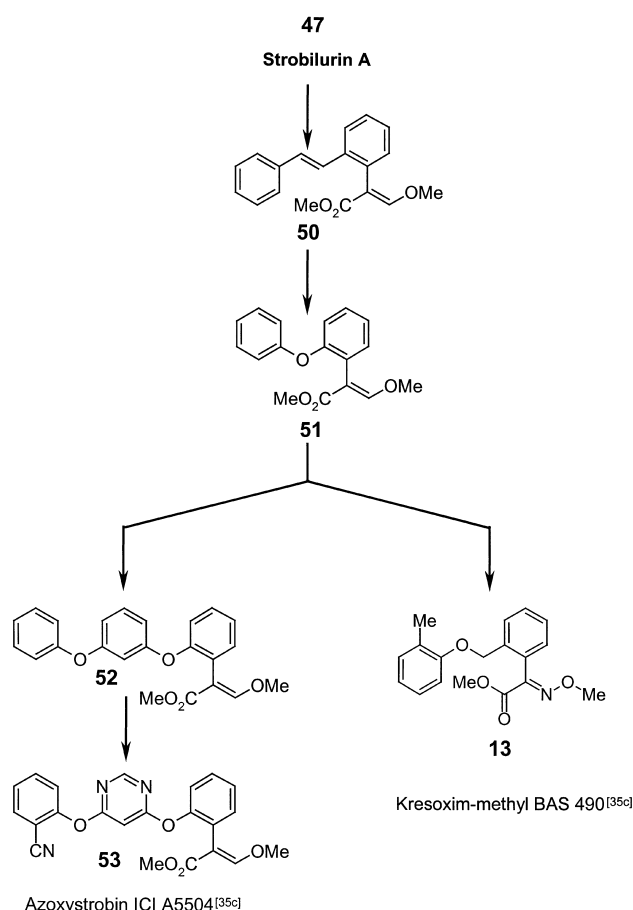


Figure 15. Evolution of the structure of Strobilurin A to Azoxystrobin und Kresoxim-methyl.

products: Azoxystrobin from Zeneca and Kresoxim-methyl from BASF. Interestingly, in the latter the methoxyacrylate toxophore is replaced by the bioisosteric methoximinoacetate residue. Both can be used in a very flexible way against a broad spectrum of fungi, in a wide range of crops such as rice, grapes for wine, fruit, and vegetables. This is a new promising class of fungicide which in the new millennium may gain equal prominence to that enjoyed by the azoles.

The neonicotinoids and methoxyacrylates have been selected here as being representative for a variety of other new developments in the area of active-ingredient research in the crop-protection industry. These examples illustrate just how important the element of chance is at the “cradle” of a new class of active ingredient following its birth, and how much a concerted scientific effort can promote the success of the optimization process to arrive at the ideal solution. The whole

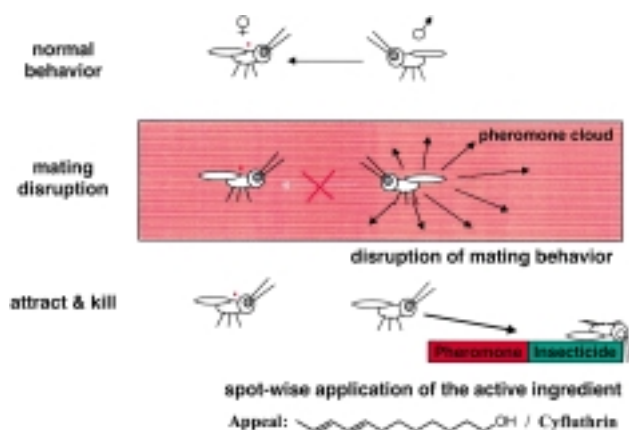


Figure 16. Behavioral manipulation of insects—the “Attract and Kill” strategy.

Attract and Kill

- Advantages:

- intelligent combination of pheromone and insecticide
- species specific
- targeted application
- no spray drift
- protects beneficial organisms

- Disadvantages:

- each formulation combats only one species
- difficulties in acceptance of a new concept by growers



Aim: Bring the insect to the insecticide!

Figure 17. Attract and kill—a novel way to control insect pests.

The advantages are self evident: Only the tiniest amounts of pheromone are required and even the application rate of the insecticide used is markedly lower than in conventional spray applications. The method is highly selective, with only the major pest species which is responsive to the particular pheromone applied being controlled. It is a method therefore which should find acceptance in situations where a single pest species dominates and for high-price crops, such as fruit or grapes for wine. Two new products of this type are Sirene (Novartis)^[71] and Appeal (Bayer).^[72]

For the control of plant parasitizing nematodes, the concept of using so-called hatch-inducing factors is very attractive. Inducing synchronized hatching of nematodes at a time in which the host plant is not yet present or in combination with a non-persistent nematicide could provide a very good and practicable solution to the nematode problem.

6.2. Induced Immunity

Substances which enhance the immune defence of the host plant against pathogens, thereby helping to reduce the amount of fungicides applied, stand on the divide between chemical and biological methods of controlling pests and pathogens. Under the influence of so-called elicitors, which are frequently cell wall fragments of a pathogen, the plants stimulate the production of defensive substances called phytoalexins through a signal cascade, thereby preventing infection.^[73] To date a variety of purely synthetic substances are known which can act as resistance inducers. One such product, known by the trade-name Bion, was commercialized by the Novartis company in 1996.^[74]

The wide field of allelopathic substances produced by plants to confer a competitive advantage upon themselves in their habitat can here only be mentioned but not commented on in further depth. The border zone between chemistry and biology addressed here is scientifically highly interesting and will surely provide many surprises yet.

6.3. Biological Products

In journalism at least, much is promised of biological products, occasionally even to the extent that they will largely replace chemically based products. This view is, however, unrealistic and partly also politically motivated wishful thinking. Only in a few cases has it, so far, been possible to complete the transformation from basic knowledge into crop-protection strategies used in practice. Apart from economic obstacles such as the limited market potential, there have been specific problems with the reliability of action, which have prevented a rapid development with a major impact on the crop-protection market.

6.3.1. *Bacillus thuringiensis* Products

By far the majority of insecticides sold under a biological crop-protection label are *Bacillus thuringiensis* (Bt) products.^[75] They have a market volume of about 200 million DM today, therefore, about 2 % of the insecticides market. By way of comparison, in the USA 18% of the area of agricultural land used to cultivate maize in 1998 was already planted with a Bt maize cultivar, 14 % was planted with Bt cotton and 4 % was planted with Bt potatoes.^[76] In future, Bt products will increase in importance, especially through expansion of their possible field of application by means of genetically modified bacteria. The number of species of insect which can be controlled using Bt products has in the meantime increased in an impressive way. The spectrum of activity of Bt products

reaches beyond the Lepidoptera market to encompass Coleoptera and Diptera as well (Table 7).

Regardless of the undoubted success enjoyed by Bt products, their use is limited almost exclusively to high-value crops and niche markets. The reasons for this lie in the biology of

Table 7. Commercial Bt products.

Pest	market or target organism	strain or source	product	company
Lepidoptera	vegetables	<i>Bacillus thuringiensis</i> (B. t.) <i>kurstaki</i>	Bactospeine	Novo
	fruit		Biobit	Novo
	cotton		Condor	Ecogen
	maize		Cutlass	Ecogen
	forestry		Dipel	Abbott
			Forey	Novo
			Javelin	Sandoz
		<i>Pseudomonas fluorescens</i>	MVP	Mycogen
	<i>Plutella xylostella</i>	<i>B. t. aizawai</i>	Cen Tari	Abbott
		transconjugate (B. t. <i>kurstaki</i> /B. t. <i>aizawai</i>)	Florbac	Novo
Coleoptera	potatoes	<i>B. t. tenebrionis</i>	M-One	Mycogen
	vegetables		Trident	Sandoz
	forestry		Novodor	Novo
		transconjugate (B. t. <i>kurstaki</i> /B. t. <i>aizawai</i>)	Foil	Ecogen
		<i>Pseudomonas fluorescens</i>	M-Trak	Mycogen
Diptera	mosquito	<i>B. t. israelensis</i>	Acrobe	ACC
	blackfly		Bactimos	Novo
			Skeetal	Novo
			Teknar	Sandoz
			Vectobac	Abbott

Bacillus thuringiensis and in the properties of its insecticidal crystalline toxins, such as:

- Very selective action which is limited to a few species
- Inadequate longevity of action on leaves
- Inadequate longevity of action in the soil, due to microbial degradation

These serious disadvantages of Bt products demand more frequent applications, more complicated forms of application, and intensive monitoring and controlling of the crop to determine the optimum timing for applications. In other words, the costs and the expenditure of time are generally higher for a Bt application than for conventional insecticides. For this reason Bt products are used above all in organic farming and in the production of fresh vegetables. Still only sporadically, if at all, are Bt products used by growers of the most important crops such as maize, cereals, cotton, and oil seeds.

When looked at it detail however, the application of the Bt toxins, “packed-up” in the dead bacterium so to speak, is nothing other than chemical pest control, albeit using a highly specific active ingredient. Whether or not the very low level of toxicity to warm-blooded animals can also be guaranteed when genetically modified bacteria are used for toxin production must certainly still be investigated carefully. In the meantime, there have also already been reports of resistance to Bt products.^[77]

6.3.2. Other Biological Products

The true and original arsenal of biological control agents for combating pests and pathogens consists of living bacteria, viruses, fungi, nematodes, and predatory insects which can parasitize and kill the pest organisms.^[78] The intelligent use of such principles is a stimulating challenge time and again. These methods of control are particularly appealing when used together with chemical agents or semiochemicals in the context of an integrated concept for crop protection. In using biological products, one should however acknowledge their limits:

- Narrow spectrum of activity due to high host specificity
 - Reliable effect only under controlled conditions
 - Efficacious only up to certain levels of infestation
- These constraining factors have the following consequences:
- The use of biological products alone is normally insufficient
 - Regions with extreme weather conditions are unsuitable for the use of biological products
 - Reliable control cannot be guaranteed under intense infestation

In cases where these constraints are acceptable, such as under controlled conditions in a greenhouse or in the relatively stable microclimate of the soil, biological products are an interesting alternative.

In the meantime bio-insecticides based on the entomopathogenic fungi of the geni *Beauveria*, *Metharhizium*, and *Verticillium* have been developed by various companies (Table 8).

A very important point in the development of biological products is the question of registration. As the markets in question are generally small, development is only worthwhile if the expenditure incurred is substantially lower than that for a synthetic pesticide. Here there is still no coherent legislation and a hesitant stance on the part of the regulatory authorities.

7. Transgenic Plants

Through a process of breeding and selection based on the available gene pool, man has created the crops of today over the course of many thousands of years. Without the intervention of humans these would therefore not exist, as they would not have been capable of surviving had nature been left to its own devices. It was only in the middle of the nineteenth century that Gregor Mendel introduced scientific rigour into the process of plant breeding which until then had been based on intuition and visual, phenotypic selection.^[79] The rapid progress made in green biotechnology in the last two decades, today makes it possible to introduce desired properties (traits) into the plant in a targeted way by the transfer of particular genes. Illustrated in Table 9 are some of the landmarks on the way from Mendel’s laws of inheritance to the first commercially available transgenic crop plants.^[80]

While the use of gene and biotechnology is now widely accepted in the sphere of human health, green biotechnology has still had to battle against a strong headwind of opposition, particularly recently.^[81] As in the case of synthetic pesticides,

Table 8. Examples of entomopathogenic fungi (commercial products and products in development).

Fungus	product and/or company	indication	formulation
<i>Aschersonia aleurodis</i>	Koppert (Netherlands)	<i>Trialeurodes vaporariorum</i> <i>Bemisia tabaci</i> (whitefly)	wettable powder
<i>Beauveria bassiana</i>	Naturalis/Troy Bioscience (USA)	whitefly, thrips, white grubs	liquid formulation
<i>Beauveria bassiana</i>	Conidia/AgrEvo (Germany, Columbia)	<i>Hypothenemus hampei</i> (coffee berry beetle)	suspendible granules
<i>Beauveria bassiana</i>	Mycontrol-WP/Mycotech Corp. (USA)	<i>Trialeurodes vaporariorum</i> <i>Bemisia tabaci</i> (whitefly)	wettable powder
<i>Beauveria bassiana</i>	Ostrinil/Natural Plant Protection (France)	<i>Ostrinia nubilalis</i> (European corn borer)	microgranules of mycelium
<i>Beauveria brongniartii</i>	Betel/Natural Plant Protection (France)	<i>Hopochelus marginalis</i>	microgranules of mycelium
<i>Metarhizium anisopliae</i>	Bio-Path/EcoScience (USA)	<i>Blattella germanica</i> (German cockroach)	conidia on a medium placed in trap/chamber
<i>Metarhizium anisopliae</i>	Biogreen/Biocare Technology Pty. Ltd (Australia)	<i>Adoryphorus couloni</i> (redheaded cockchafer)	conidia produced on grain
<i>Metarhizium anisopliae</i>	Bilogic;Bio1020/Bayer AG (Germany)	<i>Otiorynchus sulcatus</i> (black vine weevil)	granules of mycelium
<i>Verticillium lecanii</i>	Mycotal/Koppert (Netherlands)	<i>Trialeurodes vaporariorum</i> <i>Bemisia tabaci</i> (whitefly) <i>Frankliniella occidentalis</i> (thrips)	wettable powder
<i>Verticillium lecanii</i>	Vertalec/Koppert (Netherlands)	<i>Aphidus</i> sp. (aphids)	wettable powder

Table 9. Significant landmarks in the story of transgenic crop plants.

1865	Mendelian laws	Mendel
1869	first discovery of DNA in cells, called “nuclein”	Miescher
1944	DNA identified as the substance involved in genetic inheritance	Avery, MacLeod, and McCarty
1953	discovery of the double-helix structure of DNA	Watson and Crick
1965	complete description of the genetic code	(1961–1965)
1969	restriction enzymes	Arber, Cohen, and Boyer
1977	sequencing of DNA (in vitro)	Maxam, Gilbert, Sanger
1982	first transgenic plants	Horsch, Rogers, and Fraley; Chilton; Schell
1987	first field trials with transgenic plants	
1994	first commercially available transgenic crop plant	
From 2000	many crop plants with many new characteristics (stacked traits)	

one of the principle arguments is the threat of polluting the “natural” foodstuffs and the environment with unknown risks for health and species diversity. The following two statements illustrate the incompatibility of views for and against gene technology. “Genetic engineering has the potential to become the most highly refined but grossest form of bestiality yet discovered”^[82] and “Applied to gene technology in medicine and agriculture, the comparative risk analysis—risk of using transgenic organisms vs risk of deliberately not introducing gene technology—is so much in favour of employing transgenic systems that it would be inhuman to refrain from using gene technology.”^[83]

Regrettably one must conclude that, in their enthusiasm for what can be achieved and over the expected economic gains to be had, industrial supporters of green biotechnology have somewhat lost touch with the interests of the food industry and the consumer. Alarmed by the massive resistance on the part of consumers, well-known companies in the foodstuffs sector, who initially had a positive view of the matter, have now stated that they must have the approval of consumers before they can process ingredients derived from transgenic plants.^[84]

A more in depth discussion of arguments for and against would be beyond the scope of this review. However, the analogy is unmistakable: on the one hand, gentle natural products and “natural” crops, on the other hand, hard synthetic chemicals and genetically modified plants. A widely

misunderstood nature is often elevated to an unchanging God-given principle and is placed in opposition to technological progress.

Let us, however, return to the main theme of this paper. The potential of green biotechnology extends far beyond the application to crop protection. It is important to differentiate between the so-called “input traits” such as herbicide resistance, resistance to insects, fungi, and viruses, stress-tolerance, improved photosynthetic performance, and nitrogen fixation and the “output traits” such as the accumulation of constituents of value for human and animal nutrition, human health, or industrial use (proteins, amino acids, oils, carbohydrates, vitamins, vaccines, and valuable pharmaceutical substances). Linked to the key word “quality”, the reduction of toxic or allergenic food components should also be mentioned (Table 10).^[80] The currently fashionable “buzzwords” here are nutraceuticals, novel food, functional food, and pharming.

Green biotechnology can therefore build new bridges linking the chemical industry with the foodstuffs industry through seed companies. On the march from agrochemicals to “novel foods” by way of the seed, the Monsanto company has been a pioneer for a number of years now.^[85] The company has concentrated on biotechnology in a very radical way. Other leading crop-protection companies have been far more cautious in this respect and have conducted such activities as an addition, without calling their core business in chemical crop protection into question.

Table 10. Predicted development of transgenic properties.

Today	2005	2010
herbicide tolerance	non-Bt insecticides	wide ranging protection of crop plants
Bt genes	fungicidal proteins	reductions in fertilizer application rates
viral coat proteins	resistance gene	increased stress tolerance
more valuable oils	carbohydrates	improvements in photosynthetic yield
lysine	proteins/amino acids	increased knowledge about relevant genes (genome analyses)
ripening	fatty acids/oils	industrial enzymes
	polymers	new polymers
	modified secondary metabolites	new secondary metabolites
	gene stacking	pharmaceuticals
		nutritive supplements

thuriangiensis protein crystals in maize and cotton. The notion that the Bt toxin does not have to be applied to the surface of the plant by means of a spray application, but instead could be produced as a secondary metabolite by a suitably modified crop plant is, of course, captivating. Several of the drawbacks pertaining to classical Bt products mentioned above are thereby obviated (see Section 6.3.1.).

7.1. Herbicide-Resistant Crops

The transgenic plants introduced onto the market so far almost all possess properties which are exclusively relevant to crop protection. The development in the USA has been impressive. As shown in Figure 18, the area cultivated with

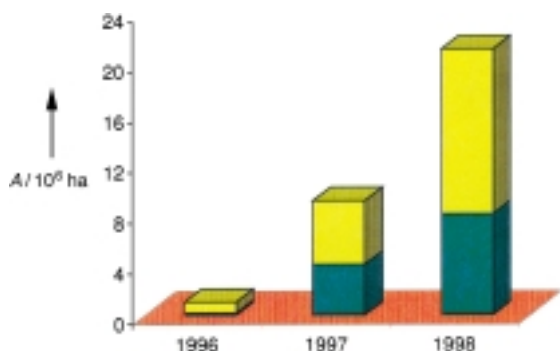


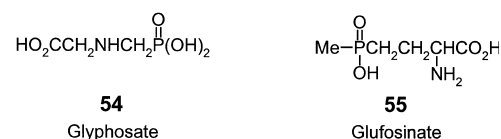
Figure 18. Estimate of the area under cultivation with transgenic crop plants in the USA. Yellow = herbicide resistance, green = insect resistance.

herbicide- and insect-resistant crops increased more than tenfold between 1996 and 1998 and already accounts for a significant proportion of the total area cultivated with maize, soya, and cotton. The herbicide resistance sector predominates and has already caused considerable turbulence in the American herbicides market.^[80]

Glyphosate-resistant, “Roundup Ready” varieties of soya, maize, and cotton account by far for the largest slice of the market, followed some way behind by glufosinate-resistant Liberty Link cultivars of maize, soya, and rape. The resistant varieties which, it is thought, will be introduced to the market in the next few years are summarized in Table 11.

7.2. Insect-Resistant Crops

Insect-resistance in crop plants is still synonymous today with the expression of *Bacillus*



In 1996, Bt cotton (Bollgard) with resistance to *Heliothis* and Bt potatoes with resistance against the Colorado potato beetle were first introduced in the USA. Since then Bt maize resistant to the European corn borer has come onto the market (Table 12).^[80] Unless the inherent problem of resist-

Table 12. Crop plants with resistance to insects.

Resistance	Company	Crop plant
Lepidoptera	AgrEvo	cereals
Lepidoptera	Novartis	cereals
Lepidoptera	Monsanto	cotton
Coleoptera	Monsanto	potatoes
Colorado beetle	Monsanto	potatoes
Lepidoptera	Monsanto	tomatoes

Table 11. Crop plants with resistance to herbicides: Commercial products and development products from 1996–2002.

Resistance	company	crop	method
Roundup Ready (Glyphosate)	Monsanto	soya, cotton	gene technology
Liberty Link, BastaR	AgrEvo (PGS)	rape, maize, soya	gene technology
imidazolines	ACC	maize	selection in cell cultures
sulfonyl ureas	DuPont	soya	selection in cell cultures
bromoxynil	Calgene (Monsanto)	cotton	gene technology
sethoxydim	BASF	maize	selection in cell cultures
Roundup Ready (Glyphosate)	Monsanto	rape, maize, rice	gene technology
Roundup Ready (Glyphosate)	Monsanto	sugar beet, rice	gene technology
imidazolines	ACC	maize, soya, rape	gene technology
sulfonyl ureas	DuPont	maize, soya, rape	gene technology

ed. Fungus-resistant genetically modified crops still lag far behind.

Naturally, the approaches listed here cannot provide solutions covering all the problems of crop protection. Impressive as the perspectives for plant gene technology may be, it is certain that the technology will not be able to produce a plant with only positive features, including the genetic potential for high yield, and, at the same time, one which is resistant to every conceivable pest and pathogen. The war against pest organisms, which are able time and again to adapt to changing conditions, will never end and in principle cannot be won. The very valuable genetically modified plants will need particular protection with the result that novel active ingredients will then certainly be required.

8. Formulation and Application

So far this article has only dealt with the huge effort invested in the search for new active ingredients and new modes of action with improved biological and ecological properties. Besides the actual active ingredient with its intrinsic properties, the form which the application takes is also of utmost importance. The formulation plays an important part in determining the efficacy of an active ingredient against the target organism and also affects its environmental impact. By means of oral and intravenous application a comparatively high level of bioavailability can be achieved for medicines. The scope in this respect is much more limited for agents of crop protection. An intelligent formulation is, therefore, all the more important.

With respect to improved bioavailability, lower risk for the user, and reduced environmental impact, the industry today expends considerable effort in formulation research. The declared aim is to establish a rational basis for the optimization of compositions, a process which in the past was, by and large, conducted empirically. Listed below are a few trends:

- Regulated droplet size with the aim of achieving a more targeted application (avoidance of the spray drifting)
- Formulations which are either low in, or even free from, solvents (suspensions instead of emulsifiable concentrates)
- Low dust formulations (water dispersed granulates instead of powders)
- Improved seed coating treatments (such as gels)
- Use of additives (optimization of activity)
- New, innovative types of formulation and application techniques (attract and kill, trunk application, pills, nanoparticles)
- Slow-release formulations (microencapsulation, etc.)
- Recyclable containers
- Water-soluble packaging
- Direct injection-based dilution and application of the spray mixture, all within a synchronized procedure (no remains of the spray mixture)

Following the theme of reduced risks for the user, the trend in formulations today is away from emulsifiable concentrates

and water-dispersible powders towards suspended concentrates and water-dispersible granules. The advantages for the user are obvious—no risks from dust during preparation of the spray mixture and less solvent introduced into the environment.

Another type of formulation which in future will increase in importance is the seed-coating treatment. Here too, the advantages are self evident: the active ingredient is only found where activity is desired, the seed-treatment process itself is carried out professionally by specialists, and the user is hardly exposed to the active ingredient at all. Figure 19 shows the dramatic differences in area of coverage achieved during the

Application	Type of product	Area treated [m ²]
Seed-coating treatment	seed coating	approx. 58
Furrow treatment	granulate	approx. 500
Broadcast spray	sprayable product	10 000



Figure 19. The treated area seen in relation to the method of application used, illustrated by the example of NTN 33893 (Imidacloprid, 7).

treatment of a hectare plot of arable land, when the application was by means of broadcast spray treatment, furrow treatment, or by application of the coated-seed treatment.

Within the remit of the article it is only possible to expand upon a few of the mentioned trends in formulation technology. Significant progress is also constantly being made in the fields of machine technical development and packaging technology.

Methods for the diagnosis and prediction of pest and predator infestation have improved considerably in the last few years. Thanks to powerful computers, a mass of various measured data can now rapidly be channeled into guiding the appropriate actions. The timing, intensity, and type of such actions can be optimized in relation to the location and variety of the crop involved. Viewed from the perspective of economic damage thresholds, the following maxim can be applied: as much as necessary but as little as possible! Through schooling and training of users, this concept is increasingly becoming the principle focus of companies acting in a responsible manner.

9. Outlook

So, there is then a great deal involved in the subject: “Innovation in Crop Protection: Trends in Research”. While

the aim of this review article is to give an overview which is as up-to-date as possible, the subject can not nearly be dealt with exhaustively. As is to be expected from the background of the authors, the examples chosen come largely from fields of research which are centered around chemistry. Colleagues from other disciplines would almost certainly have chosen somewhat different emphases.

Regarding agricultural practice, the available technologies will develop in an evolutionary way; revolutionary changes are not to be expected. In the final analysis, the strength of this prediction is unlikely to be diminished even by increases in the cultivation of transgenic crops.

One concept supported by all crop-protection companies and regulatory authorities is that of integrated crop production and crop protection; that is, the involvement and use of all possible relevant measures which help to maintain a productive, environmentally and resource-friendly agricultural industry. In this regard, chemical crop protection is one of the possible measures which has proven to be particularly efficient. Increasingly it will be joined by agricultural, breeding (gene technological), biological, and physical approaches. The disciplines involved are well prepared for this task.

However, one aspect above all must be considered: A responsible, targeted use of the available methods assumes an ever better level of technical expertise and training on the part of the user. It is becoming clear that there is an enormous need for action on the part of state institutions and industry in this regard. At the same time however, the widening chasm between industrialized and developing nations is growing ever more obvious. Without huge amounts of support from industrialized nations, the latter will hardly be able manage the step into the high-tech world that is modern crop protection.

All the research efforts described here can, in the final analysis, only be of use if society is prepared to accept the results. The illogical and unfortunate contrast which sadly seems deeply rooted in the consciousness of the general public, between biological and chemical, between nature as a benign entity and the evil of chemistry must, with the utmost care, be resolved responsibly by those of us in the scientific community. The demands for "zero risk" must be exposed for what they are: a nonsense endangering the progress of humanity.

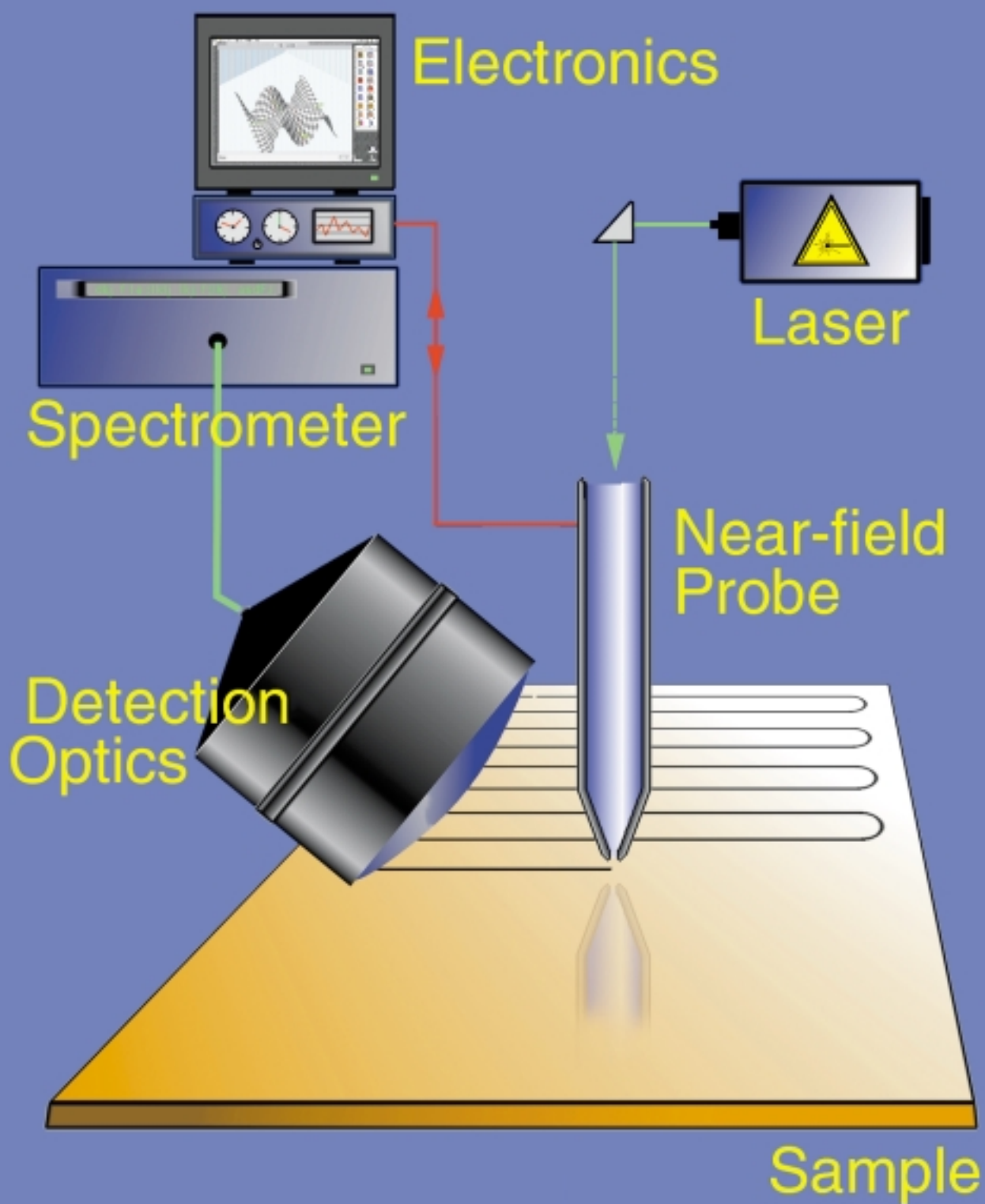
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- [1] J. Swift, *Gulliver's Travels*, Part II, Purnell, Maidenhead **1975**, chap. VII.
- [2] N. E. Borlaug, *Plant Tissue Cult. Biotechnol.* **1997**, 3, 119–127.
- [3] N. E. Borlaug, *Trans. N. Y. Acad. Sci.* **1958**, 20, 278–295.
- [4] H. Schlör in *Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel*, Vol. 2 (Ed.: R. Wegler), Springer, Berlin, **1970**, p. 47.
- [5] H. Martin, *Die wissenschaftlichen Grundlagen des Pflanzenschutzes*, 5. ed., VCH, Weinheim, **1967** (Translated and amended by K. J. Schmidt).
- [6] R. C. Roark, *J. Econ. Entomol.* **1943**, 36, 720–724.
- [7] W. Krämer in *Pflanzenschutz- und Schädlingsbekämpfungsmittel* (Ed.: K. H. Büchel), Thieme, Stuttgart, **1977**, p. 137.
- [8] C. Fest, K. J. Schmidt in *Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel*, Vol. 1 (Ed.: R. Wegler), Springer, Berlin, **1970**, pp. 246–453.
- [9] M. Eto, *Organophosphorous Pesticides: Organic and Biological Chemistry*, CRC, Cleveland, OH, **1974**.
- [10] K. Mellanby, *The DDT Story*, The British Crop Protection Council, Farnham, Surrey, **1992**.
- [11] R. G. Beatty, *The DDT Myth*, John Day, New York, **1973**.
- [12] H. Röchling in *Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel*, Vol. 1 (Ed.: R. Wegler), Springer, Berlin, **1970**, pp. 121–129.
- [13] R. Wegler, L. Eue in *Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel*, Vol. 5 (Ed.: R. Wegler), Springer, Berlin, **1977**, pp. 180–191.
- [14] W. Mackenzie, *Agrochemical Service, Update of the Countries Section* **1998**, 12, 4.
- [15] R. Carson, *Silent Spring*, Houghton Mifflin, Boston, **1962**.
- [16] a) D. Martinetz, K. Lohs, *Gift: Magie und Realität, Nutzen und Verderben*, Callwey, München, **1986**; b) W. H. Blackwell, *Poisonous and Medicinal Plants*, Prentice Hall, Englewood Cliffs, NJ, **1990**.
- [17] K. Naumann, *Chem. Unserer Zeit* **1993**, 27, 33–41.
- [18] D. G. Crosby, N. Aharonson, *Tetrahedron* **1967**, 23, 465–472.
- [19] V. R. Karekar, A. M. Mujumdar, S. S. Joshi, J. Dhuley, S. L. Shinde, S. Ghaskadbi, *Arzneim. Forsch.* **1996**, 46, 972–975.
- [20] R. C. Beier, *Rev. Environ. Contam. Toxicol.* **1990**, 113, 47–137.
- [21] G. S. Stoewsand, *Food Chem. Toxicol.* **1995**, 33, 537–543.
- [22] a) A. R. Saghir, J. W. Cowan, J. P. Salji, *Nature (London)* **1966**, 211, 87; b) J. W. Cowan, A. R. Saghir, J. P. Salji, *Aust. J. Biol. Sci.* **1967**, 20, 683–685.
- [23] S. E. Shephard, D. Gunz, C. Schlatter, *Food. Chem. Toxicol.* **1995**, 33, 257–264.
- [24] a) S. Babu, T. V. Madhavan, K. R. Rao, *Indian J. Med. Res.* **1967**, 55, 1226–1230; b) B. B. Kahn, G. I. Shulman, R. A. DeFronzo, S. W. Cushman, L. Rossetti, *J. Clin. Invest.* **1991**, 87, 561–570.
- [25] S. R. R. Musk, I. T. Johnson, *Carcinogenesis* **1993**, 14, 2079–2083.
- [26] D. L. J. Opdyke, *Food Cosmet. Toxicol.* **1974**, 12, 385–388; D. L. J. Opdyke, *Food Cosmet. Toxicol.* **1979**, 17, 277.
- [27] J. Stetter, *Regul. Toxicol. Pharmacol.* **1993**, 17, 346–370.
- [28] D. A. Evans in *Pesticide Chemistry and Bioscience, The Food-Environment Challenge* (Eds.: G. T. Brooks, T. R. Roberts), Royal Society of Chemistry, Cambridge, **1999**, pp. 3–24.
- [29] a) W. K. Moberg, B. Cross, *Pestic. Sci.* **1990**, 29, 241–246; b) H. M. Brown, J. C. Cotterman in *Chemistry of Plant Protection*, Vol. 10 (Ed.: J. Stetter), Springer, Berlin, **1994**, pp. 49–77.
- [30] a) D. L. Shaner, S. L. O'Connor, *The Imidazolinone Herbicides*, CRC, Boca Raton, FL, **1991**; b) M. Los, G. Berkelhammer, D. L. Shaner, B. K. Singh, T. M. Brady, B. Cross, R. F. Doehner, J. Finn, D. L. Ladner, *ACS Symp. Ser.* **1998**, 686, 8–37.
- [31] a) J. M. Beraud, J. Clauement, A. Montury, *Brighton Crop Prot. Conf. Weeds* **1991**, 1, 51–56; b) M. P. Prisbylla, B. C. Onisko, J. M. Shribbs, D. O. Adams, Y. Liu, M. K. Ellis, T. R. Hawkes, L. C. Mutter, *Brighton Crop Prot. Conf. Weeds* **1993**, 2, 731–738.
- [32] K. Shiokawa, S. Tsuboi, S. Kagabu, K. Moriya in *Eighth International Congress of Pesticide Chemistry, Options 2000* (Eds.: N. N. Ragsdale, P. C. Kearney, J. R. Plimmer), American Chemical Society, Washington, DC, **1995**, pp. 49–58.
- [33] a) F. Colliot, K. A. Kukorowski, D. W. Hawkins, D. A. Roberts, *Brighton Crop Prot. Conf. Pests Dis.* **1992**, 1, 29–34; b) J. E. Casida, D. A. Pulman in *Advances in the Chemistry of Insect Control III* (Ed.: G. G. Briggs), The Royal Society of Chemistry, Cambridge, **1994**, pp. 36–51; c) N. Hamon, R. Shaw, H. Yang, *Proc. Beltwide Cotton Conf.* **1996**, 2, 759–765.
- [34] a) H. Mattioda, G. Pinochet, J.-M. Faugeron, *Phytoma* **1994**, 462, 43–45; b) A. C.-T. Hsu, T. T. Fujimoto, T. S. Dhadialla, *ACS Symp. Ser.* **1997**, 658, 206–219.
- [35] a) T. Anke, H. J. Hecht, G. Schramm, W. Steglich, *J. Antibiot.* **1979**, 32, 1112–1117; b) J. M. Clough, P. J. de Fraine, T. E. M. Fraser, C. R. A. Godfrey, *ACS Symp. Ser.* **1992**, 504, 372–383; c) Review: H. Sauter, W. Steglich, T. Anke, *Angew. Chem.* **1999**, 111, 1417–1438; *Angew.*

- Chem. Int. Ed.* **1999**, *111*, 1328–1349; d) Famoxate: D. B. Jordan, R. S. Livingston, J. J. Bisaha, K. E. Duncan, S. O. Pember, M. A. Piccollelli, R. S. Schwartz, J. A. Sternberg, X.-S. Tang, *Pestic. Sci.* **1999**, *55*, 105–118.
- [36] R. Nyfeler, P. Ackermann, *ACS Symp. Ser.* **1992**, *504*, 395–404.
- [37] a) M. C. Cramp, J. Gilmour, L. R. Hatton, R. H. Hewett, C. J. Nolan, E. W. Parnell, *Proc. Br. Crop Prot. Conf. Weeds* **1985**, *1*, 23–28; b) C. F. A. Kyndt, M. T. F. Turner, J. Rognon, *Proc. Br. Crop Prot. Conf. Weeds* **1985**, *1*, 29–34; c) P. Wightman, C. Haynes, *Proc. Br. Crop Prot. Conf. Weeds* **1985**, *1*, 171–178; d) M. C. Cramp, J. Gilmour, L. R. Hatton, R. H. Hewett, C. J. Nolan, E. W. Parnell, *Pestic. Sci.* **1987**, *18*, 15–28.
- [38] L. F. Lee, G. L. Stikes, L. Y. L. Sing, M. L. Miller, M. G. Dolson, J. E. Normansell, S. M. Auinbau, *Pestic. Sci.* **1991**, *31*, 555–568.
- [39] a) J. Secor, C. Cseke, W. J. Owen, *ACS Symp. Ser.* **1989**, *389*, 265–276; b) J. Secor, C. Cseke, W. J. Owen, *Brighton Crop Prot. Conf. Weeds* **1989**, *1*, 145–154.
- [40] a) R. Neumann, W. Guyer, *10th Int. Congr. Plant Prot. Proc. Conf.* **1983**, *1*, 445–451, [*Chem. Abstr.* **1985**, *102*, 57756]; b) T. Haga, T. Toki, Y. Tsujii, R. Nishiyama, *Nippon Noyaku Gakkaishi* **1992**, *17*, 103–113.
- [41] Experimental results of Bayer AG.
- [42] G. Levitt, *ACS Symp. Ser.* **1991**, *443*, 16–31.
- [43] J. Stetter in *Residuos de Plaguicidas 96* (Ed.: A Valverde), Universidad de Almeria-IEA, Almeria, **1998**, pp. 89–123.
- [44] H. Kubinyi, *3D QSAR in Drug Design, Theory, Methods and Applications*, Escom, Leiden, **1993**.
- [45] a) C. Hansch, H. Gao, *Chem. Rev.* **1997**, *97*, 2995–3059; b) *Rational Approaches to Structure, Activity, and Ecotoxicology of Agrochemicals* (Eds.: W. Draber, T. Fujita), CRC, Boca Raton, FL, **1992**.
- [46] a) R. D. Cramer III, D. E. Patterson, J. D. Bunce, *J. Am. Chem. Soc.* **1988**, *110*, 5959–5967; b) G. L. Dust, *Quant. Struct. Act. Relat.* **1998**, *17*, 419–426.
- [47] G. R. Marshall, C. D. Barry, H. E. Bossard, R. A. Dammkoehler, D. A. Dunn, *ACS Symp. Ser.* **1979**, *112*, 205–226.
- [48] G. Jäger in *Pflanzenschutz- und Schädlingsbekämpfungsmittel* (Ed.: K. H. Büchel), Thieme, Stuttgart, **1977**, pp. 169–172.
- [49] a) L. Burgaud, B. Delahousse, J. Deloraine, M. Guillot, M. Riottot, R. J. Cole, *Proc. Br. Weed Control Conf.* **1974**, *2*, 801–808; b) J. W. Dicks, *Pestic. Sci.* **1978**, *9*, 59–62.
- [50] a) E. Hacker, K. Bauer, H. Bieringer, H. Kehne, L. Willms, *Brighton Crop Prot. Conf. Weeds* **1995**, *1*, 73–78; b) M. Hess, E. Rose, *Brighton Crop Prot. Conf. Weeds* **1995**, *2*, 763–768.
- [51] a) R. Wegler, L. Eue in *Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel*, Vol. 8 (Ed.: R. Wegler), Springer, Berlin, **1982**, pp. 51–54; b) S. Yamato, Y. Suzuki, M. Katagiri, H. Ohkawa, *Pestic. Sci.* **1995**, *43*, 357–358.
- [52] G. Theodoridis, J. S. Baum, F. W. Hotzman, M. C. Manfredi, L. L. Maravetz, J. W. Lyga, J. M. Tymonko, K. R. Wilson, K. M. Poss, M. J. Wyle, *ACS Symp. Ser.* **1992**, *504*, 134–146.
- [53] a) R. A. Fuchs, R. Schröder in *Pflanzenschutz- und Schädlingsbekämpfungsmittel* (Ed.: K. H. Büchel), Thieme, Stuttgart, **1977**, pp. 5–10; b) K. Nagata, T. Ikeda, T. Shono, *Pestic. Sci.* **1999**, *55*, 452–456.
- [54] a) H. Takahashi, J. Mitsui, N. Takakusa, M. Matsuda, H. Yoneda, J. Suzuki, K. Ishimitsu, T. Kishimoto, *Brighton Crop Prot. Conf. Pests Dis.* **1992**, *1*, 89–96; b) H. Takahashi, N. Takakusa, J. Suzuki, T. Kishimoto, *Nippon Noyaku Gakkaishi* **1998**, *23*, 193–200.
- [55] a) J. Stetter in *Pflanzenschutz- und Schädlingsbekämpfungsmittel* (Ed.: K. H. Büchel), Thieme, Stuttgart, **1977**, pp. 15–22; b) L. J. Lawrence, J. E. Casida, *Life Sci.* **1984**, *35*, 171–178; c) O. Takaaki, H. I. Yamamura, E. Malatynska, M. Ikeda, H. Laird, J. C. Palmer, J. E. Casida, *J. Pharmacol. Exp. Ther.* **1988**, *244*, 802–806.
- [56] a) T. Kato in *Chemistry of Plant Protection*, Vol. 1 (Eds.: G. Haug, H. Hoffmann), Springer, Berlin, **1986**, pp. 1–24; b) D. W. Krämer in *Chemistry of Plant Protection*, Vol. 1 (Eds.: G. Haug, H. Hoffmann), Springer, Berlin, **1986**, pp. 25–64.
- [57] a) E. Ammermann, F. Loecher, G. Lorenz, B. Janssen, S. Karbach, N. Meyer, *Brighton Crop Prot. Conf. Pests Dis.* **1990**, *2*, 407–414; b) I. M. Y. Kwok, R. T. Loeffler, *Pestic. Sci.* **1993**, *39*, 1–11.
- [58] a) P. Reinecke, H. Kaspers, H. Scheinpflug, G. Holmwood, *Proc. Br. Crop Prot. Conf. Pests Dis.* **1986**, *1*, 41–46; b) K. H. Kuck, W. Thielert, *Pflanzenschutz-Nachr. Bayer (Engl. Ed.)* **1987**, *40*, 133–152; c) D. Berg, L. Born, K. H. Büchel, G. Holmwood, J. Kaulen, *Pflanzen-schutz-Nachr. Bayer (Engl. Ed.)* **1987**, *40*, 111–132; d) D. Berg, K. H. Büchel, G. Holmwood, W. Krämer, R. Pontzen, *ACS Symp. Ser.* **1990**, *421*, 184–198.
- [59] S. Watkins, *R&D Strategies In The Pesticide Industry*, Agrow Reports, PJB Publications, Richmond, Surrey, **1999**, p. 48.
- [60] a) F. Balkenhohl, C. von dem Bussche-Hünnefeld, A. Lansky, C. Zechel, *Angew. Chem.* **1996**, *108*, 2436–2488, *Angew. Chem. Int. Ed. Engl.* **1996**, *108*, 2288–2337.
- [61] S. B. Soloway, A. C. Henry, W. D. Kollmeyer, W. M. Padgett, J. E. Powell, S. A. Roman, C. H. Tieman, R. A. Corey, C. A. Horne in *Advances in Pesticide Science*, Vol. 2 (Eds.: H. Geissbühler, G. T. Brooks, P. C. Kearney), Pergamon, Oxford, **1979**, pp. 206–217.
- [62] W. Leicht, *Pflanzenschutz-Nachr. Bayer (Ger. Ed.)* **1996**, *49*, 71–86.
- [63] a) C. Methfessel, *Pflanzenschutz-Nachr. Bayer (Ger. Ed.)* **1992**, *45*, 369–380; b) M.-Y. Liu, J. E. Casida, *Pestic. Biochem. Physiol.* **1993**, *46*, 40–46; c) R. Zwart, M. Oortgiesen, H. P. M. Vijverberg, *Pestic. Biochem. Physiol.* **1994**, *48*, 202–213.
- [64] T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell, J. W. Daly, *J. Am. Chem. Soc.* **1992**, *114*, 3475–3478.
- [65] I. Minamida, K. Iwanaga, T. Tabuchi, I. Aoki, T. Fusaka, H. Ishizuka, T. Okauchi, *J. Pestic. Sci. (Int. Ed.)* **1993**, *18*, 41–48.
- [66] K. Odaka, K. Kinoshita, T. Wakita, S. Shiraishi, K. Oonuma (Mitsui Toatsu Chemicals), JP-A 07173157, **1995** (Priority 26.10.93) [*Chem. Abstr.* **1995**, *123*, 256770].
- [67] a) P. Maienfisch, L. Gsell, A. Rindlisbacher, *Pestic. Sci.* **1999**, *55*, 351–355; b) T. Gobel, L. Gsell, O. F. Huter, P. Maienfisch, R. Naef, A. C. O'Sullivan, T. Pitterna, T. Rapold, G. Seifert, M. Senn, H. Szczepanski, D. J. Wadsworth, *Pestic. Sci.* **1999**, *55*, 355–357.
- [68] a) W. F. Becker, G. von Jagow, T. Anke, W. Steglich, *FEBS Lett.* **1981**, *132*, 329–333; b) U. Brandt, U. Haase, H. Schägger, G. von Jagow in *Wege zu neuen Produkten und Verfahren der Biotechnologie, Dechema Monographien*, Vol. 129 (Eds.: T. Anke, U. Onken), VCH, Weinheim, **1993**, pp. 27–38.
- [69] a) B. A. M. Schwalge, Dissertation, University of Bonn, **1986**; b) S. Zapf, Dissertation, University of Kaiserslautern, **1994**; c) M. Engler, Dissertation, University of Kaiserslautern, **1997**; d) U. Schirmer, S. Karbach, E. H. Pommer, E. Ammermann, W. Steglich, B. A. M. Schwalge, T. Anke (BASF AG), DE-B 3519280 A1, **1985** (Priority: 30.5.85) [*Chem. Abstr.* **1987**, *106*, 101890].
- [70] a) M. S. Mayer, J. R. McLaughlin, *Handbook of Insect Pheromones and Sex Attractants*, CRC, Boca Raton, FL, **1991**; b) *Insect Pheromone Research, New Directions* (Eds.: R. T. Cardé, A. K. Minks), Chapman & Hall, New York, **1997**, pp. 1–684; c) P. E. Howse, I. D. R. Stevens, O. T. Jones, *Insect Pheromones and their Use in Pest Management*, Chapman & Hall, London, **1998**, pp. 1–369.
- [71] a) M. Angst, F. Gugumus, G. Rist, M. Vogt, J. Rody (Ciba Geigy AG), EP-B 376 888 A1, **1990** (Priority 28.12.88) [*Chem. Abstr.* **1991**, *115*, 24383]; b) “Control of Codling Moth (*Cydia Pomonella*) With Sirene CM, A Novel Attract & Kill Formulation”: D. Hofer, P.-J. Charmillot, M. Angst presented at the XXth International Congress Of Entomology, Florence, Italy, **1996**; c) P.-J. Charmillot, D. Pasquier, A. Scalco, D. Hofer, *Rev. Suisse Viti. Arboric. Hortic.* **1997**, *29*, 111–117.
- [72] a) P. Lösel, G. Penners, J. Weissmüller (Bayer AG), DE-B 4446344 A1, **1996** (Priority 23.12. 94) [*Chem. Abstr.* **1996**, *125*, 79429]; b) P. Lösel, G. Penners, M.-G. Cianciulli-Teller (Bayer AG), DE-B 19528529 A1, **1997** (Priority 3.8.95) [*Chem. Abstr.* **1997**, *126*, 140996]; c) “Field Studies On Factors Affecting The Efficacy And Mode Of Action Of The Attract And Kill Strategy For The Control Of The Codling Moth”: P. M. Lösel, R. J. Potting, G. W. Thwaite, A. Elbert, D. Nicoll, J. Scherkenbeck presented at the VIth European Congress of Entomology, České Budějovice, Czech Republic, **1998**.
- [73] a) J. B. Harborne, *Biochem. Syst. Ecol.* **1999**, *27*, 335–367; b) F. Schröder, *Angew. Chem.* **1998**, *110*, 1271–1274; *Angew. Chem. Int. Ed.* **1998**, *110*, 1213–1216; J. Ebel, A. Mithöfer, *Planta* **1998**, *206*, 335–348.
- [74] a) W. Kunz, R. Schurter, T. Maetzke, *Pestic. Sci.* **1997**, *50*, 257–282; b) H. Kessmann, M. Oostendorp, T. Staub, J. Goerlach, L. Friedrich, K. Lawton, J. Ryals, *Brighton Crop Prot. Conf. Pests Dis.* **1996**, *3*, 961–966; c) H. Kessmann, M. Oostendorp, W. Ruess, T. Staub, W. Kunz, J. Ryals, *Pestic. Outlook* **1996**, *6*, 10–13.
- [75] D. J. Ellar, *BCPC Symp. Proc. (Microbial Insecticides: Novelty or Necessity?)* **1997**, *68*, 83–100.

- [76] L. P. Gianessi, J. E. Carpenter, *Agricultural Biotechnology: Insect Control Benefits*, National Center for Food and Agricultural Policy, Washington, DC, **1999**.
- [77] a) F. Gould, A. Martinez-Ramirez, A. Anderson, J. Ferre, F. Silva, W. J. Moar, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7986–7990; b) F. Gould, A. Anderson, A. Reynolds, L. Bumgarner, W. Moar, *J. Econ. Entomol.* **1995**, *88*, 1545–1559; c) B. E. Tabashnik, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3488–3490.
- [78] Bacteria: a) G. B. Dunphy, J. M. Webster, *Int. J. Parasitol.* **1988**, *18*, 729–737; Viruses: b) T. Hanzlik, P. Christian, K. Gordon, *Pestic. Outlook* **1999**, *10* (1), 22–26; c) F. Moscardi, *Annu. Rev. Entomol.* **1999**, *44*, 257–289; Fungi: d) A. E. Hajek, R. J. St. Leger, *Annu. Rev. Entomol.* **1994**, *39*, 293–322; e) G. Zimmermann, *Pflanzenschutz-Nachr. Bayer (Ger. Ed.)* **1992**, *45*, 113–128; f) K. Stenzel, *Pflanzenschutz-Nachr. Bayer (Ger. Ed.)* **1992**, *45*, 143–158; Nematodes: g) H. K. Kaya, R. Gaugler, *Annu. Rev. Entomol.* **1993**, *38*, 181–206; Predatory insects: h) M. Samish, J. Rehacek, *Annu. Rev. Entomol.* **1999**, *44*, 159–182.
- [79] a) R. Knippers, K. P. Schäfer, E. Fanning, *Molekulare Genetik*, 3. Ed., Thieme, Stuttgart, **1982**, p. XV; b) J. Falbe, M. Regitz, *Römp Lexikon Chemie*, Vol. 4, 10. Ed., Thieme, Stuttgart, **1998**, p. 2587.
- [80] J. S. McLaren, *Pestic. Outlook* **1998**, *9* (6), 36–41.
- [81] Destruction of fields: a) *Landeszeitung Lüneburg* from 8.4.1999; Allergies: b) J. A. Nordlee, S. L. Taylor, J. A. Townsend, L. A. Thomas, R. K. Bush, *N. Engl. J. Med.* **1996**, *334*, 688–692; c) S. B. Lehrer, G. Reese, *Int. Arch. Allergy Immunol.* **1997**, *113*, 122–124; Monarch butterfly: d) J. E. Losey, L. S. Rayor, M. E. Carter, *Nature* **1999**, *399*, 214.
- [82] C. Johnson, *The Green Dictionary, Key Words, Ideas And Relationships For The Future*, Macdonald Optima, London, **1991**, p. 120.
- [83] H. Mohr in *Pesticide Chemistry – Advances in International Research, Development and Legislation* (Ed.: H. Frehse), VCH, Weinheim, **1991**, p. 28.
- [84] a) M. Busch in *Die Welt* from 17.7.1999; b) T. Fischermann in *Die Zeit* from 6.5.1999; c) P. Brabeck-Letmathe in *Lebensmittelzeitung* from 24.6.1999.
- [85] A. Beer, *Agrow Reports*, Vol. 294 (12.12.), PJB Publications Ltd, Richmond, Surrey, **1997**, pp. 6–9.
- [86] D. Bowen, T. A. Rocheleau, M. Blackburn, O. Andreev, E. Golubeva, R. Bhartia, R. H. French-Constant, *Science* **1998**, *28*, 2013–2032.



Scanning Near-Field Optical Microscopy and Spectroscopy as a Tool for Chemical Analysis

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Research into the nanometer-scale region is currently of relevance in many branches of modern science and engineering, such as in microelectronics, supramolecular chemistry, and in a biological context. A great deal of attention is given to the design of molecular devices, usually towards understanding the function of existing “molecular machines”. Central to this task are powerful diagnostic tools capable of recording chemical information with spatial resolution in the nanometer range. While elemental analysis of surfaces with a lateral resolution of a few dozen nanometers is almost routine, analysis of molecular species with a resolution of $<1\ \mu\text{m}$ is very difficult. Scanning-tunneling and

atomic-force microscopies usually do not give any chemical information. By combining scanning near-field optical microscopy (SNOM)—the “optical member” of the family of scanning-probe microscopies—with optical spectroscopy, it is possible to obtain molecular information from sample areas as little as 50 nm in diameter. In SNOM, a light source is scanned above the object of interest at a distance of a few nanometers. In the optical near field, the illuminated area is not subject to the Abbé diffraction limit, but merely by the size of the illuminating source. High quality SNOM probes can be reproducibly prepared by a chemical-etching method. These etched probes have an optical transmission

up to 1000-fold higher than commercial (pulled) SNOM tips and can withstand higher laser power. This last advantage allows not only high resolution optical imaging, but also localized spectroscopic investigations of surfaces and even optical “nanosampling” by pulsed-laser ablation. The ablated material can be transported over a considerable distance, which opens the possibility for its subsequent analysis with a complementary, highly sensitive analytical method, such as mass spectrometry.

Keywords: analytical methods • mass spectrometry • optical near-field microscopy • optical spectroscopy • surface analysis

1. Introduction

A great breakthrough in surface science was the development of the scanning-probe microscopy techniques, led by the development of the scanning-tunneling microscope (STM), which earned Heinrich Rohrer and Gerd Binnig the 1986 Nobel Prize in Physics.^[1–3] Soon thereafter, a host of related methods were developed, all based on piezoelectric scanning of a very sharp probe relative to the sample, such as atomic-force microscopy (AFM), scanning electrochemical microscopy, scanning magnetic microscopy, and many others. They are sometimes collectively referred to as the “children of the STM”.^[4]

Of particular interest to applications in molecular science is scanning near-field optical microscopy (SNOM),^[5–8] the “optical” version among the children of the STM. SNOM delivers light to spots of less than 100 nm diameter and thereby holds great promise for molecular analysis on the nanometer scale. An optical resolution of as little as 12 nm has been claimed by Betzig and Trautman^[9] but only after deconvolution of the size of the near-field optical probe. This spatial resolution has not been reproduced lately by other researchers. Delivering light to such small spots is not possible by conventional optical methods, such as by a focussed laser beam. It is also impossible to resolve features separated by less than $\lambda/2$ by conventional optical microscopy, where λ is the wavelength of the illuminating light. This limitation is imposed by the Abbé diffraction limit. At visible wavelengths, a resolution approaching 200–250 nm should be theoretically possible but, except for confocal schemes employing oil-immersion lenses and therefore subject to other limitations, a resolution better than 0.5–1 μm is rarely achieved. This diffraction limit is the reason that short-wavelength ultra-

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violet light or even X-rays, where λ is smaller still, find increasing use in microelectronics and other applications of lithography but at the cost of more expensive optics and instrumentation.

The possibility of illumination in the visible, in the near ultraviolet, or in the infrared range onto a spot smaller than the diffraction limit permits the application of a wide range of spectroscopic methods to problems in chemistry, biology, materials science, and other fields in which the molecular composition of nanoscale objects is of interest. Analyzing the elemental composition with a resolution less than 100 nm is now established and a routine procedure with scanning Auger microscopy, some secondary-ion mass spectrometers, and certain forms of electron-microprobe analysis.^[10] These methods typically operate under ultrahigh vacuum conditions, often require special sample pretreatment, and rely on focussing an ion or electron beam to a spot < 100 nm in size, which is detrimental for most molecular species. The chemical analysis of the *molecular* composition with such a resolution was impossible in practise until now, and is just starting to be explored by a combination of SNOM technology with spectroscopic methods. A large advantage of the SNOM methods is that they can operate under atmospheric pressure.

2. Experimental Section

Commercially available near-field scanning optical microscopes (models Lumina and Aurora, ThermoMicroscopes (formerly Topometrix), Santa Clara, CA (USA) form the basic units of our instrumentation (Figure 1). Laser light penetrating through the nanometer-sized aperture of the probe illuminates the surface under investigation. The sample stage is scanned relative to the tip in order to maintain a fixed

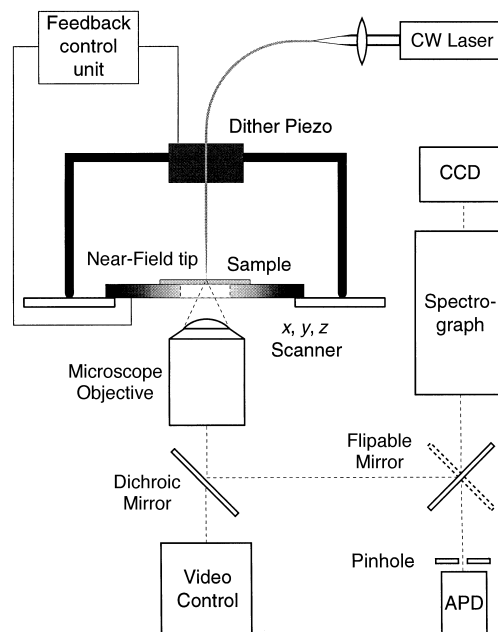


Figure 1. Equipment for scanning near-field microscopy. The sample is mounted on a piezoelectric x,y,z scanning stage. The SNOM tip is held either by a dither piezo (shown) or by a tuning fork (not shown). Shear force damping is used for controlling the tip-sample distance through a feedback loop. Light from a continuous-wave (CW) laser is coupled onto the blunt end of the fiber, illuminating the sample through the SNOM tip. Scattered light is collected either in transmission (shown) or in reflection (not shown) using conventional far-field optics. Detection uses either an avalanche photodiode (APD) or by an optical spectrograph equipped with a charge-coupled device (CCD). The entrance slit of the spectrograph or a pinhole mounted in front of the APD further reduce stray light.

optical path. The emitted or scattered light from the sample is collected with high numerical aperture microscope objectives either in transmission or in reflection (not shown in Figure 1).

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Collected light is then filtered and projected onto a sensitive detector for fluorescence detection or, alternatively, onto the entrance slit of an optical spectrograph. The latter is optimized for a greater amount of transmitted light. In our arrangement, a transmission holographic-grating spectrograph with a high numerical aperture was used (Holospec f/1.8i, Kaiser Optical Systems, Ann Arbor, MI (USA)).

To maintain a constant tip-to-sample distance, an optically detected shear-force feedback loop is most frequently used,^[11, 12] although other methods have been employed.^[13–17] For the feedback loop, the tip dithers at its resonance frequency. The dithering amplitude decreases dramatically when the tip is within 5–15 nm of the sample surface. The reasons for the shear force damping near the surface are still not fully understood; probably a combination of contact, frictional, and adhesive forces induced by a thin water film on the sample surface are responsible, at least under ambient conditions.^[18, 19] In the “constant gap” mode, a feedback loop maintains a constant distance, normally < 10 nm, between the probe and surface. For flat samples, scans in “constant height mode” are advantageous to avoid topographic artifacts.^[20] Such scans are performed in the absence of a distance feedback loop once the tip–sample distance has been established.

The probe is the most delicate component of any SNOM equipment. In this review, we will be dealing exclusively with “aperture SNOM probes”, that is, tips with an apex smaller than the wavelength of the illuminating light, and made of a tapered glass fiber coated with metal except at the apex.^[12, 21] Other probes are also used, including point-source emission probes^[22] or apertureless probes.^[23–28] The important properties of aperture SNOM probes are good transparency, a well defined circular aperture, no light leaking through defects in the metal coating, and a high optical-damage threshold. Several methods have been proposed to prepare the tapered glass core probes. Adiabatic pulling of optical fibers during heating with a CO₂ laser,^[29, 30] similar to preparing a pipette, is currently the most popular method. In another case, tiny splinters of glass as tetrahedral SNOM tips are used.^[31, 32] Several groups are employing focused ion-beam milling to create very well defined apertures at the tip apex.^[33, 34] Recently, microfabricated tips have been described in the literature^[35–38] and such tips will become commercially available soon.

A very practical method for the preparation of probes is based on etching glass fibers at the meniscus between hydrofluoric acid (etching fluid) and a protective organic overlayer.^[39] Etched fiber probes usually provide a higher optical throughput due to larger cone angles than the pulled probes,^[38–42] but a high yield of good tips produced by etching is often not obtained. A more reproducible and efficient method, called “tube etching”, was recently developed in our laboratory.^[43] By this method, the protective polymer coating of the optical fiber is retained, and a tip forms due to a convective mechanism inside the polymer jacket. The method is tolerant to perturbations during preparation such as vibrations and temperature changes. The resulting tips are produced reproducibly and in high yield with large cone angles and smooth aluminum coating. It should also be noted

that the composition of the glass is crucial for obtaining good etching results and also to avoid large background signals during SNOM experiments arising, for example, by Raman scattering of the fiber material itself (see Section 3.2).

The left side of Figure 2 shows an electron micrograph of an aluminum-coated SNOM tip produced by the tube-etching method. The large opening angle is immediately apparent

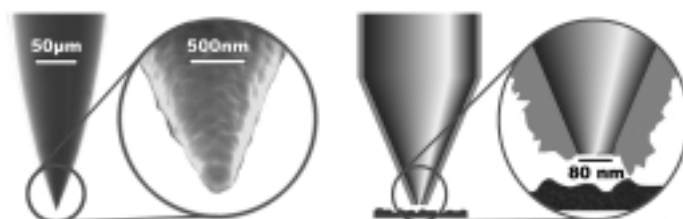


Figure 2. Left: Electron micrograph of a SNOM tip produced by the tube-etching method and a closeup of the apex region of the same tip. Right: Representation of the origin of differences between optical and topographic images.

and, in the closeup, an aperture of well below 100 nm diameter is visible. On the right side, the difference between the “topographic tip” and the “optical tip” is illustrated. Sometimes a grain of the metal coating will protrude such that the center of the tapered optical fiber and that of the topographic tip can be separated by several tens of nanometers. If this is the case, the offset of the topographic and the optical images can lead to a misinterpretation of the combined SNOM and topographic data.

3. Results and Discussion

3.1. Fluorescence Imaging with Etched Optical-Fiber Tips

Fluorescence is by far the most widespread and easily implemented mode for chemical imaging by near-field optical methods, although the resolution offered by modern confocal fluorescence-microscopy techniques is often sufficient.^[44] A fluorescent tag must be attached to the molecules of interest, unless their natural fluorescence can be exploited. Due to high signal intensity, it is even possible to image single fluorescent molecules with SNOM technology. Fascinating applications, involving single molecule fluorescence detection, have been described by Betzig,^[45] Xie,^[46–48] and van Hulst.^[49]

A very promising area for the use of SNOM in combination with fluorescence detection is the study of biological nanostructures, in particular, parts of cells. An example from our laboratory is shown in Figure 3A. In this application, dog kidney (Madine–Darby canine kidney, MDCK) cells were grown on a flat silica slide using glutaraldehyde and lysed such that the interior of their cell walls became accessible to SNOM imaging.^[50] The actin filaments of the cytoskeleton were stained selectively with a rhodamine-labeled phalloidin; they appear green in the image.

Figure 3 shows immediately that much finer features are resolved with SNOM. Specialized laser-scanning confocal-

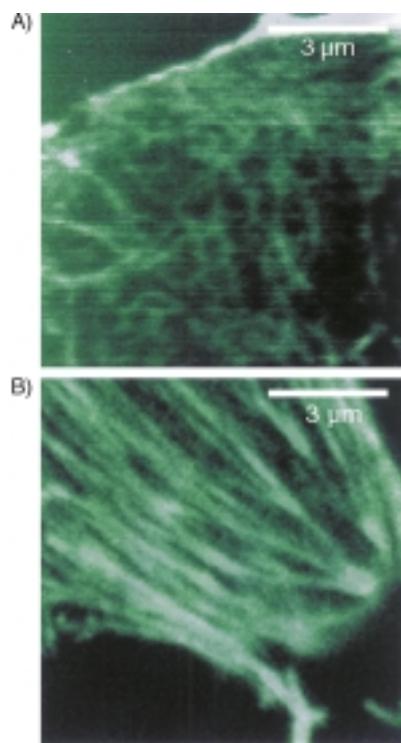


Figure 3. A) SNOM fluorescence image of the cytoskeleton of Madine–Darby canine kidney cells stained with a fluorescent dye (see text for details). B) Confocal micrograph image of the same sample (different area).

microscopy techniques push the resolution below 200 nm by, for example, using two-photon excitation. However, the lateral resolution in Figure 3B is about 300 nm, typical for confocal fluorescence microscopes. Actin filament bundles can be resolved by confocal microscopy but the resolution in the SNOM image (Figure 3A) considerably exceeds that of the confocal-microscope image (Figure 3B).

Near-field optical detection is not strictly limited to surfaces. This is illustrated in Figure 4. Here, a sample consisting of fluorescent latex spheres embedded in a polyvinyl butyral (PVB) thin film was imaged by SNOM using fluorescence detection in transmission mode. The spheres had a diameter of 288 nm and were impregnated with a fluorescent dye that is easily excited with the 488 nm line of an Ar-ion laser. Spheres that are located on the surface of the polymer matrix appear with sharp contours both in the topographic and in the SNOM/fluorescence image, albeit with a larger apparent diameter of about 375 nm. This allows determination of the tip diameter by deconvolution, in this case an optical tip diameter of ≤ 100 nm may be deduced.

Other latex spheres appear blurred in the optical image at locations where no protrusions were apparent in the simultaneously recorded topographic image. This can be attributed to latex spheres that are not located directly at the surface but are embedded deeper in the PVB film. Detection to a depth of at least 150 nm is therefore possible.

A disadvantage of fluorescence imaging is the need for a fluorescent moiety or tag to be employed, which is not always possible. A further shortcoming is that fluorescence spectra are broad and structureless, to reveal little chemical informa-

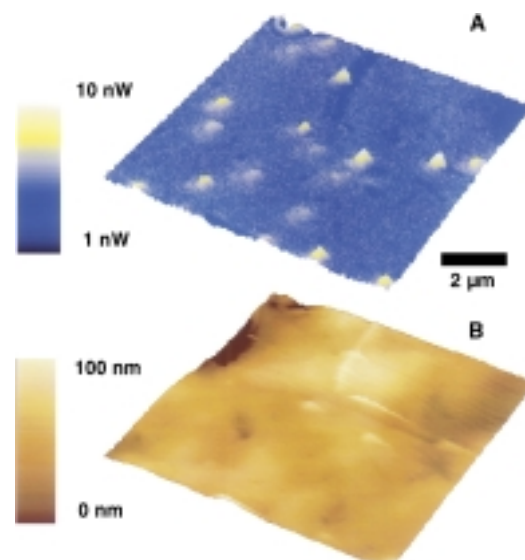


Figure 4. A) SNOM/fluorescence image and B) topographic image of a $10 \times 10 \mu\text{m}$ area of a PVB film containing fluorescent microspheres. The spheres had a diameter of 288 nm. The fluorescence was excited with the 488 nm line of an Ar-ion laser.

tion. It would be, at best, very difficult to identify unknown molecules based solely on fluorescence. Other optical spectroscopies are better suited for this purpose.

3.2. Near-Field Vibrational Spectroscopy

Vibrational spectroscopy is very useful to identify unknown molecules due to its high chemical-information content. It is, however, difficult to combine infrared spectroscopy with SNOM for two reasons: 1) Fiber materials in the mid-infrared region, where most molecular vibrations are detected, are not readily available and are difficult to handle; 2) The discrepancy between the desired resolution and the diffraction limit is accentuated due to the much longer excitation wavelength. Nevertheless, there are some very interesting, even heroic, attempts to use SNOM methods for imaging the mid-infrared range.^[51] Keilmann et al.^[28] and Boccara et al.^[25, 26] use an apertureless approach: An AFM tip is used for field enhancement within the focus of a fixed-frequency infrared laser. A resolution of $\lambda/600$, about 17 nm, was claimed with this approach.^[25] However, true IR spectroscopy with a resolution on the order of a few nanometers has not been achieved yet.

An alternative to IR spectroscopy is Raman spectroscopy. The difficulty lies here with the exceedingly low scattering cross-section, such that its combination with SNOM seems hopeless at first. However, there are certain advantages of Raman over IR spectroscopy: Suitable optical fibers are readily available, there is a large choice of very sensitive detectors, and it is possible to use resonance-excitation techniques. Finally, Raman cross-sections are increased by orders of magnitude if surface enhancement (SERS) is employed. It is relatively straightforward to combine surface-enhanced spectroscopies with SNOM to improve the overall photon yield.

Several research groups have begun to implement Raman microscopes where the excitation uses an aperture SNOM probe.^[52–62] The alternative is again to use apertureless probes: A very sharp tip, usually a metallic STM or AFM tip, can be used for field enhancement inside the diffraction-limited focus of the illuminating laser beam. The position of the tip can be temporally modulated in order to employ lock-in detection schemes.^[23, 24] Resolution to 10 Å has been claimed using this method, albeit not with Raman scattering. It is, however, still unclear where the optical contrast originates from. For example, it is possible that light reflected off the side of the probe contributes more to the overall signal than radiation from the apex of the probe. A shadowing effect can also occur, which may even cause a reversal of the optical contrast. Clever schemes, for example using two-photon excitation, can be used to circumvent these problems.^[27]

Figure 5 shows an image of a silver SERS substrate that was specially designed to contain isolated islands of silver without any topographic substructure.^[63] The substrate was then coated with an adlayer of rhodamine 6G (R6G). First, the

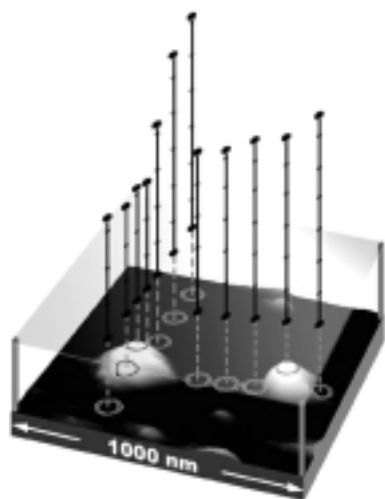


Figure 5. Shear-force topographic image of a substrate with a SERS coating. For near-field Raman measurements, a monolayer of rhodamine 6G is applied. At various locations, marked by the circles, near-field SERS measurements were made. The circles are 70 nm in diameter and represent the optical resolution obtained with the SNOM tip. The pole heights above the transparent plane denote the relative signal intensity of the Raman peak at 1650 cm^{-1} . The signals are normalized against the average Raman intensity measured on unmetallized glass slides. Each segment of the poles corresponds to an 1000-fold signal enhancement.

surface morphology was measured using the shear-force feedback-loop mechanism. The SNOM probe was then parked at selected positions of the depicted $1 \times 1 \mu\text{m}$ area and the Raman spectrum of the R6G layer was measured. Each measured position in Figure 5 is depicted by a white circle, the diameter of which (about 70 nm) indicates the approximate optical resolution obtained by the SNOM tip. Each position was illuminated for one minute through the SNOM tip, to yield the characteristic Raman spectrum of R6G. The optical resolution of the tip was determined by inherent spectral variation in the Raman spectra of adjacent locations; scanning electron micrographs of the used SNOM

tips confirmed this estimate of 70 nm. Assuming a monolayer coverage of the dye, a spot size of this diameter corresponds to a sensitivity of less than 100 R6G molecules!

As a measure for the Raman enhancement factor (EF), the normalized and background-corrected Raman peak intensities at 1650 cm^{-1} were selected, which correspond to an aromatic C–C stretch vibration of R6G. The magnitude of this band was directly compared with the average value obtained for unmetallized, R6G-coated substrates. The calculated EFs are superimposed on the topographic image of Figure 5. Each segment of the poles corresponds to an additional 1000-fold signal enhancement. The EFs lay between 2000 to well above 6000, with over a 50% variation observed in the Raman intensity for two points separated by less than 100 nm. Interestingly, the overall EF measured by far-field optics was higher still, around 11 000. We still cannot fully explain the discrepancy between the enhancement factors determined by near-field and far-field measurements. Perhaps “hot spots” with much higher EFs exist on the substrate, which cannot be found in the near field. Alternatively, cumulative effects of neighboring islands may play a role. A further possibility is that the aluminum coating of the SNOM tip deteriorates the EF near the silver islands by some electrostatic interaction as soon as the SNOM tip is in proximity.

We have also demonstrated high resolution Raman imaging is possible with the near-field optics. Figure 6A shows the shear-force topographic image and Figure 6B–D the optical images of a $2 \times 2 \mu\text{m}$ area of a SERS-coated substrate, which consisted of silver-coated Teflon microspheres with an overall diameter of 300 nm.^[61] The shear-force topography im-

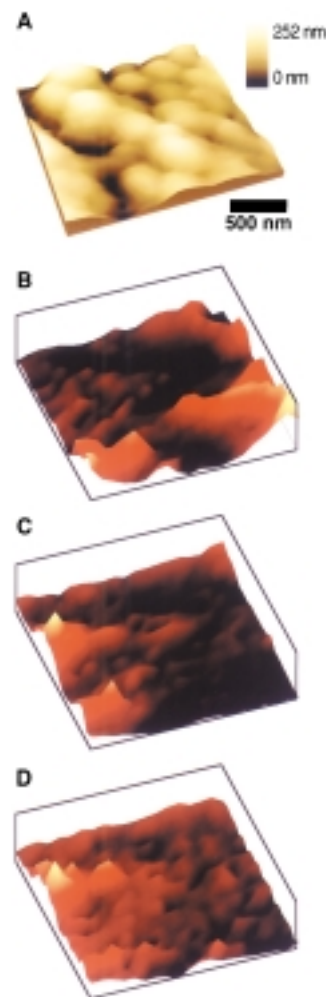


Figure 6. SNOM-SERS imaging of a BCB-labelled DNA sample deposited on a substrate consisting of monodisperse silver spheres for SERS enhancement. Lateral dependence of: A) shear-force topography; B) the Raman signal intensity at 800 cm^{-1} (glass); C) the near-field surface-enhanced Raman intensity at 1641 cm^{-1} (BCB) before normalization; and D) the near-field surface-enhanced Raman intensity at 1641 cm^{-1} after normalization. In images (B)–(D), the pixel size was only $100 \times 100 \text{ nm}$ and, for each graph, the intensity of one line of the complete Raman spectrum was plotted. In images (C) and (D), the contrast between the lowest and the highest Raman intensity was about a factor of three.

age shows that aggregated silver spheres are clearly resolved and their spatial distribution is quite irregular. This substrate was coated with a monolayer of a 18-mer DNA fragment labelled with brilliant cresyl blue (BCB).^[62] To achieve a reasonable acquisition time for the entire image, we measured the near-field Raman spectra as an array of only 20×20 data points. This acquisition time was twenty times longer compared to normal SNOM imaging times. Nevertheless, for an aperture smaller than 100 nm, it is the tip size and not the step size that limits the optical resolution. This represents a slight undersampling that we accept in order to obtain the entire spectral information within a reasonable measurement time. The image of the Raman spectrum feature at 800 cm^{-1} (Figure 6B), which stems from the glass of the SNOM tip itself, is at least partially dependent upon the topography. From the comparison of Figures 6A and B, it is obvious that the correlation of the topographic information and the reflected light from such a highly corrugated sample is by no means trivial. In particular, an interpretation of the image and an estimation of the optical resolution is difficult if topographic coupling or shadowing effects^[20] occur.

The image in Figure 6C shows the lateral intensity dependence of the Raman band from the BCB label (at 1641 cm^{-1}). By comparing Figures 6B and 6C, it is apparent that Raman signals from the label and from the glass tip show some correlation, such as the increased intensity in the lower left corner. However, the Raman signal stems from the BCB-labelled DNA, whereas the glass scattering signal originates entirely from the SNOM tip. The bands from the glass therefore should be proportional to the local reflectivity of the surface. The Raman scattered signal is subject to the same reflectivity changes as the glass signal. If, for instance, a particle between the illuminated spot and the collection optics decreases the reflectivity, the Raman signal is attenuated in exactly the same fashion and extent. In other words, the topography of our sample is encoded in the reflectivity signal, such that the topographic effects can be removed from the Raman signal by normalizing it against the intensity of the glass band. The effect of this normalization is demonstrated in Figure 6D, in which the Raman intensity of the 1641 cm^{-1} BCB band at each position after dividing the data in Figure 6C by the intensity of Figure 6B. A comparison of Figures 6D and 6C shows that instead of two bright spots, the corrected image shows only one spot of high SERS intensity. At the position in the lower left, an enhanced sample reflectivity is responsible for the increased Raman signal, whereas the bright spot on the upper left remains after the normalization.

In the corrected Raman images at all frequencies, we observe a small, highly SERS-active region of about 400 nm diameter on the center-left side of the image. If we assume a uniform distribution of the sample on the surface, this increase in intensity can only be attributed to a particularly strong enhancement by the substrate ("hot spots"), although concentration fluctuations leading to a variation of Raman signals cannot entirely be excluded. Interestingly, the maximum SERS enhancement does not coincide with the position of a silver particle but, in fact, the location of the maximum enhancement seems to be at a position where a gap between

the silver spheres is visible (Figure 6A). We have to be aware of the possibility that the topographic and the SNOM-Raman image may not be in perfect congruence. It has been postulated that "hot spots" may be located on SERS substrates where gaps or cavities exist.^[64] In previous work by Emory and Nie,^[56] there are hints for such "hot spots" but they were unable to image these sites directly. In contrast, the SNOM method allows directly visualization of "hot spots" because of the simultaneous recording of sample topography and Raman intensity at each data point; this is particularly straightforward if low-density silver-island films are used as SERS substrates.^[63]

It is interesting to compare the relative magnitude of Raman scattered light and fluorescence signals. The cross sections found for SERS on "hot spots" was of the same order of magnitude as that of the fluorescence signals!^[56, 65] Upon absorption of a single photon, an individual molecule will either undergo a Raman scattering event or fluoresce, but not both. Photobleaching processes are intimately linked with fluorescence and therefore should not affect the Raman emission process. This is demonstrated in Figure 7, where three consecutive spectra of R6G deposited on a silver-island SERS substrate are shown. The intensity of the fluorescence background decreases due to photobleaching, while that of the Raman bands remains constant, in agreement with the expectation. The reason for this behavior may be that "hot spots" on the SERS substrate coax the adsorbed molecules into undergoing Raman scattering rather than fluorescence, thereby protecting them from photobleaching. The average coverage of the SERS substrate by the dye was slightly more than one monolayer; apparently only a fraction of the adsorbed R6G molecules are associated with these protective sites but are responsible for quite strong SERS signals. This again supports the idea that only a few "hot spots" exhibit a very large Raman enhancement and are responsible for the overall intensity observed.

Are SERS methods the only hope to obtain Raman spectra excited by near-field optical probes? The answer to this question is, as demonstrated in Figure 8, a clear "no". The near-field Raman spectrum of a chemical-vapor deposited (CVD) diamond film shows a sharp feature at 1330 cm^{-1} , which is the well known Raman band of diamond. Raman scattering from graphitic carbon is expected around

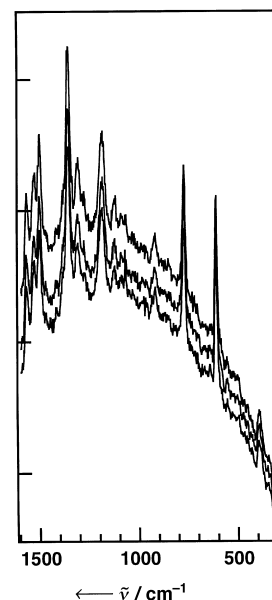


Figure 7. Raman lines superimposed on a fluorescence background in the optical spectrum of a monolayer of rhodamine 6G deposited on a silver SERS substrate (excitation at 488 nm). The spectra were recorded consecutively (from top to bottom), using an integration time of 5 min per spectrum. Slow photobleaching is observed for the fluorescence background, while the intensity of the Raman lines remains constant.

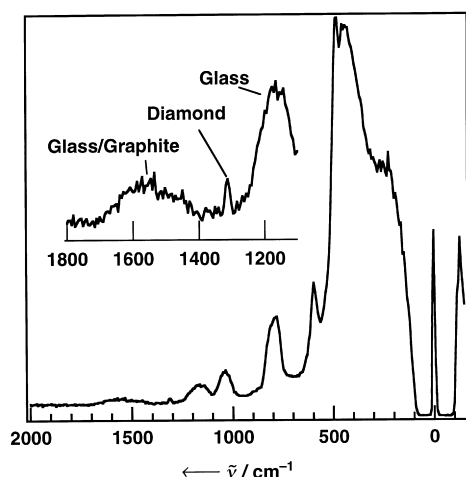


Figure 8. Near-field Raman spectrum of a diamond film produced by CVD methods. The excitation wavelength was 488 nm and the laser line was filtered out in front of the spectrograph by a holographic notch filter. Strong bands due to Raman scattering from the SERS tip itself dominate the spectrum (glass). Inset: the sharp band at 1330 cm^{-1} is assigned to diamond, and the broad feature at $\approx 1600\text{ cm}^{-1}$ is due partially to glass and partially to graphitic carbon.

1600 cm^{-1} , but unfortunately a fairly strong residual signal from the long progression of glass Raman bands, originating from the tip itself, overlaps with the sp^2 carbon band. This is partially due to the low Raman intensity of the sample, but the Raman emission from the glass of the SNOM tip depends strongly on the glass fiber material used. Our latest generation

of SNOM tips exhibit much lower Raman scattering in this spectral region, such that, in principle, the purity of the film could be measured at any given location.

The exquisite resolution in the z direction makes the SNOM an attractive tool to study the interface between two liquids. In particular, molecular orientation at such interfaces may be studied by the anisotropies of the Raman scattering tensor using polar-filtered exciting light and detection. For the isotropic part of the scattering tensor of the fully symmetric vibrational modes, a strong decrease in signal intensity is expected if the polarizers are crossed. In contrast, for anisotropic modes, some intensity will still be detected even for crossed polarizers. This is illustrated in Figure 9. At an interface, the orientation of the investigated mole-

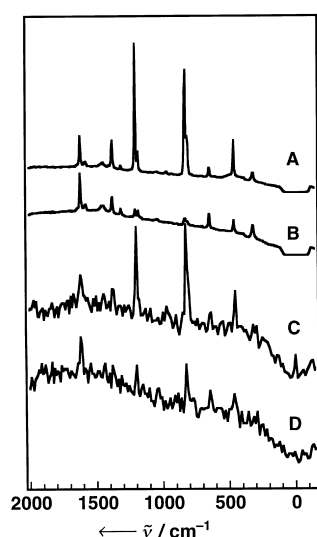


Figure 9. Raman spectra recorded in liquid *p*-xylol using polar-filtered exciting light and detection. A) Far-field spectrum with parallel polarizers; B) Far-field spectrum with perpendicular polarizers; C) Near-field spectrum with parallel polarizers; D) Near-field spectrum with perpendicular polarizers. The absolute intensities for each spectrum are not directly comparable. The laser power was $10\text{ }\mu\text{W}$ (at $\lambda = 488\text{ nm}$) and the signal, averaged over 10 min, was used.

cules will not be randomly oriented and the structure of an interface can be studied through symmetry considerations.

Figure 9A and 9B are far-field Raman spectra of liquid *p*-xylol, while 9C and 9D are near-field Raman spectra of the same. Over the transition from parallel to crossed orientation of the polarizers in the excitation and detection beam path, the expected intensity decrease is observed for many Raman modes, for example, the strong aromatic-ring vibrations at 820 and 1200 cm^{-1} . In contrast, the band at about 1600 cm^{-1} remains strong even when crossed polarizers are used, indicating large anisotropic components in its scattering tensor. Figure 9 shows impressively that the SNOM tip used for recording the near-field data preserves the polarization quite well.

3.3. Laser Ablation through SNOM Tips

The high transmission SNOM tips that we use permit not only high resolution optical imaging and spectroscopic investigations of surfaces, but also optical “nanosampling” by pulsed-laser ablation through the tip.^[66] The laser-ablated material can be transported over a considerable distance and analyzed in a second step by a highly sensitive analytical method such as mass spectrometry. A schematic of this concept is shown in Figure 10.

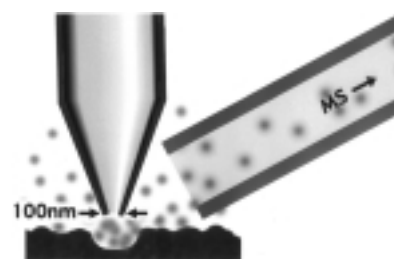


Figure 10. Schematic representation of the coupling of laser ablation through the SNOM tip with mass-spectrometric analysis of the vaporized material.

This localized ablation has already been described and a crude realization has appeared in the literature.^[67] The authors guided a sharpened optical fiber over a transmission electron microscopy (TEM) grid inside the ion source of a mass spectrometer and performed a matrix-assisted laser-desorption/ionization (MALDI) mass-spectrometry experiment. The TEM grid was covered with an acetylcholine sample embedded in a 2,5-dihydroxybenzoic acid matrix. The advantage of this experiment over earlier MALDI studies, where the laser light was also brought in by an optical fiber,^[68, 69] remains unclear. The spatial resolution was of several micrometers, far greater than what is possible with the SNOM method.

Several mechanisms are possible for laser-induced ablation through SNOM tips.^[70] The photochemical mechanism assumes that the energy is deposited in the sample via optical absorption and ablation follows as a consequence of breaking chemical bonds. The photothermal mechanism is similar but assumes the absorbed optical energy would be converted into

heat, which causes ablation from the locally heated spot. A ballistic mechanism is also possible, in which the material from the tip is sputtered onto the sample and material ablates from the surface. A ballistic mechanism is the most likely explanation for an experiment carried out on an anthracene surface using a completely metallized SNOM tip,^[41] since, in this case, no photons reach the sample and therefore a sputter process, either by metal atoms from the tip or by material adsorbed on its surface, was believed to be the reason for the ablation process. Fourth, one also has to take transient thermal expansion^[71–73] into account as a possible mechanism for the creation of surface indentations, but it is likely to be negligible.^[70]

We now discuss an experiment that gave clear results about the ablation mechanism of a rhodamine B film. The sample was irradiated with laser pulses either at its optical adsorption maximum (“on resonance”, 532 nm) or nearby this point (“off resonance”, 650 nm).

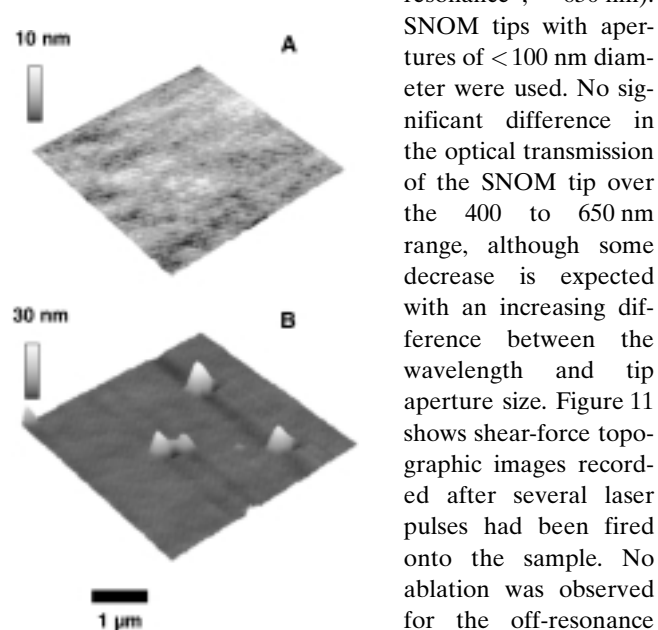


Figure 11. Shear-force topographic images of a rhodamine B film after transmitting laser pulses through the SNOM tip at A) 2.1 μJ at $\lambda = 650$ nm, far from the maximum absorption of rhodamine B, and B) 1.4 μJ at $\lambda = 532$ nm (B), a wavelength near the maximum absorption. The total topographic contrast in the z direction is A) 10 nm and B) 30 nm.

SNOM tips with apertures of < 100 nm diameter were used. No significant difference in the optical transmission of the SNOM tip over the 400 to 650 nm range, although some decrease is expected with an increasing difference between the wavelength and tip aperture size. Figure 11 shows shear-force topographic images recorded after several laser pulses had been fired onto the sample. No ablation was observed for the off-resonance wavelength (Figure 11 A; $\lambda = 650$ nm, 2.1 μJ per pulse). In contrast, well defined, ≈ 70 nm diameter (FWHM), 5 nm deep holes were created when the laser operated at a wavelength that is strongly absorbed by

the sample (Figure 11 B; $\lambda = 532$ nm, 1.4 μJ per pulse). This clearly points to an optical ablation mechanism, either photochemical or photothermal. Energy transfer by absorption of photons appears to be more efficient than by a ballistic process. The photon energy used here is not sufficient for inducing bond dissociation in rhodamine molecules, in contrast to ablation experiments with ultraviolet laser radiation, where photochemical dissociation is often used to rationalize the observed phenomena.^[74] We therefore suggest that a photothermal mechanism is responsible for the ablation of the rhodamine film: The absorbed photon energy is converted to

vibrational excitation, which causes a rapid temperature increase and leads to thermal desorption of the molecules.

Another interesting observation in Figure 11 is that the ablated material is redeposited on the sample surface close to the ablation crater. The distribution of redeposited material was not symmetric in this experiment but was always to the left side of the crater and independent of the scan direction. In other SNOM ablation experiments, the distribution was more symmetric. The origin of the directionality appears to be the tip itself: The apex of a SNOM tip is frequently not symmetric and the ablated material is ejected in a specific direction.

Transport over micrometer distances was observed in several cases. The result of such an experiment is shown in Figure 12.^[70] Here, an anthracene crystal surface was continuously irradiated with a pulsing laser (6 ns, 532 nm, 50 μJ) operated at a 20 Hz repetition rate over a 1×2 μm area. A shear-force image of a larger area was then recorded without

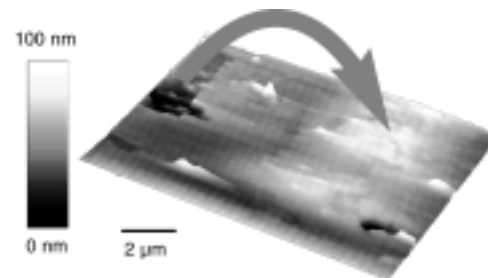


Figure 12. Transport of material over many micrometers: A topographic image of a 5×8 μm area of an anthracene film crystal surface, previously irradiated in a 1×2 μm trench in the upper left corner with ablating laser pulses (pulse: 6 ns pulse; wavelength: 532 nm; pulse energy: 50 μJ ; repetition rate: 20 Hz). Total topographic contrast in the z direction is 100 nm. Adapted from ref. [70].

irradiation. An approximately 1 μm wide trench with well defined edges could be detected in the upper-left corner of the image, while a 2 μm wide, 60 nm high mound was found about 5 μm away running roughly parallel to the trench. We interpret this again as vaporization followed by redeposition of material in a preferred direction. The direction of deposition is most likely determined by an asymmetry of the tip. For collection of ablated molecules, for example by a vacuum interface to a mass spectrometer, this asymmetry could be an advantage if the SNOM tip could be arranged to direct the ablated material towards the collector.

From Figure 12 we also estimate that only a few, larger chunks of material are ablated. Most of the anthracene is found in the form of a diffuse and structureless mound, which is expected if the molecules are redeposited from the gas phase. We have also shown that the laser-ablated molecules do not decompose.^[70] We conclude that material transport does not take place by a mechanical dragging from the SNOM tip, but instead by vaporization and redeposition in a direction dictated by the asymmetry of the tip.

4. Conclusions and Outlook

Near-field imaging based on aperture probes can now provide spectroscopic data with a lateral resolution on the

order of 50 nm. Some improvements in resolution may still be feasible but the aperture SNOM will probably never reach a resolution of 1 nm or less, which is possible by STM and AFM. However, as shown in this article, SNOM can provide a wealth of chemical information, which, in our opinion, more than compensates for the lack in spatial resolution.

High quality, high transmission SNOM tips can now be produced by various methods. The chemical etching method described here is one of the most easily implemented and provides high quality SNOM tips in a good yield. The “tube etched” tips can also withstand higher laser energies^[75] and are therefore brighter near-field light sources.

We have shown examples of fluorescence and SERS imaging in the optical near-field with a lateral optical resolution of less than 100 nm, relatively short exposure times, and high signal-to-noise ratios. The different origins of the reflectivity and Raman signals can be used for an intensity correction of the Raman images by a simple normalization. This procedure corrects topographic artifacts present in standard SNOM images and can always be used if the detection of the interesting compound can be made at a different wavelength.

Surface enhancement of optical transitions appears to be due to a small number of “hot spots”. With the combined optical and topographic information available in SNOM, we have a good chance to identify the nature of these sites. Surface enhancement, however, is not always a prerequisite for obtaining near-field Raman spectra, as shown on the example of a CVD diamond film. Finally, the tip itself could be used as a platform to carry a small particle^[76, 77] that provides strong Raman enhancement. Such a “tip SERS” approach would not require the sample to be deposited on a rough silver film and thereby makes the technique more generally usable.

In the area of pulsed-laser ablation through SNOM tips, we have shown that it is possible to create highly localized (around 70 nm diameter) ablation craters on the surface of organic materials. The ablated molecules remain intact and often fly in a direction determined by an asymmetry of the SNOM tip. Material can be transported over several micrometers, collected, and analyzed by a complementary method. We have recently built an interface to a mass spectrometer for this purpose. In contrast to common laser-ablation ion sources for mass spectrometry, this interface allows the sample to be at ambient pressure. Mass spectral data of material ablated by single diffraction-limited laser pulses have been successfully recorded, and the signal-to-noise ratio was good enough to put an equivalent near-field experiment within reach.

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- [1] G. Binnig, H. Rohrer, *Helv. Phys. Acta* **1982**, *55*, 726–735.
- [2] G. Binnig, H. Rohrer, C. Gerber, E. Weibel, *Phys. Rev. Lett.* **1982**, *49*, 57–59.
- [3] G. Binnig, H. Fuchs, C. Gerber, H. Rohrer, E. Stoll, E. Tosatti, *Europhys. Lett.* **1986**, *1*, 31–36.
- [4] R. Pool, *Science* **1990**, *247*, 634–636.
- [5] D. W. Pohl, W. Denk, M. Lanz, *Appl. Phys. Lett.* **1984**, *44*, 651–653.
- [6] A. Lewis, M. Isaacson, A. Harootunian, A. Muray, *Ultramicroscopy* **1984**, *13*, 227–231.
- [7] D. W. Pohl, U. C. Fischer, U. T. Dürig, *Scanning Microscopy Technologies and Applications*, Washington, DC, **1988**, pp. 84–90.
- [8] E. Betzig, M. Isaacson, A. Lewis, K. Lin, *Scanning Microsc. Technol. Appl.* **1988**, *897*, 91–99.
- [9] E. Betzig, J. K. Trautman, *Science* **1992**, *257*, 189–195.
- [10] D. Brune, R. Hellborg, H. J. Whitlow, O. Hunderi, *Surface Characterization*, WILEY-VCH, Weinheim, **1997**.
- [11] R. Toledo-Crow, P. C. Yang, Y. Chen, M. Vaez-Iravani, *Appl. Phys. Lett.* **1992**, *60*, 2957–2959.
- [12] E. Betzig, P. L. Finn, J. S. Weiner, *Appl. Phys. Lett.* **1992**, *60*, 2484–2486.
- [13] M. Garcia-Parajo, E. Cambril, Y. Chen, *Appl. Phys. Lett.* **1994**, *65*, 1498–1500.
- [14] K. Karrai, R. D. Grober, *Ultramicroscopy* **1995**, *61*, 197–205.
- [15] J. W. P. Hsu, M. Lee, B. S. Deaver, *Rev. Sci. Instrum.* **1995**, *66*, 3177–3181.
- [16] J.-K. Leong, C. C. Williams, *Appl. Phys. Lett.* **1995**, *66*, 1432–1434.
- [17] J. Barenz, O. Hollricher, O. Marti, *Rev. Sci. Instrum.* **1996**, *67*, 1912–1916.
- [18] H. Heinzelmann, D. W. Pohl, *Appl. Phys. A* **1994**, *59*, 89–101.
- [19] C. Durkan, I. V. Shvets, *J. Appl. Phys.* **1996**, *80*, 5659–5664.
- [20] B. Hecht, H. Bielefeldt, Y. Inouye, D. W. Pohl, L. Novotny, *J. Appl. Phys.* **1997**, *81*, 2492–2498.
- [21] D. W. Pohl, *Adv. Opt. Electron Microsc.* **1991**, 243–312.
- [22] N. Kuck, K. Lieberman, A. Lewis, *Appl. Phys. Lett.* **1992**, *61*, 139–141.
- [23] F. Zenhausern, M. P. O’Boyle, H. K. Wickramasinghe, *Appl. Phys. Lett.* **1994**, *65*, 1623–1625.
- [24] F. Zenhausern, Y. Martin, H. K. Wickramasinghe, *Science* **1994**, *269*, 1083–1085.
- [25] A. Lahrech, R. Bachelot, P. Gleyzes, A. C. Boccard, *Opt. Lett.* **1996**, *21*, 1315–1317.
- [26] A. Lahrech, R. Bachelot, P. Gleyzes, A. C. Boccard, *Appl. Phys. Lett.* **1997**, *71*, 575–577.
- [27] E. J. Sánchez, L. Novotny, X. S. Xie, *Phys. Rev. Lett.* **1999**, *82*, 4014–4017.
- [28] B. Knoll, F. Keilmann, *Nature* **1999**, *399*, 134–137.
- [29] E. Betzig, J. K. Trautman, T. D. Harris, J. S. Weiner, R. L. Kostelak, *Science* **1991**, *251*, 1468–1470.
- [30] G. A. Valaskovic, M. Holton, G. H. Morrison, *Appl. Opt.* **1995**, *34*, 1215–1228.
- [31] U. C. Fischer, J. Koglin, H. Fuchs, *J. Microsc. (Oxford)* **1994**, *176*, 231–237.
- [32] J. Koglin, U. C. Fischer, H. Fuchs, *J. Biomed. Opt.* **1996**, *1*, 75–78.
- [33] J. A. Veerman, A. M. Otters, L. Kuipers, N. F. van Hulst, *Appl. Phys. Lett.* **1998**, *72*, 3115–3118.
- [34] S. Pilevar, K. Edinger, W. Atia, I. Smolyaninov, C. Davis, *Appl. Phys. Lett.* **1998**, *72*, 3133–3135.
- [35] S. Münster, S. Werner, C. Mihalcea, W. Scholz, E. Oesterschulze, *J. Microsc.* **1997**, *186*, 17–22.
- [36] W. Noell, M. Abraham, K. Mayr, A. Ruf, J. Barentz, O. Hollricher, O. Marti, P. Günther, *Appl. Phys. Lett.* **1997**, *70*, 1236–1238.
- [37] D. Drews, W. Noell, W. Ehrfeld, M. Lacher, K. Mayr, O. Marti, C. Serwatzy, M. Abraham, *SPIE Conference on Materials and Device Characterization in Micromachining*, Santa Clara, CA, **1998**, pp. 76–83.
- [38] T. Yatsuya, M. Kourogi, M. Ohtsu, *Appl. Phys. Lett.* **1998**, *73*, 2090–2092.
- [39] P. Hoffmann, B. Dutoit, R.-P. Salathé, *Ultramicroscopy* **1995**, *61*, 165–170.
- [40] L. Novotny, D. W. Pohl, B. Hecht, *Opt. Lett.* **1995**, *20*, 970–972.
- [41] D. Zeisel, S. Nettesheim, B. Dutoit, R. Zenobi, *Appl. Phys. Lett.* **1996**, *68*, 2491–2492.

- [42] M. N. Islam, X. K. Zhao, A. A. Said, S. S. Mickel, C. F. Vail, *Appl. Phys. Lett.* **1997**, *71*, 2886–2888.
- [43] R. Stöckle, V. Deckert, C. Fokas, R. Zenobi, B. Hecht, B. Sick, U. P. Wild, *Appl. Phys. Lett.* **1999**, *75*, 160–162.
- [44] J. Pawley, *Handbook of Biological Confocal Microscopy*, Plenum, New York, **1995**.
- [45] J. K. Trautman, J. J. Macklin, L. E. Brus, E. Betzig, *Nature* **1994**, *369*, 40–42.
- [46] X. S. Xie, R. C. Dunn, *Science* **1994**, *265*, 361–364.
- [47] R. C. Dunn, G. R. Holtom, L. Mets, X. S. Xie, *J. Phys. Chem.* **1994**, *98*, 3094–3098.
- [48] X. S. Xie, J. K. Trautman, *Ann. Rev. Phys. Chem.* **1998**, *49*, 441–480.
- [49] A. G. T. Ruiter, J. A. Veerman, M. F. Garcia-Parajo, N. F. van Hulst, *J. Phys. Chem. A* **1997**, *101*, 7318–7323.
- [50] U. Ziegler, A. Vinckier, P. Kernen, D. Zeisel, J. Biber, G. Semenza, H. Murer, P. Groscurth, *Mol. Biol. Cell* **1998**, *9*, 600.
- [51] M. K. Hong, S. Erramilli, P. Huie, G. James, A. Jeung, *SPIE* **1996**, *2863*, 54–63.
- [52] D. P. Tsai, A. Othonos, M. Moskovits, *Appl. Phys. Lett.* **1994**, *64*, 1768–1770.
- [53] C. L. Jahncke, M. A. Paesler, H. D. Hallen, *Appl. Phys. Lett.* **1995**, *67*, 2483–2485.
- [54] C. L. Jahncke, H. D. Hallen, M. A. Paesler, *J. Raman Spectrosc.* **1996**, *27*, 579–586.
- [55] D. A. Smith, S. Webster, M. Ayad, S. D. Evans, D. Fogherly, D. Batchelder, *Ultramicroscopy* **1995**, *61*, 247–252.
- [56] S. R. Emory, S. Nie, *Anal. Chem.* **1997**, *69*, 2631–2635.
- [57] Y. Narita, T. Tadokoro, T. Ikeda, T. Sakai, S. Mononobe, M. Ohtsu, *Appl. Spectrosc.* **1998**, *52*, 1141–1144.
- [58] S. J. Stranick, L. J. Richter, R. R. Cavanagh, *J. Vac. Sci. Technol. B* **1998**, *16*, 1948–1952.
- [59] D. Zeisel, B. Dutoit, V. Deckert, T. Roth, R. Zenobi, *Anal. Chem.* **1997**, *69*, 749–754.
- [60] D. Zeisel, V. Deckert, R. Zenobi, T. Vo-Dinh, *Chem. Phys. Lett.* **1998**, *283*, 381–385.
- [61] T. Vo-Dinh, *Surface-Enhanced Raman Scattering*, Elsevier Science, **1995**.
- [62] V. Deckert, D. Zeisel, R. Zenobi, T. Vo-Dinh, *Anal. Chem.* **1998**, *70*, 2646–2650.
- [63] R. Stöckle, V. Deckert, C. Fokas, D. Zeisel, R. Zenobi, *Vibr. Spectrosc.* **2000**, *22*, 39–48.
- [64] M. Moskovits, *J. Mod. Phys.* **1985**, *57*, 783–826.
- [65] K. Kneipp, Y. Wang, H. Kneipp, I. Itzkan, R. R. Dasari, M. S. Feld, *Phys. Rev. Lett.* **1996**, *76*, 2444–2446.
- [66] G. Krausch, J. Mlynek, *Microelectron. Eng.* **1996**, *32*, 219–228.
- [67] D. Kossakowski, S. D. O'Connor, M. Widmer, J. D. Baldeshwieler, J. L. Beauchamp, *Ultramicroscopy* **1998**, *71*, 111–115.
- [68] J. D. Hogan, D. A. Laude, *The 38th ASMS Conference on Mass Spectrometry and Allied Topics*, Tucson, Arizona, **1990**, pp. 848–849.
- [69] K. Dreisewerd, M. Schürenberg, M. Karas, F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes* **1995**, *141*, 127–148.
- [70] B. Dutoit, D. Zeisel, V. Deckert, R. Zenobi, *J. Phys. Chem. B* **1997**, *101*, 6955–6959.
- [71] M. Stähelin, M. A. Bopp, G. Tarrach, A. J. Meixner, I. Zschokke-Gränacher, *Appl. Phys. Lett.* **1996**, *68*, 2603–2605.
- [72] H. J. Mamin, *Appl. Phys. Lett.* **1996**, *69*, 433–435.
- [73] C. Lienau, A. Richter, T. Elsaesser, *Appl. Phys. Lett.* **1996**, *69*, 325–327.
- [74] D. Singleton, G. Paraskevopoulos, R. S. Taylor, *Chem. Phys.* **1990**, *144*, 415–423.
- [75] R. Stöckle, N. Schaller, V. Deckert, C. Fokas, R. Zenobi, *J. Microsc.* **1999**, *194*, 378–382.
- [76] K. Sasaki, *J. Vac. Sci. Technol. B* **1997**, *15*, 2786–2790.
- [77] R. Stöckle, Y.-D. Suh, V. Deckert, R. Zenobi, *Chem. Phys. Lett.* **2000**, *318*, 131–136.

On Closed-Shell Interactions, Polar Covalences, d Shell Holes, and Direct Images of Orbitals: The Case of Cuprite**

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1. Introduction

During the last four decades of the previous century, efforts had been undertaken by “intellectual communities” (see, for example, Feyerabend^[1]) to surpass the “rational enlightenment” and the “critical rationalism” of Popper^[2] and others, though critiques of this epistemic relativism have never been silenced (for example, Sokal^[3]). The “postmodern” relativism had been prepared by Thomas Kuhn’s^[4] thesis that the historical development of science is a succession of incompatible viewpoints.

Fitting well into the popular “relativistic” convictions on differing viewpoints,^[1, 4] an experimental investigation on cuprite (Cu_2O) of combined X-ray and electron scattering has recently been interpreted^[5, 6a] regarding the four topics of this essay’s title. These authors “provide the first experimental verification of the controversial hypothesis that both ionic and covalent bonding occurs”^[5c] between copper and oxygen; they “demonstrate the existence of (covalent) bonding between pairs of copper”,^[5a, b] $\text{Cu}^+ \cdots \text{Cu}^+$, “with short metal–metal distances” of 3 Å,^[5a, c] which “is likely to make them re-write the chemistry textbooks”,^[5b] since “[X-ray diffraction is] unable to give details about the shape of the charge distribution”,^[6a] they claim “it is the first time that we have ever seen an orbital at this level of accuracy”,^[5c] namely “an unusual d-orbital hole”^[6a] in Cu^+ , and the “correspondence [between] the textbook d_{z^2} orbital is striking”.^[6a] These statements were enthusiastically presented during the last weeks of 1999 in many highly esteemed periodicals^[6] such as *Nature*, and *Chemical and Engineering News*^[6c] rated it among

the five most important chemical-research results of the last year. It seems to us necessary to refresh some textbook knowledge and we also include the results of some additional quantum-chemical calculations.^[7]

2. Polar Cu–O Bonding in Cuprite

Cuprite has received considerable interest because of it is a parent compound of the high temperature superconductors and of its interesting crystal structure.^[5, 8–11] Oxygen ions form a tetrahedral diamondlike lattice and a Cu^+ ion sits between each neighboring pair of O^{2-} ions to form the common linear $[\text{OCuO}]^{3-}$ dumb-bell unit. The Cu–O distance of 185 pm corresponds to the summation of the effective ionic radii^[12] of about 135 pm for O^{2-} (coordination number CN=4) and of about 50 pm for Cu^+ (CN=2). Because of the long O–O distances ($2 \times 185 = 370$ pm), this lattice has large holes, leaving enough space for a second, equivalent, interlacing lattice without requiring the lattices being explicitly bound together. The Cu^+ ions form a face-centered cubic lattice, that is, every unit cell contains eight Cu_4 tetrahedra each of volume $a^3/24$ (unit cell length $a = 427$ pm, $d(\text{Cu}–\text{Cu}) = 302$ pm), to leave four octahedral holes (volume = $a^3/6$) between them. One quarter of the Cu_4 tetrahedra is occupied by O atoms in the space group $Pn\bar{3}m$ (No. 224/2), while the other tetrahedra and all of the octahedra are empty.

Since the early development of quantum chemistry in the 1930s, the ideas of a partial charge transfer between ions and atoms, and of polar covalence between atoms of different electronegativity ($\chi_{\text{Cu}} = 1.8$; $\chi_{\text{O}} = 3.5$), have become adopted by the chemical community.^[13] Accordingly, effective charges of about $\text{Cu}^{0.5+}$ and $\text{O}^{1.0-}$ are expected. We have performed quantum chemical calculations of copper–oxygen structures (Figure 1) at the DFT (density functional theory) and SCF-MP2 levels of approximation, including relativistic corrections and using extended basis sets.^[7] We have found Mulliken and natural partial charges are $\text{Cu}^{(0.5 \pm 0.1)+}$ and $\text{O}^{(1.0 \pm 0.2)-}$.

Empirical electron-density determination by X-ray crystallography has become a mature field, exploiting synchrotron radiation and high-speed data collection devices, single crystal and powder diffraction, and electron and neutron scattering combined with theoretical techniques, a field competently reviewed in recent years.^[14, 15] Despite the point of view published in *Nature*,^[6a] Cu_2O has been the subject of several

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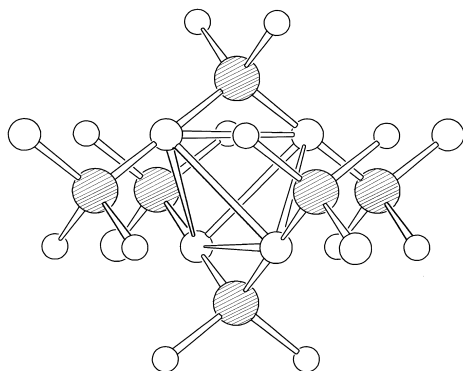


Figure 1. Copper–oxygen structures from the cuprite lattice. In the center lies the empty Cu_4 tetrahedron (smaller, open circles) at the $6d$ Wyckoff D_{2d} position, surrounded by six oxygen atoms (large, shaded circles). Every oxygen atom is surrounded tetrahedrally by four copper atoms. During calculation, variable numbers of the outer copper atoms were replaced by point charges in the range $[0, +\frac{1}{2}]$ in a symmetric manner to generate a neutral system.

experimental electron-density determinations,^[8–11] from which effective charges of $\text{Cu}^{(0.45–0.65)+}$ and $\text{O}^{(0.9–1.3)-}$ were deduced. The recent work^[5] is a good example of a state-of-the-art synergistic interaction of different techniques and of possible pitfalls. Remarkably, the effective charges reported therein ($\text{Cu}^{1.0+}$ and $\text{O}^{2.0-}$) differ significantly from the convergent collection of experimental and theoretical results.

3. The d Hole in $d^{10} \text{Cu}^+$ and the Effective Radius of Cu^+

In some of the literature on Cu_2O ,^[5, 9, 10, 16] it was mentioned that the Cu–O distance is short in comparison to the effective ionic radii. Similar comments can be found in the literature for some other compounds (see the discussion in ref. [17]). Such statements are often based on ionic radii which are either obsolete or the strong variation with coordination number had not been considered. The near-degeneracy of the nd and the $(n+1)s$ and $(n+1)p$ orbitals, and the linear symmetry of diagonally coordinated “coinage metal”(i) atoms are known^[16–19] to hybridize the nd_{z^2} and $(n+1)s$ orbitals to a σ -like orbital (Figure 2), and result in M^I ions with a slightly

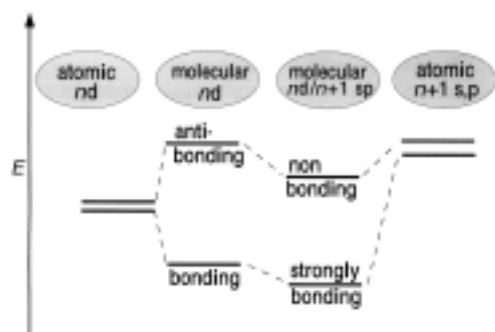


Figure 2. Schematic orbital energy diagram for atomic nd and $(n+1)s$, and molecular $d-d$ and $dsp-dsp$ orbitals.

oblate shape. All experimental and theoretical investigations of $[\text{XCuX}]^{3-}$ units ($\text{X} = \text{chalcogen}$) yield “short” internuclear distances, for example 185 pm for $[\text{OCuO}]^{3-}$.^[17, 19]

We have calculated^[7] the following natural atomic orbital (AO) populations on the “ $\text{Cu}^{0.5+}$ ” ion in Cu_2O as $d^{9.5}s^{0.75}p^{0.25}$. Zuo et al.^[5] reported $d^{9.8}s^{0.2}p^0$, that is, a less pronounced 3d hole and significantly less density in the 4sp shell. In either case, there is an electron deficiency in the formally closed 3d shell despite the 4sp electron excess on the (formally) singly charged copper ion. This was already well known from other experimental^[8–11] and theoretical investigations.^[16–19] The total electron density in Cu_2O and the differential electron density of Cu_2O with respect to Cu^{1+} and O^{2-} ions—the latter being stabilized in a positive lattice of point charges—are shown in Figures 3 to 5, where our SCF-MP2 results are compared with a common experimental $\Delta\rho$ map.^[5, 9–11]

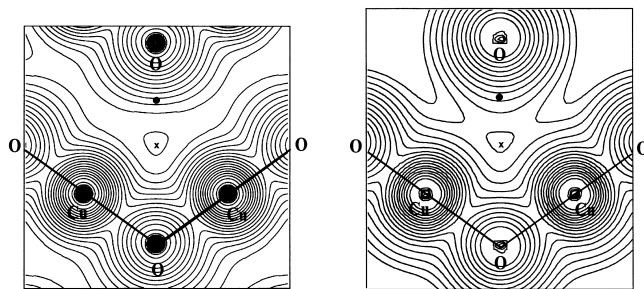


Figure 3. Total electron density in cuprite. Straight lines indicate the linear O–Cu–O units, and the dot (•) indicates the two copper atoms of the Cu_4 unit which lie above and below the plane. Contour line values are $0.05 \times 1.5^n \text{ e } \text{\AA}^{-3}$ ($n = 0, 1, 2, \dots$). Left: Experimental values of Lippmann and Schneider.^[11] Right: Our SCF-MP2 calculation. Note the similarity in the density minimum in the center (x) of the Cu_4 tetrahedra, which is at about $0.05 \text{ e } \text{\AA}^{-3}$ in the experiment and $0.07 \text{ e } \text{\AA}^{-3}$ in the calculation. Since the calculation refers to a cluster and not to an infinite crystal, the calculated electron density diminishes to zero outside.

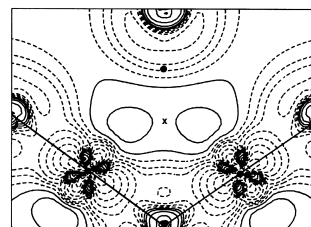


Figure 4. Calculated (SCF-MP2) difference electron density between cuprite (native cuprous oxide) and ions $\text{Cu}^+/\text{O}^{2-}$. Contour line values are $\pm 0.025 \times 2^n \text{ e } \text{\AA}^{-3}$ ($n = 0, 1, 2, \dots$); negative contours are dashed. Note the saddle point, rather than a maximum in the center of Cu_4 (*), $\Delta\rho < 0.05 \text{ e } \text{\AA}^{-3}$.

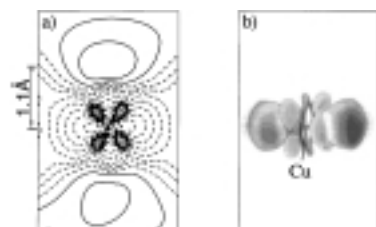


Figure 5. a) Calculated difference density around Cu in cuprite (see legend of figure 4). Note the d_σ hole on the horizontal axis the difference density of opposite, positive sign between the d_σ lobes, and the diffuse, extended positive lobes at the top and bottom (belt) due to d_σ -s hybridization. b) The respective computer graphic generated from ref. [5] without positive lobes above and below the horizontal axis.

4. Orbitals in “Nature”

In quantum mechanics, N -electron systems such as molecules or crystals are described by the many-electron wavefunction $\Psi(x_1, \dots, x_N)$, the density kernel $D(x_1, \dots, x_N, x_1', \dots, x_N')$ or, in the framework of DFT, by the single-particle probability $\rho(x_1)$, the so-called density. The x_i terms represent the canonical variables (position, momentum, angular momentum, and so forth) of one of the equivalent particles (electrons). The functions Ψ or D may (not necessarily must) be represented with the help of arbitrarily chosen single-electron orbitals φ_j . Within the ab initio approaches, the total density may then be represented by [Eq. (1)], where n_j is the occupation value of orbital φ_j . The orbitals are not all uniquely defined, only their sum is. In other words, orbitals represent arbitrary pieces of the total density (concerning some subtleties, see ref. [20]). Two points should be remembered:

$$\rho(r) = \sum_j n_j |\varphi_j(r)|^2 \quad (1)$$

Firstly, for some systems it is possible to choose the φ_j such that $n_j \approx 1$ for the first $j = 1 \dots N$ orbitals and $n_j \approx 0$ for the infinitely many other φ_j ($j > N$). In such cases the molecular orbital (MO) approximation is a reasonable approximation for many purposes [Eq. (2)]. For the DFT representation, there holds similarly for most systems [Eq. (3)], however, see ref. [21]. Now, let us compare two related N -electron systems a and b (for example, Cu_2O and $2\text{Cu}^+ + \text{O}^{2-}$). The so-called electron difference density is given in [Eq. (4)].

$$\rho(r) \approx \sum_j^N 1 \times |\varphi_j^{\text{MO}}(r)|^2 \quad (2)$$

$$\rho(r) = \sum_j^N 1 \times |\varphi_j^{\text{DFT}}(r)|^2 \quad (3)$$

$$\begin{aligned} \Delta\rho^{\text{ab}} &= \rho^{\text{a}} - \rho^{\text{b}} = \sum_j^N |\varphi_j^{\text{a,DFT}}|^2 - \sum_j^N |\varphi_j^{\text{b,DFT}}|^2 \\ &\approx \sum_j^N |\varphi_j^{\text{a,MO}}|^2 - \sum_j^N |\varphi_j^{\text{b,MO}}|^2 \end{aligned} \quad (4)$$

Secondly, often, orbital reorganization is small, which means that for all but few j , such as all j except $j = N$, $\varphi_j^{\text{a,DFT}} \approx \varphi_j^{\text{b,DFT}}$ and/or $\varphi_j^{\text{a,MO}} \approx \varphi_j^{\text{b,MO}}$. Then the electron difference density is given in [Eq. (5)]. The quantity $\Delta\rho^{\text{ab}}$

$$\Delta\rho^{\text{ab}} \approx |\varphi_N^{\text{a,DFT}}|^2 - |\varphi_N^{\text{b,DFT}}|^2 \approx |\varphi_N^{\text{a,MO}}|^2 - |\varphi_N^{\text{b,MO}}|^2 \quad (5)$$

represents the difference of two positive quantities and integrates to zero over the whole space; the positive and negative regions of the electron difference density cancel. If ionization is considered, either $\varphi_N^{\text{b,DFT}}$ or $\varphi_N^{\text{b,MO}}$ describe the ionized electron, which is distributed over the whole space with vanishing electron density everywhere and therefore [Eq. (6)] follows. The (not everywhere positive) differential

$$\Delta\rho^{\text{ion}} \approx |\varphi_N^{\text{a,MO}}|^2 \approx |\varphi_N^{\text{a,DFT}}|^2 \quad (6)$$

density $\Delta\rho^{\text{ion}}$ can often, but not always, be approximated by a single, everywhere positive, orbital density. If bonding in a

crystal is considered and one compares the experimentally investigated compound with the theoretically computed atoms or some selected ions, then $\varphi_N^{\text{b,MO}}$ or $\varphi_N^{\text{b,DFT}}$ is an atomic (ionic) orbital and $\varphi_N^{\text{a,MO}}$ or $\varphi_N^{\text{a,DEF}}$ is a localized Wannier orbital over the unit cell of the crystal. Then $\Delta\rho^{\text{bond}}$ is the difference of two localized charge distributions.

In summary, if the two systems compared can be well approximated by a finite number of appropriately chosen orbitals, and if most of these orbitals of the two systems can be chosen to be very similar, then the electron difference density of the two systems—for instance an experimentally investigated real one and a chosen theoretical reference—can be well approximated by the difference of the two chosen orbital densities. Thirdly, if furthermore one of these two orbitals is rather diffuse, then the difference density is dominated by the shape of the other orbital density map. Finally, and extending still further, if this orbital has large values on one atom only, $\Delta\rho$ is similar that of an atomic hybrid. This special situation does occur quite often for transition-metal compounds and a number of such cases, of levels of accuracy in the range of several $0.1 \text{ e } \text{\AA}^{-3}$ down to a very few $0.01 \text{ e } \text{\AA}^{-3}$, can be found in the literature.^[14, 15] An early experimental example, where the $\Delta\rho$ looked like “a textbook picture of a d atomic orbital”, was published in ref. [22], and one of the more recent ones is presented beautifully as a computer-generated graphic in *Nature*^[5] (Figure 5b). Such cases are correctly described not as an orbital in nature but as a case where one has found two systems for which conditions 1) to 4) could be fulfilled.

5. The Shape of Orbitals

Orbital densities $|\varphi|^2$ are real, positive functions in three-dimensional space. The respective orbital functions themselves may have positive or negative “phases” (or even complex phases in magnetic fields, or quaternionic phases^[23] in the case of relativistic spin-orbit coupling). These functions may be represented by contour surface graphics or in two dimensions by contour line maps. The contour lines or envelopes of an orbital cannot touch each other, because there exist regions of lower density and a zero surface between the lobes. These lobes are typically somewhat extended; see the AO density representations in Figure 6a. Both σ and π bonds can be formed due to the possibility of overlapping in different directions.

In the case of nonhybridized atomic orbitals, the orbital functions can be represented as a product $\varphi(r, \vartheta, \varphi) = R(r)Y(\vartheta, \varphi)$ of a radial factor $R(r)$ and an angular factor $Y(\vartheta, \varphi)$, where r, ϑ, φ are the polar coordinates with respect to the nucleus at the origin. One may then represent symbolically the topology of the orbital by “polar graphs” $r = |Y(\vartheta, \varphi)|$ or $r = |Y(\vartheta, \varphi)|^2$. In these cases, the symbolic lobes necessarily touch each other tangentially (Figure 6b). There is now no longer any direct relation between the (experimental or theoretical) orbital density distributions and these symbolic orbital pictures.

A further abstraction is found in many textbooks, where the lobes of the symbolic orbital pictures are represented slimmer still (Figure 6c) so that “metaphoric” pictures of molecular

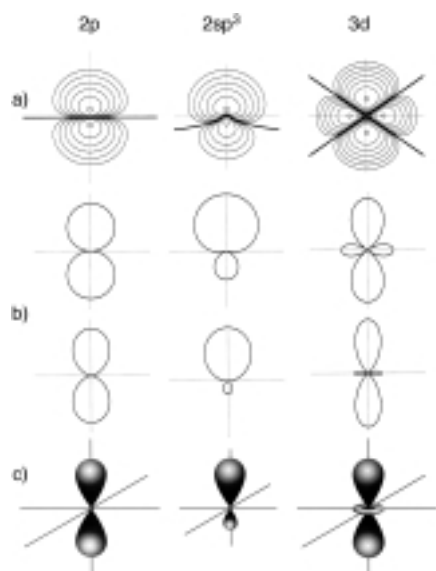


Figure 6. Plots and pictures of carbon 2p, carbon 2sp³, and chromium 3d orbitals. a) Contour plots, where the bold lines represent the zero contours; b) polar graphs (above: $r = |Y|$, below: $r = Y^2$); c) symbolic, textbook style, orbital pictures.

bonding can be generated in a transparent, non-overcrowded manner without explicitly showing the orbital overlaps, which are mandatory for covalent bonding. Of course, no experimental difference densities, independent of the choice of any reasonable reference densities, will resemble those textbook orbitals. Some textbook authors even call the latter orbital pictures (Figure 6c) “misleading”.

6. Closed-Shell Interactions

Closed-shell bonding is a controversial topic under active research for a number of years (see ref. [24]). Closed-shell atoms are distinguished by outer valence configurations of ns^2 , np^6 , or nd^{10} (for examples: ns^2 : He, Be, Ba, Au⁺; np^6 : O²⁻, Rb⁺, Xe; nd^{10} : Pd, Cu⁺, Au⁺). Four types of forces clarify closed-shell–closed-shell bonding: 1) the dispersion attraction $F \sim \alpha^2/R^6$ or $F \sim \nu^2/R^6$ (force F , internuclear distance R , and electric polarizabilities α , and atomic volumes ν of the two interacting atoms); 2) electrostatic effects, such as the ion-induced dipole attraction $F \sim \alpha/R^4$; 3) the common closed-shell repulsion (according to the Pauli exclusion principle), which acts as the electronic densities (with both spin directions) of different atoms begins to overlap; 4) specific overlapping orbital interference effects, which contribute to a lowering of the “Pauli repulsion” by hybridization of nearly degenerate orbitals such as 2s with 2p or 3d with 4s. In the case of point 4), the equilibrium interatomic distance may become rather short and the attractive forces (points 1) and, in the case of ions, 2)) become relatively large.

The weakest closed-shell–closed-shell attraction occurs in He₂, where α or ν are especially small and the minimum in the electronic potential-energy curve is just deep enough (calculated $D_e = -0.09$ kJ mol⁻¹ at $R_e = 3$ Å)^[25] to support the zero-vibrational level with dissociation energy D_0 as small as 10⁻⁵ kJ mol⁻¹.^[26] The (so far) strongest closed-shell attraction has been predicted to occur for an atomic–anionic system

with especially large α values, namely Ba–Au⁻ with a D_0 of about 140 kJ mol⁻¹.^[27]

An exciting, large class of substances is formed by clusters of Au^I molecular compounds such as A.^[28] These aurophilic systems exhibit Au⁺–Au⁺ closed-shell bond energies in the range 20–50 kJ mol⁻¹.^[24, 29] There exist two extreme viewpoints concerning the mechanism of the aurophilic attraction. The earlier, semiempirical calculations (by Hoffmann, Mingos, and Schmidbaur, among others^[30]) exaggerated the orbital stabilization of the nd^{10} – nd^{10} repulsive Pauli interaction on account of nd – $(n+1)s(p)$ hybridization and attributed the bonding to the lower energies of the bonding and antibonding d type MOs (Figure 2). The other point of view is that only the dispersion interaction (electron correlation) is responsible for Au^I–Au^I bonding.^[24] In this context, one may cite the s^2 – s^2 interaction in Be₂.^[31] Because of s – p hybridization, strong (Pauli-principle derived) repulsion between the two 2s² shells of Be occurs only at comparatively short distances, $R < 2$ Å. Although there is no bonding at all at the noncorrelated SCF MO level, the repulsion at $R > 2$ Å is weak enough to be compensated by the van der Waals attraction, which yields a potential curve minimum of $D_e = -10$ kJ mol⁻¹ at $R_e = 2.45$ Å.

A recent analysis of bonding in several Au^I cluster compounds^[32] indicates that, not unexpectedly, the synergetic cooperation of orbital interference and electron correlation is responsible for Au^I–Au^I bonding, too. We stress that such bonds are not characterized by pronounced “bond densities” between the atoms. This is expected, since electron correlation is an electron-pair phenomenon, which has only a small influence on the one-electron density distribution. Furthermore, Pauli repulsion (partially quenched by hybridization) is characterized by a slight reduction of electronic density in the overlap region.

7. Closed-Shell Cu–Cu Bonding in Cuprite?

A long time ago, O’Keeffe had calculated the Madelung constants and showed that the electrostatic interaction of the two point-charge lattices in Cu₂O is slightly repulsive.^[33] One obtains a value of the order 2 kJ mol⁻¹ for the effective charges mentioned in Section 2. While there exist several indications of a structural instability for the oxygen sublattice,^[34, 35] the Cu₄O₂ double lattice is still stable. Accordingly there must be either a topologically unexpected electronic-charge distribution in Cu₂O, with possibly far-reaching implications for high temperature superconductors, as postulated from time to time,^[5, 6a, 8] or the traditional dispersion and higher-order polarization effects are large enough (estimated to be a few kJ mol⁻¹) to overcome the electrostatic repulsion.

One may still speculate whether in addition some orbital interaction contributes to the stability of cuprite. In contrast to several experimental and theoretical investigations,^[9–11, 16] an older investigation by Fischer et al.^[8] and recently Zuo et al. “for the first time”,^[5] stated a significant electron difference density of about 0.2 e Å⁻³ over a volume of about 1 Å diameter in the center of the empty Cu₄ tetrahedra, containing about 0.1 e at each of the six $6d$ [$\frac{1}{4}\frac{1}{4}\frac{3}{4}$] D_{2d} positions.

The authors of the paper in *Nature*^[5] themselves believe that they have excluded all extinction, absorption, and other intensity errors, well known to be serious problems in cuprite studies.^[9] Neither statistical and possible systematic $\Delta\rho$ uncertainties are mentioned, nor uncertainties for the Wyck-off-6d position, which is expected to be especially sensitive. Another open question is whether an artifact of the theoretical refinement procedure has created the above-mentioned density feature. The Cu₄ hole is situated in a shortened octahedron of two axially positioned oxygen atoms (at 213 pm distance) and four equatorial ones (at 302 pm). At these distances, the single zeta basis used for the multipole refinement of the oxygen centers^[5] has appreciable density tails. Two other explanations for the Cu₄ density feature, however, may be ruled out: 1) due to oxygen atomic displacements in the percent range, this seems too large despite the exper-

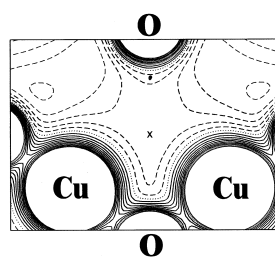


Figure 7. Electrostatic potential in the Cu₄ tetrahedron in cuprite. Note the flatness of the saddle in the center (*). Contour line values differ by 1 V; repulsive contours are dashed; zero potential is dotted. The Maddung potential is similarly flat and small in the central region.

For the defectless system, we calculate a positive difference density in a trigonally distorted diffuse belt around the Cu d¹⁰ shell, due to the d-s orbital hybridization, corresponding to the density deficiency on the orthogonal Cu-O axis. The local symmetry of Cu is D_{3d}, which is nicely reflected in the “Copper–Oxygen Bond” of ref. [5], although the diffuse belt is missing (see Figure 5b).

However, we do not find a pronounced density maximum but a minimum (in agreement with the band structure calculations of ref. [5]) at the center of Cu₄ and we do not find there more than a 0.045 e Å⁻³ difference density although we have included also an extended basis at the center (see Figures 3 and 4). A significant accumulation of electron density between the Cu⁺ cores will only occur at Cu–Cu distances of ≤ 2.5 Å, comparable to the ones in the Cu₂ molecule (222 pm) or in Cu metal (256 pm). As an example, we show the difference density of the calculated [CuF]₂ dimer **B** at different Cu–Cu distances in Figure 8. Upon decreasing the proximity to about 2.5 Å, the d-s hybridization density belts around copper do not remarkably influence each other; in cuprite, however, the Cu proximity is 3 Å.

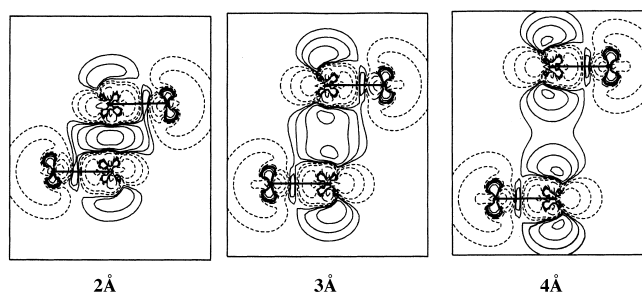
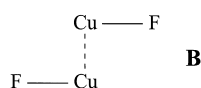


Figure 8. Electron density difference of calculated [CuF]₂ (structure **B**) with respect to Cu⁺ and F⁻ for Cu–Cu distances of 2–4 Å. Contour line values are $\pm 0.01 \times 2^n \text{ e Å}^{-3}$ ($n=0,1,2,\dots$); negative contour lines are dashed.

8. Conclusion

There seems as yet to exist no proof of significant Cu⁺–Cu⁺ closed-shell bonding at such large distances as found in cuprite, nor of a significant bonding (difference) density for Cu⁺–Cu⁺. No strongly founded doubts have arisen over the last decades against the chemical concept of a partially ionic covalency in Cu^I–O bonds. The effective charges are about one half of the formal ones, if one chooses the usual charge definitions. (Concerning the problematic choice of unit for the effective charges on atoms in molecules and crystals, see ref. [36]). There is about one half of an electron missing in the Cu d_z orbital, but about one electron is found in the 4sp shell of the formal Cu⁺ ion. There are many cases found in the literature, especially for transition metal compounds, where an appropriately defined difference electron density is dominated in some region of space by the difference between two atomic-orbital densities, or even by a single orbital density, if also the orbital densities are appropriately chosen. The symbolic textbook orbital plots are intrinsically different from orbital density-contour plots.

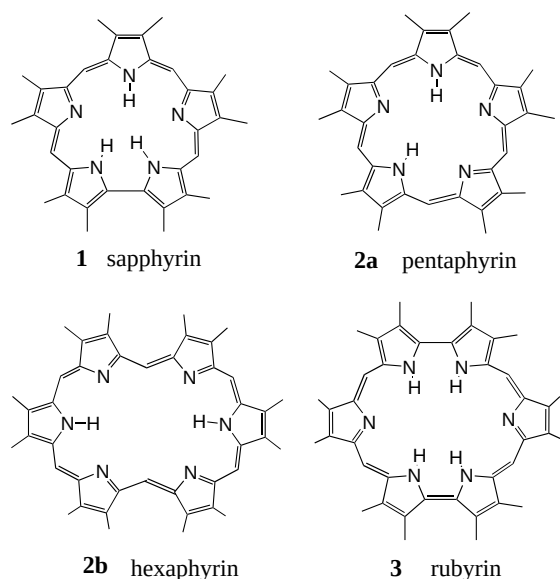
- [1] P. K. Feyerabend, *Against Method. Outline of an Anarchistic Theory of Knowledge*, Humanities Press, London, **1975**; P. K. Feyerabend, *Wider den Methodenzwang. Skizze einer anarchistischen Erkenntnistheorie*, Suhrkamp, Frankfurt/Main, **1976**.
- [2] K. R. Popper, *Logik der Forschung*, Springer, Wien, **1934/1935**; K. R. Popper, *The Logic of Scientific Discovery*, Hutchinson, London, **1959**.
- [3] A. Sokal, J. Bricmont, *Impostures Intellectuelles*, Odile Jacob, Paris, **1997**; A. Sokal, J. Bricmont, *Fashionable Nonsense*, Picador, New York, **1998**; A. Sokal, J. Bricmont, *Eleganter Unsinn*, Beck, München, **1999**; see also www.physics.nyu.edu/faculty/sokal.
- [4] T. S. Kuhn, *The Structure of Scientific Revolutions*, University Press, Chicago, **1962**; T. S. Kuhn, *Die Struktur wissenschaftlicher Revolutionen*, Suhrkamp, Frankfurt/Main, **1967**.
- [5] a) J. M. Zuo, M. Kim, M. O'Keeffe, J. C. H. Spence, *Nature* **1999**, *401*, 49; see also the authors' homepages and links therein and b) www.asu.edu/asunews/Releases/Cuprite.htm and c) <http://www.clasdean.la.asu.edu/news/cuprite.htm>.
- [6] a) C. J. Humphreys, *Nature* **1999**, *401*, 21; b) P. Yam, *Sci. Am.* **1999**, *281* (5), 28; K. Leutwyler, www.sciam.com/explorations/1999/092099 cuprite; c) P. Zurer, *Chem. Eng. News* **1999**, November 29, 38; d) A. G. Samuelson, *Curr. Sci. India* **1999**, *77*, 1131; e) *Phys. World* **1999**, October, 5; f) V. Drach, *Phys. Unserer Zeit* **1999**, *29*, 263; g) M. Rauner, *Phys. Bl.* **1999**, *55* (II), 17; h) S. Albus, *Nachr. Chem. Tech. Lab.* **1999**, *47*, 1298.
- [7] “A Theoretical Investigation of Cuprite”: S. G. Wang, W. H. E. Schwarz, unpublished results.

- [8] a) D. Mullen, K. Fischer, *Z. Kristallogr.* **1981**, 156, 85; b) K. Eichhorn, J. Spilker, K. Fischer, *Acta Crystallogr. Sect. A* **1984**, 40, C160.
- [9] R. Restori, D. Schwarzenbach, *Acta Crystallogr. Sect. B* **1986**, 42, 201.
- [10] a) A. Kirfel, K. Eichhorn, *Acta Crystallogr. Sect. A* **1990**, 46, 271; b) A. Kirfel, H. G. Krane (Hamburger Synchrotronstrahlungslabor/Deutsches Elektronen-Synchrotron), *HASYLAB/DESY Annual Report*, **1999**.
- [11] a) T. Lippmann, J. R. Schneider, *J. Appl. Crystallogr.* **2000**, 33, in press; b) T. Lippmann, J. R. Schneider, *HASYLAB/DESY Annual Report*, **1998**; www-hasylab.desy.de/science/annual_reports/1998/part1/contrib/24/1298.pdf.
- [12] a) R. D. Shannon, *Acta Crystallogr. Sect. A* **1976**, 32, 751; b) R. D. Shannon, *Struct. Bonding Cryst.* **1981**, 2, 53.
- [13] a) L. Pauling, *J. Am. Chem. Soc.* **1932**, 54, 3570; b) L. Pauling, *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, NY, **1939**; L. Pauling, *Die Natur der Chemischen Bindung*, Verlag Chemie, Weinheim, **1962**; c) R. T. Sanderson, *Polar Covalence*, Academic Press, New York, **1983**.
- [14] P. Coppens, *X-Ray Charge Densities and Chemical Bonding*, Oxford University Press, Oxford, **1997**; V. G. Tsirelson, R. P. Ozerov, *Electron Density and Bonding in Crystals*, Institute of Physics, Bristol, **1996**.
- [15] M. A. Spackman, *Annu. Rep. Prog. Chem. C* **1997**, 94, 177; M. A. Spackman, *Annu. Rep. Prog. Chem. C* **1994**, 91, 175.
- [16] P. Marksteiner, P. Blaha, K. Schwarz, *Z. Phys. B* **1986**, 64, 119.
- [17] M. S. Liao, W. H. E. Schwarz, *Acta Crystallogr. Sect. B* **1994**, 50, 9.
- [18] L. E. Orgel, *J. Chem. Soc.* **1958**, 4186.
- [19] M. S. Liao, W. H. E. Schwarz, *J. Alloys Compd.* **1997**, 246, 2.
- [20] W. H. E. Schwarz, B. Müller, *Chem. Phys. Lett.* **1990**, 166, 621; W. H. E. Schwarz, A. Langenbach, L. Birlenbach, *Theor. Chim. Acta* **1994**, 88, 233, and references therein.
- [21] S. G. Wang, W. H. E. Schwarz, *J. Chem. Phys.* **1996**, 105, 4641.
- [22] B. Rees, *Acta Crystallogr. Sect. A* **1976**, 32, 48.
- [23] N. Rösch, *Chem. Phys.* **1983**, 80, 1.
- [24] P. Pykkö, *Chem. Rev.* **1997**, 97, 597; *Unkonventionelle Wechselwirkungen in der Chemie metallischer Elemente* (Ed.: B. Krebs), VCH, Weinheim, **1992**.
- [25] T. Van Mourik, T. H. Dunning, *J. Chem. Phys.* **1999**, 111, 9248, and references therein.
- [26] F. Luo, G. C. McBane, G. Kim, C. F. Giese, W. R. Gentry, *J. Chem. Phys.* **1993**, 98, 3564; T. Korona, H. L. Williams, R. Bukowski, B. Jeziorski, K. Szalewicz, *J. Chem. Phys.* **1997**, 106, 5109.
- [27] R. Wesendrup, P. Schwerdtfeger, *Angew. Chem.* **2000**, 112, 938; *Angew. Chem. Int. Ed.* **2000**, 39, 907.
- [28] P. Pykkö, Y. F. Zhao, *Angew. Chem.* **1991**, 103, 622; *Angew. Chem. Int. Ed. Engl.* **1991**, 30, 604; O. D. Häberlen, N. Rösch, *J. Phys. Chem.* **1993**, 97, 4970.
- [29] H. Schmidbaur, *Chem. Soc. Rev.* **1995**, 391.
- [30] A. Dedieu, R. Hoffmann, *J. Am. Chem. Soc.* **1978**, 100, 2074; R. Hoffmann, *Angew. Chem.* **1982**, 94, 725; *Angew. Chem. Int. Ed. Engl.* **1982**, 21, 711; K. P. Hall, D. M. P. Mingos, *Prog. Inorg. Chem.* **1984**, 32, 237; D. G. Evans, D. M. P. Mingos, *J. Organomet. Chem.* **1985**, 295, 389; A. Görling, N. Rösch, D. E. Ellis, H. Schmidbaur, *Inorg. Chem.* **1991**, 30, 3986.
- [31] For example, C. Sosa, J. Noga, R. J. Bartlett, *J. Chem. Phys.* **1988**, 88, 5974.
- [32] "On the Aurophilic Interaction": S. G. Wang, W. H. E. Schwarz, unpublished results.
- [33] M. O'Keeffe, *J. Chem. Phys.* **1963**, 38, 3035.
- [34] M. Ivanda, D. Waasmaier, A. Endriss, J. Ihringer, A. Kirfel, W. Kiefer, *J. Raman Spectrosc.* **1997**, 28, 487; reference [10a].
- [35] J. Hallberg, R. C. Hanson, *Phys. Status Solidi* **1970**, 42, 305.
- [36] J. Meister, W. H. E. Schwarz, *J. Phys. Chem.* **1994**, 98, 8245.

Giant Porphyrinoids: From Figure Eights to Nanomolecular Cavities

Timothy D. Lash*

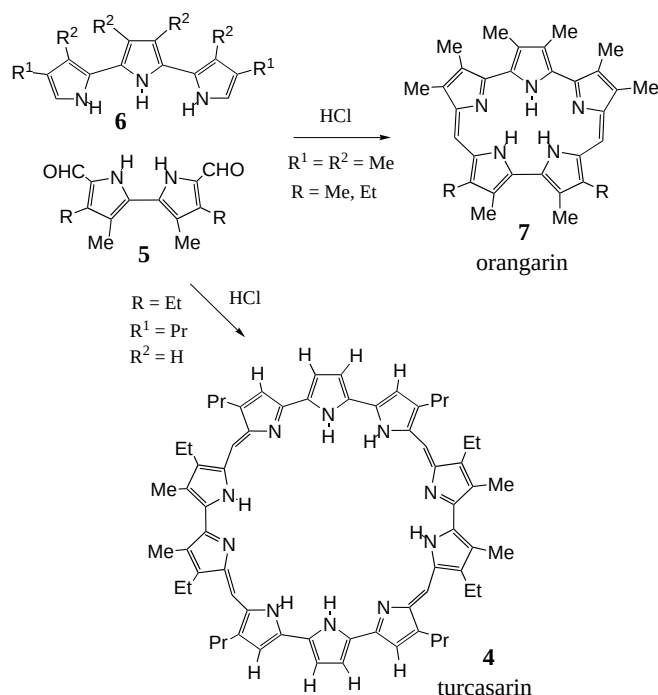
Expanded porphyrinoids with five or more conjugated pyrrolic rings have been known for well over 35 years.^[1] Sapphyrin (**1**) and its analogues, pentapyrrolic structures with four carbon bridges, were originally discovered by accident during R. B. Woodward's investigations into the total synthesis of Vitamin B₁₂.^[1, 2] These macrocyclic systems were further studied by Woodward and Johnson and later, in more detail, by other workers. Further examples of penta- and hexapyrrolic structures were described during the 1970's, 1980's, and early 1990's, including analogues of pentaphyrin (**2a**),^[3] hexaphyrin (**2b**),^[4] and rubyrin (**3**).^[5] The early studies



in this area mostly focused upon the aromatic characteristics of these conjugated oligopyrroles and to a lesser extent on their metallation chemistry.^[1a, b] However, more recent observations, most notably by Sessler and co-workers,^[1d, 2c] have discerned many additional intriguing properties for these systems, including selective anion binding. In addition, the

unique properties of the expanded porphyrins have led to potential biomedical applications.^[1b, d]

Perhaps surprisingly, no larger oligopyrrolic structures were described in the literature until 1994 when Sessler and coworkers reported the synthesis of a decapyrrolic macrocycle **4**, named turcasarin for the deep turquoise color that it exhibits in solution.^[6] The synthesis of **4** made use of a "MacDonald-type" condensation,^[7, 8] where a bipyrrrole dialdehyde **5** is condensed with a terpyrrole **6** in the presence of an acid catalyst (Scheme 1). Interestingly, hexamethylterpyrrole underwent a "3+2" condensation with **5** to give the



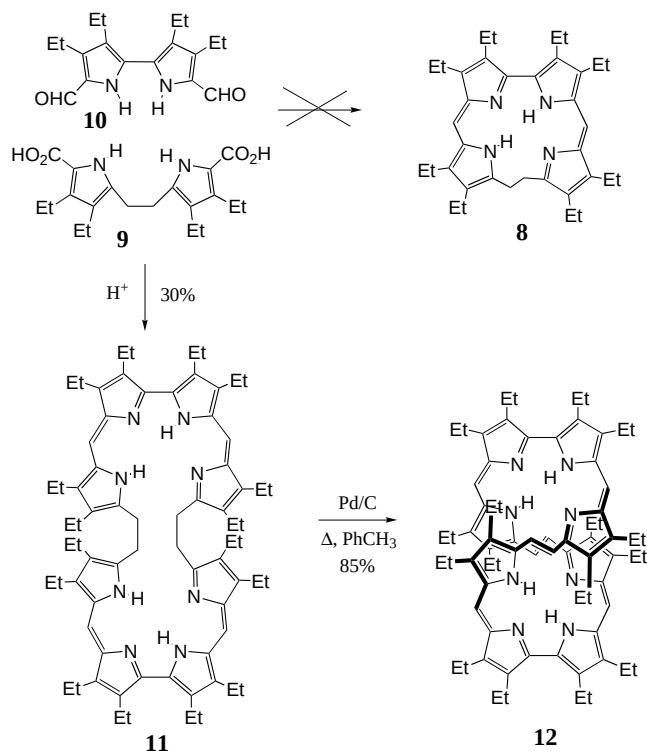
Scheme 1. Sessler's synthesis of turcasarin **4**.

nonaromatic pentapyrrolic system of orangarin (**7**) as the exclusive macrocyclic product,^[9] while the dialkylterpyrrole with R¹ = Pr, R² = H underwent a "3+2+3+2" condensation to afford decapyrrolic turcasarin (**4**) instead.^[6] This demonstrates that the peripheral substituents exert a critical influence on these cyclizations and may, under certain circumstances, facilitate the formation of higher order systems. The NMR data for **4** was consistent with a nonaromatic

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fully conjugated macrocycle that possesses C_2 symmetry and exists in a twisted “figure eight” conformation. This analysis was confirmed in the solid state for the tetrahydrochloride $[H_4(4)]^{4+} 4Cl^-$ by X-ray crystallography. The point of “ribbon crossing” for the figure eight structure showed a separation between the pyrrolic units of 3.268 Å. The presence of two “hemipentaphyrin” cavities indicated that this structure might be able to form bimetallic complexes and some evidence for the generation of a bis-uranyl chelate was noted.^[6] The figure eight conformations are also chiral and interconvert slowly on the NMR timescale, suggesting the possibility of isolating conformational enantiomers for this system.^[6] While these possibilities have so far been little explored, a dioxaturcasarin has recently been prepared.^[1d]

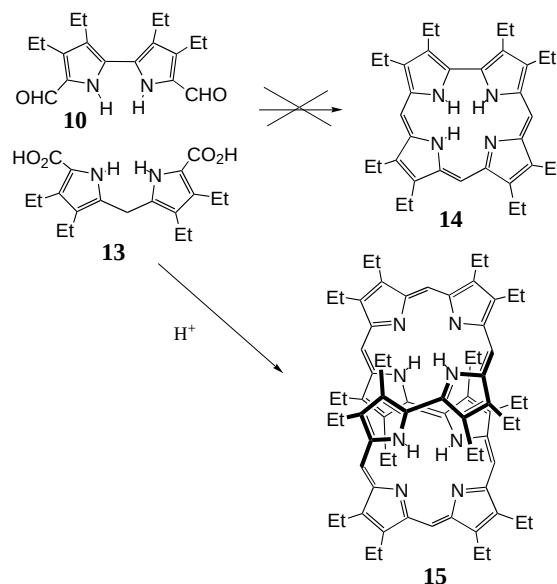
In relation to their exploration of porphyrin isomers,^[10] Vogel and co-workers attempted to prepare a dihydrocorrphycene **8** by a MacDonald “2+2” condensation of 1,2-di(2-pyrrolyl)ethane (**9**) with a bipyrrroledialdehyde **10** (Scheme 2).



Scheme 2. Vogel's synthesis of [36]octaphyrin-(2.1.0.1.2.1.0.1) **12**.

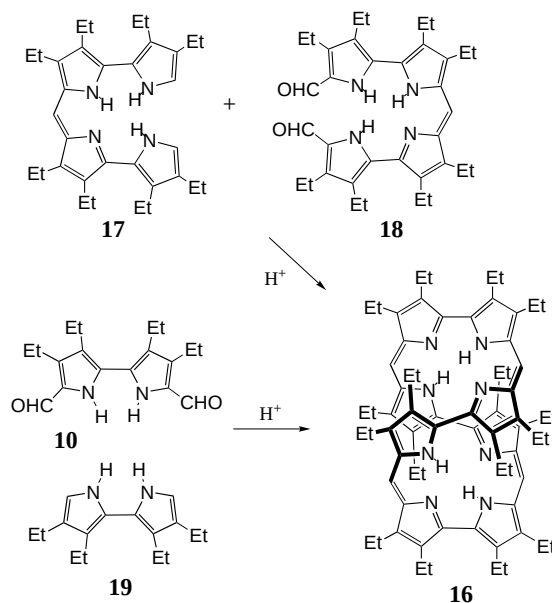
Instead of isolating the expected tetrapyrrole **8**, an octapyrrolic “2+2+2+2” condensation product **11** was obtained.^[11] Dehydrogenation with Pd/C in refluxing toluene afforded the fully conjugated albeit nonaromatic [36]octaphyrin **12** (Scheme 2) in 85% yield. The NMR spectra for **11** and **12** indicated the presence of diastereotopic CH_2 groups and figure eight conformations were postulated for these structures. This was confirmed for **12** by X-ray crystallography. The barrier to conformational racemization for **12** must be high as the ethyl substituents remained diastereotopic by proton NMR spectroscopy even at 378 K.

In related studies,^[11] acid-catalyzed condensation of **13** with **10** failed to give corrole (**14**), the “2+2” condensation product, but instead generated the “2+2+2+2” product [34]octaphyrin **15** (Scheme 3). Again this system favors a



Scheme 3. Vogel's synthesis of [34]octaphyrin-(1.1.1.0.1.1.1.0) **15**.

figure eight conformation as demonstrated by NMR spectroscopy and X-ray crystallography. A third octapyrrolic system **16** was prepared^[12] by the “4+4” condensation of tetrapyrrole **17** with the related dialdehyde **18** in the presence of TFA, although better yields were subsequently obtained by treating bipyrrrole **19** with **10** (Scheme 4). X-ray crystallography indicates that [32]octaphyrin **16** also exists as a figure eight structure, although this system appears to be far more dynamic and the barrier to racemization is predicted to be relatively low.^[12]



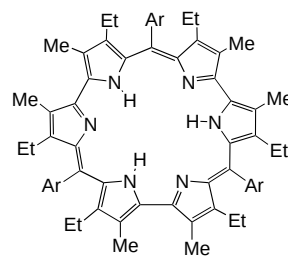
Scheme 4. Vogel's syntheses of [32]octaphyrin-(1.0.1.0.1.0.1.0) **16**.

Octaphyrins **12**, **15**, and **16**, together with the tetrahydrooctaphyrin **11**, potentially provide two porphyrin-sized cavities for metal complexation. This offers the possibility of forming mononuclear, homobinuclear, and heterobinuclear metal complexes. This potential has recently been exploited in the preparation of all three categories of metal chelates for **16**, while nickel(II), palladium(II), and copper(II) homobinuclear complexes of **12** have also been synthesized.^[13, 14] Although the full details of this work have as yet not been published, the electrochemical properties of these metallooctaphyrins have been examined.^[13]

All of the octaphyrins described above, together with turcasarin (**4**), exist in chiral figure eight conformations and the possibility exists that these might be separated into the individual enantiomers. This has recently been accomplished for **12** and the related tetrahydrooctaphyrin **11**.^[14] Octaphyrin **12** was easily resolved by HPLC on a chiral column and the individual enantiomers characterized by circular dichroism. The enantiomers of **12** proved to be optically stable at room temperature and no racemization was observed in *n*-hexane even after several hours at 60 °C. The derivative **11** was also resolved by HPLC at 15 °C, although racemization occurred at room temperature. Nonetheless, even in this case the barrier to racemization was shown to be > 85 kJ mol⁻¹.^[14] In addition to these exciting developments, the Pd-Pd complex of **11** and the Pd-Pd and Cu-Cu chelates of **12** have also been resolved. These results point the way to potential applications for these novel chiral molecules as catalysts for asymmetric syntheses.

The assembly of four or more components by the MacDonald condensation can potentially be applied to condensations involving other pyrrolic building blocks such as 1,2-di(2-pyrrolyl)ethanes, dipyrrolylketones, dipyrrolylsulfides, and 2,2-di(2-pyrrolyl)propanes.^[15] Although the resulting pyrrolic macrocycles may not be fully conjugated, this approach has led to the generation of a dodecapyrrolic system by cross-condensation of a bipyrroledialdehyde with a 2,2-di(2-pyrrolyl)propane.^[15]

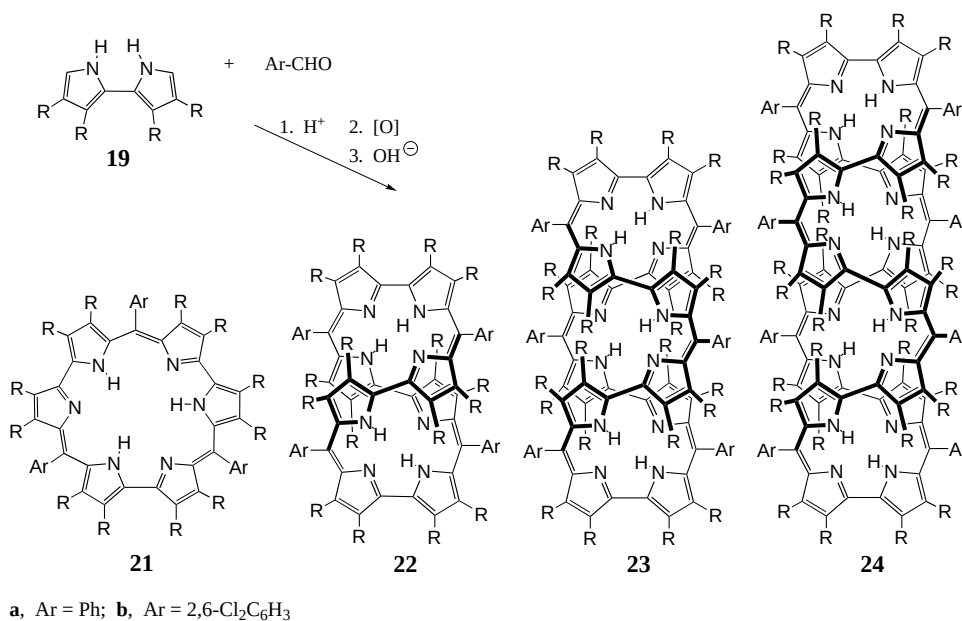
The Rothmund reaction provides the most straightforward method for porphyrin synthesis^[16] in that a simple pyrrole is condensed with an aldehyde to generate the porphyrin macrocycle. Within the last years, this chemistry has been shown to generate other macrocyclic species such as N-confused porphyrins,^[17] corroles,^[18] sapphyrins,^[19] and hexaphyrins.^[20] Sessler and co-workers demonstrated that a tetraalkylbipyrrole underwent a Rothmund-type condensation to produce the hexapyrrolic system rosarin (**20**) in excellent yields.^[21] As was the case for the MacDonald syntheses described above, the nature of the substituents of bipyrrole and aldehyde might be



20, Ar = Ph, *o*-NO₂C₆H₄, *p*-NO₂C₆H₄, *p*-H₃COC₆H₄

expected to influence the structures of the resulting macrocyclic products. Although the formation of a tetrapyrrolic product is disallowed due to steric constraints, the generation of higher cyclic oligomers of the rosarin type is clearly a possibility.

In an important new study, Setsune et al. report^[22] the synthesis of octa-, dodeca- and hexadecacyclopyrroles by exploiting this aspect of the Rothmund chemistry (Scheme 5). Reaction of tetraethylbipyrrole **19** with benzaldehyde in the presence of 0.25 equivalents of trifluoroacetic acid in dichloromethane at room temperature, followed by oxidation with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), afforded the rosarin **21a** in 60% yield together with a new purple compound corresponding to octaphyrin **22a**. X-ray analysis of **22a** indicated that the *meso*-phenyl substituents caused the ring system to be highly distorted from planarity. In order to further exaggerate this effect, the chemistry was repeated using 2,6-dichlorobenzaldehyde but in this case a third blue-colored macrocyclic product was isolated. The yield of rosarin was much reduced (17% **21b**), while octaphyrin **22b** and the new species dodecaphyrin **23b** were isolated in 19% and 5% yield, respectively. Reaction in the presence of zinc acetate produced the dodecaphyrin in 6% yield together with a second blue compound that was



a, Ar = Ph; **b**, Ar = 2,6-Cl₂C₆H₃

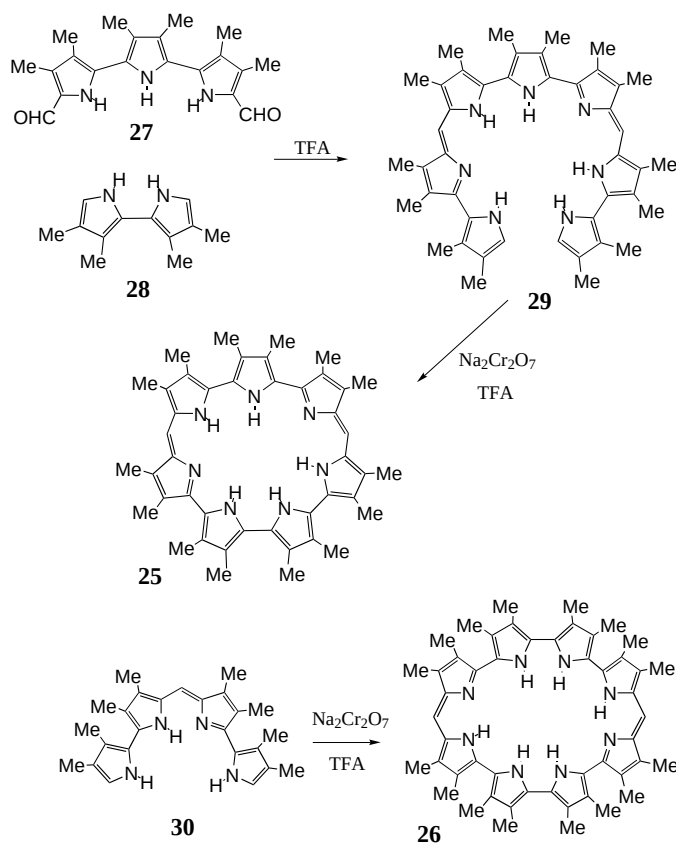
Scheme 5. Setsune's synthesis of octaphyrins **22**, dodecaphyrins **23**, and hexadecaphyrins **24**. R = Et.

identified as hexadecaphyrin **24b** (9%).^[22] These represent the largest cyclopyrrolic macrocycles described to date and the simplicity of this methodology, together with the accessibility of the bipyrrolic precursors, makes these compounds readily available for further study. Clearly, the steric bulk of the aryl substituents provides a means by which the ratio of macrocyclic products can be manipulated. The influence of the zinc acetate in directing the chemistry is also intriguing, if somewhat difficult to explain. X-ray crystallography for **23b** shows that the decapyrrolic system takes on a chiral zigzag conformation that is analogous to the figure eight structures observed previously for the octaphyrins.

The fact that macrocycles with 8, 12, or 16 pyrrole subunits can be generated by this chemistry, while 10 and 14 membered systems are not observed, may indicate that there is a preference for the alignment of four individual pyrrole rings and this structural consideration presumably guides macrocyclic ring formation. The crystal structure for **23b** differs from those of Vogel's octaphyrins in that there is a greater degree of distortion from planarity and the separation between the "upper" and "lower" strands corresponds to approx. 5 Å, while the length of the entire π -conjugated structure is 9.9 Å.^[22] Molecular models for the larger hexadecaphyrin **24b** suggest that the cylindrical cavity of this system has a diameter of approximately 1 nm.^[22] These new systems may be able to act as hosts in molecular recognition studies and anion binding, and could potentially form complexes with three or more transition metal ions.

McMurry coupling has been extensively utilized in the preparation of porphyrin analogues^[10] with CH=CH connections, but this methodology has not been applied to macrocycles with more than six pyrrole subunits. Nonetheless, this approach has been used to synthesize conjugated macrocycles with six five-membered ring subunits,^[23–25] in particular, thiophene-containing systems. Wittig chemistry has also been applied to the synthesis of larger ring systems built up from furan and thiophene rings.^[14] These approaches, particularly the former, may provide alternative routes to new porphyrinoid giants in the future.

Oxidative couplings have recently become widely applied in the synthesis of porphyrinoid systems such as sapphyrins,^[26] corroles,^[27] and expanded corroles.^[28] In a new study, Sessler et al. report^[29] the synthesis of [28]heptaphyrin **25** and [32]octaphyrin **26** (Scheme 6). Previous attempts to synthesize macrocycles with quaterpyrrole subunits from preformed quaterpyrroles had failed, and a strategy to generate this moiety by coupling bipyrrole units was explored. Acid catalyzed condensation of terpyrrole dialdehyde **27** with bipyrrole **28** afforded the open-chain heptapyrrole **29** and subsequent cyclization with sodium dichromate in TFA afforded heptaphyrin **25** in an overall 43% yield. Similarly, tetrapyrrole **30** reacted under these conditions to give octaphyrin **26** in 16% yield.^[29] These systems contain a far greater number of direct pyrrole–pyrrole linkages relative to the previously discussed porphyrinoids, and for this reason can be considered to be "contracted" expanded porphyrins.^[29] This greatly alters the conformations of these macrocycles. Although they are both nonaromatic compounds, heptaphyrin **25** is fairly planar and forms an open cavity. In the



Scheme 6. Sessler's Cr^{VI} oxidative cyclization methodology for the synthesis of [28]heptaphyrin-(1.0.0.1.0.0.0) **25** and [32]octaphyrin-(1.0.0.0.1.0.0.0) **26**.

diprotonated form [H₂(**25**)]²⁺, it can accommodate a sulfate counterion (observed by X-ray crystallography) and hence this system shows some promise for anion binding. X-ray diffraction analysis of octaphyrin **26** was carried out on the dihydrochloride [H₂(**26**)]²⁺ 2Cl[−] and, while this system greatly deviates from planarity, it also encloses a large cavity and can bind to two chloride anions.^[29] These more open structures are unlikely to form metal complexes of the type observed for Vogel's octaphyrins, but nonetheless appear to be better suited for anion binding studies. The Cr^{VI} oxidative coupling methodology also appears to be well suited for the synthesis of expanded porphyrinoids and complements the MacDonald and Rothmund routes to related systems.

Now that efficient synthetic methodologies are available to these nonaromatic cyclo-oligopyrroles, research is likely to focus on their physical and chemical properties. There is clearly a great deal of promise in selective anion binding, molecular recognition and catalysis. In addition, now that resolutions of chiral octaphyrins and their bimetallic chelates have been achieved, applications in asymmetric catalysis and enantioselective binding can be contemplated.

[1] a) A. W. Johnson in *Porphyrins and Metalloporphyrins* (Ed.: K. M. Smith), Elsevier, Amsterdam, **1975**; b) J. L. Sessler, S. J. Weghorn, *Expanded, Contracted and Isomeric Porphyrins*, Elsevier, Oxford, **1997**; c) A. Jasat, D. Dolphin, *Chem. Rev.* **1997**, 97, 2267; d) J. L. Sessler, A. Gebauer, S. J. Weghorn in *The Porphyrin Handbook*, Vol. 2

- (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, New York, **2000**.
- [2] a) V. J. Bauer, D. R. Clive, D. Dolphin, J. B. Paine III, F. L. Harris, M. M. King, J. Loder, S.-W. C. Wang, R. B. Woodward, *J. Am. Chem. Soc.* **1983**, *105*, 6429; b) M. J. Broadhurst, R. Grigg, A. W. Johnson, *J. Chem. Soc. Perkin Trans. 1* **1972**, 2111; c) J. L. Sessler, M. J. Cyr, A. K. Burrell, *Synlett* **1991**, 127.
- [3] H. Rexhausen, A. Gossauer, *J. Chem. Soc. Chem. Commun.* **1983**, 275; A. K. Burrell, G. Hemmi, V. Lynch, J. L. Sessler, *J. Am. Chem. Soc.* **1991**, *113*, 4690.
- [4] R. Charriere, T. A. Jenny, H. Rexhausen, A. Gossauer, *Heterocycles* **1993**, *36*, 1561.
- [5] J. L. Sessler, T. Morishima, V. Lynch, *Angew. Chem.* **1991**, *103*, 1018; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 977.
- [6] J. L. Sessler, S. J. Weghorn, V. Lynch, M. R. Johnson, *Angew. Chem.* **1994**, *106*, 1572; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1509.
- [7] G. P. Arsenault, E. Bullock, S. F. MacDonald, *J. Am. Chem. Soc.* **1960**, *82*, 4384.
- [8] In MacDonald-type condensations, the methine bridges are generated by α -unsubstituted pyrroles reacting with pyrrole aldehydes to generate the linking methine bridges (*meso* carbons). Although this was originally conceived as a “2+2” methodology,^[7] a related “3+1” route is also commonly utilized: T. D. Lash, *Chem. Eur. J.* **1996**, *2*, 1197. Expanded porphyrins may be synthesized by using larger oligopyrrolic precursors, or by having more than one of each condensing unit combine to form the oligopyrrolic macrocycle.^[1]
- [9] J. L. Sessler, S. J. Weghorn, Y. Hiseada, V. Lynch, *Chem. Eur. J.* **1995**, *1*, 56.
- [10] E. Vogel, *J. Heterocycl. Chem.* **1996**, *33*, 1461.
- [11] E. Vogel, M. Bröring, J. Fink, D. Rosen, H. Schmickler, J. Lex, K. W. K. Chan, Y.-D. Wu, D. A. Plattner, M. Nendel, K. N. Houk, *Angew. Chem.* **1995**, *107*, 2705; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2511.
- [12] M. Bröring, J. Jendrny, L. Zander, H. Schmickler, J. Lex, Y.-D. Wu, M. Nendel, J. Chen, D. A. Plattner, K. N. Houk, E. Vogel, *Angew. Chem.* **1995**, *107*, 2709; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2515.
- [13] J. P. Gisselbrecht, J. Bley-Eschrich, M. Gross, L. Zander, M. Michels, E. Vogel, *J. Electroanal. Chem.* **1999**, *469*, 170.
- [14] A. Werner, M. Michels, L. Zander, J. Lex, E. Vogel, *Angew. Chem.* **1999**, *111*, 3866; *Angew. Chem. Int. Ed.* **1999**, *38*, 3650.
- [15] M. Michels, L. Zanders, J. Wytke, *Proceedings of the XXIV International Symposium on Macrocyclic Chemistry* (Barcelona, Spain), July **1999**.
- [16] J. S. Lindsey in *The Porphyrin Handbook*, Vol. 1 (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, New York, **2000**.
- [17] P. J. Chmielewski, L. Latos-Grażyński, K. Rachlewicz, T. Głowiak, *Angew. Chem.* **1994**, *106*, 805; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 779; H. Furuta, T. Asano, T. Ogawa, *J. Am. Chem. Soc.* **1994**, *116*, 767; G. R. Geier III, D. M. Haynes, J. S. Lindsey, *Org. Lett.* **1999**, *1*, 1455.
- [18] R. Paolesse, L. Jaquinod, D. J. Nurco, S. Mini, F. Sagone, T. Boschi, K. M. Smith, *Chem. Commun.* **1999**, 1307; Z. Gross, N. Galili, I. Saltsman, *Angew. Chem.* **1999**, *111*, 1530; *Angew. Chem. Int. Ed.* **1999**, *38*, 1427.
- [19] P. J. Chmielewski, L. Latos-Grażyński, K. Rachlewicz, *Chem. Eur. J.* **1995**, *1*, 68.
- [20] M. G. P. M. S. Neves, R. M. Martins, A. Tomé, A. J. D. Silvestre, A. M. S. Silva, V. Félix, M. G. B. Drew, J. A. S. Cavaleiro, *Chem. Commun.* **1999**, 385.
- [21] J. L. Sessler, S. J. Weghorn, T. Morishima, M. Rosingana, V. Lynch, V. Lee, *J. Am. Chem. Soc.* **1992**, *114*, 8306.
- [22] J.-I. Setsune, Y. Katakami, N. Iizuna, *J. Am. Chem. Soc.* **1999**, *121*, 8957.
- [23] M. R. Johnson, D. C. Miller, K. Bush, J. J. Becker, J. A. Ibers, *J. Org. Chem.* **1992**, *57*, 4414.
- [24] F. Ellinger, A. Gieren, T. Hübner, J. Lex, A. Merz, R. Neidlein, J. Salbeck, *Monatsh. Chem.* **1993**, *124*, 931.
- [25] Z. Hu, J. L. Atwood, M. P. Cava, *J. Org. Chem.* **1994**, *59*, 8071.
- [26] R. Paolesse, S. Licoccia, M. Spagnoli, T. Boschi, R. G. Khoury, K. M. Smith, *J. Org. Chem.* **1997**, *62*, 5133.
- [27] R. Paolesse in *The Porphyrin Handbook*, Vol. 2 (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, New York, **2000**.
- [28] R. Paolesse, R. G. Khoury, F. D. Sala, C. D. Natale, F. Sagone, K. M. Smith, *Angew. Chem.* **1999**, *111*, 2727; *Angew. Chem. Int. Ed.* **1999**, *38*, 2577. Oxidative coupling is also presumably taking place in the syntheses of *meso*-substituted sapphyrins, rubyrins, and heterosmaragdyrins by Chandrashekar and co-workers: S. J. Narayanan, B. Sridevi, T. K. Chandrashekar, A. Vij, R. Roy, *Angew. Chem.* **1998**, *110*, 3582; *Angew. Chem. Int. Ed.* **1998**, *37*, 3394; S. J. Narayanan, B. Sridevi, A. Srinivasan, T. K. Chandrashekar, R. Roy, *Tetrahedron Lett.* **1998**, *39*, 7389; S. J. Narayanan, B. Sridevi, T. K. Chandrashekar, U. Englich, K. Ruhlandt-Senge, *Org. Lett.* **1999**, *1*, 587.
- [29] J. L. Sessler, D. Seidel, V. Lynch, *J. Am. Chem. Soc.* **1999**, *121*, 11257.

The Tumor Suppressor p53 in the Center of a Strategy Aiming at the Alleviation of Side Effects in Cancer Therapies

Lisa Wiesmüller*

Instabilities in the genetic material of somatic cells (alteration of single nucleotides, loss, exchange, or duplication of chromosome segments, lack of single chromosomes or supernumerary chromosomes) can contribute to the development of hyperproliferative cell populations and tumors, and finally to metastasis, when, as part of a multistep process, oncogenes become activated or tumor suppressors become inactivated.^[1] The importance of the maintenance of the genetic stability for tumor suppression becomes especially evident when looking at the anti-oncogenic protein p53; the natural wild-type p53 gene is inactivated in 50–60 % of all cancer types by mutation or deletion of one or two alleles.^[2, 3] Furthermore, in other tumor types epigenetic mechanisms, such as exclusion from the nucleus, accelerated degradation, or neutralization of the protein by complex formation, indirectly lead to the functional inactivation of p53.^[4] During cellular stress situations p53 can halt growth in the cell-cycle stage G1 (before DNA synthesis), more rarely in stage S (during DNA synthesis), or in stage G2/M (after DNA synthesis), or can induce active cell death, called apoptosis (Figure 1).^[5]

Apoptosis represents a protective mechanism, which eliminates cells after irreparable damage. This mechanism is critical for the avoidance of malignant tumors and for the success of chemotherapeutic treatments. Apoptosis is a cellular suicide mechanism, during which cellular structures are dissolved and proteins and chromosomes are degraded by proteases and DNases, respectively. The signals for p53-mediated growth regulation, which were identified at the molecular level, are predominantly DNA strand breaks, which for example, arise after treatment with ionising radiation. Kinases, such as Atm, which recognize a DNA-strand break, transfer phosphate groups to p53.^[6] The resulting activation of the p53 molecule allows genes with p53 recognition sequences to be switched on. The product of the

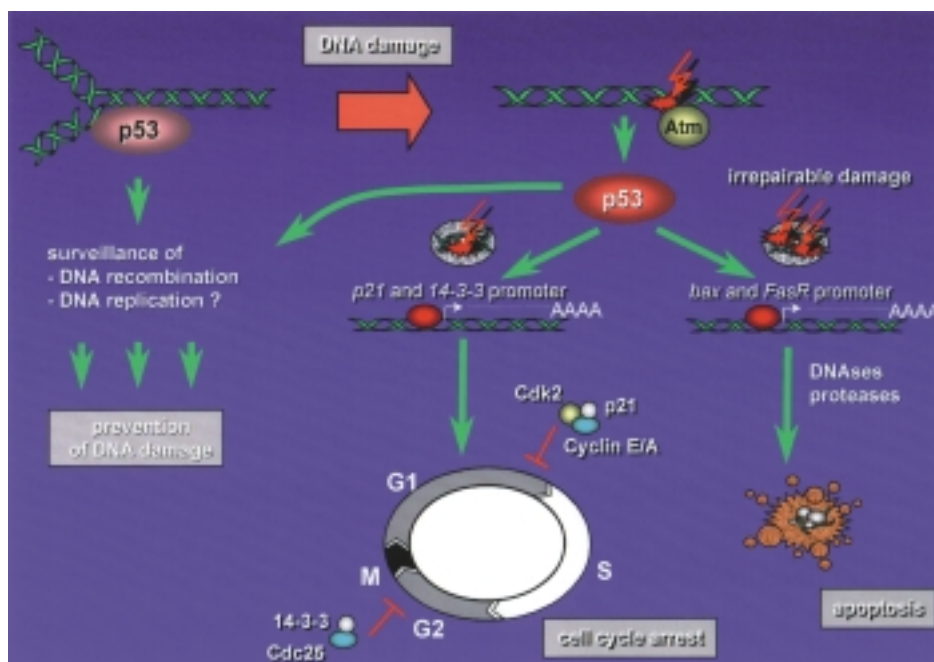


Figure 1. The role of p53 as the “Guardian of the Genome”. p53 maintains the genetic integrity during natural cellular proliferation phases by surveillance of recombination and possibly also of replication processes. This figure also comprises the p53-dependent intracellular signal-transduction pathways, which lead to growth arrest or apoptosis during cellular stress situations.

target gene *p21^{WAF}* represents an inhibitor of the G1/S cell-cycle stage specific kinases CyclinE:Cdk2 and CyclinA:Cdk2, and the duplication of damaged DNA is thereby inhibited.^[5, 7] The product of the *14-3-3* gene sequesters the phosphatase Cdc25, which is necessary for the activation of the Cdk1 kinase at the G2/M transition. Thus, 14-3-3 inhibits the mitotic division of damaged cells. The target genes which encode death receptors, such as CD95(APO-1/FasR), and a gene which encodes an antagonist of the anti-apoptotic factor Bcl2, Bax, mediate the induction of active cell death.

During the last few years it has been reported from several laboratories that, apart from growth regulatory functions, p53 actively participates in DNA repair processes, particularly recombination.^[8] Recombination processes execute DNA exchange between two regions on the genome and represent the final repair mechanism, when DNA double-strand breaks do not allow transfer of the missing information from the complementary strand by replication. By use of special test systems it could be demonstrated that p53 controls the accuracy of recombination processes, that is, it reduces the frequency of erroneous exchange events by one to two orders of magnitude.^[9] In summary, p53 guarantees the maintenance of genomic stability during natural proliferation phases and during cellular stress situations by activities in recombinative DNA repair as well as by switching on growth regulatory molecules.^[10]

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Due to this central, but also extremely complex, role of p53 as a tumor suppressor, this protein has become the most important object for cancer research since its discovery 20 years ago. In the field of diagnosis, the main goal today is to establish the functional status of p53 in tumors in order to improve the rate of curing by individual cancer therapies.^[4] For this purpose methods from gene technology, microscopy and biochemistry are applied. The importance of individual therapies is exemplified by the fact that p53-deficient colon carcinoma cells are resistant to an inhibitor of DNA synthesis, 5-fluorouracil, whereas they respond extremely sensitively towards irradiation. Taken together, it seems that, as a consequence of the individual p53 mutation and of the specific anticancer treatment applied, the impairment of DNA repair and the inability to execute apoptosis produce different cellular responses. For the development of new therapeutic strategies, scientists from basic and clinical research try to enhance or reconstitute the p53-dependent activities, which mediate apoptosis in tumor cells. To this end, scientists test the applicability of synthetic peptides for blocking the attack of p53 by the cellular protein degradation machinery or catalyzing the refolding of mutant p53 into wild-type p53.^[11, 12] According to another approach, wild-type p53 is introduced into p53-deficient cells with adenoviral or retroviral gene vehicles.^[13] The most promising strategy today (clinical trials at stages I and II) was developed by ONYX Pharmaceuticals in Richmond, California. It comprises the targeted destruction of tumor cells lacking functional wild-type p53 by viral lysis.^[14] For this purpose the adenoviral genome was manipulated in such a way that the virus can only reproduce in p53-deficient cells and, therefore, actively kills the corresponding cancer cells.

Theoretically, we would expect that the same functions which enable wild-type p53 to eliminate tumor cells, can conversely be suppressed so that normal tissues are protected from the destructive effect of genotoxic stress. With the aim of developing a corresponding strategy for alleviation of the side effects of radio- and chemotherapies with patients carrying tumors devoid of functional wild-type p53, Komarov and colleagues, in their recently published work, searched a library of 10000 synthetic chemicals for inhibitors of wild-type p53.^[15] The selection procedure chosen by the authors relies on the murine fibroblast cell line ConA, which carries wild-type p53. Additionally, the bacterial gene coding for β -galactosidase (*lacZ*) was introduced into ConA cells in combination with a synthetic DNA-recognition sequence for p53. This combination of DNA elements enhanced activity of cellular p53 protein as a transcriptional transactivator to be demonstrated 14 hours after treatment of the cells with the cytostatic agent doxorubicin. When the β -galactosidase substrate X-Gal turns blue in the cell cultures plated in 96-well plates, this indicates the activation of the *lacZ* gene by p53. When this colour change cannot be detected, this indicates a reduction of the transcriptional activity by the chemical added to the corresponding well. A water-soluble compound with a molecular mass of 367 was influencing this p53-dependent activity most prominently and was consequently named "p fifty three inhibitor" Pifitrin α (PFT α ; Figure 2). At concentrations of 10 μ M, PFT α sup-

presses the p53-dependent gene activation by more than 90% after application of doxorubicin ($\leq 0.4 \mu\text{g mL}^{-1}$) or UV irradiation ($\leq 25 \text{ J m}^{-2}$). As would be expected from this, PFT α also inhibits transcriptional transactivation of chromosomal genes with p53-recognition sequences and downstream biological processes, such as the induction of cell cycle arrest or apoptosis, according to the cell and DNA damage type. Comparison of cells which differ in their p53 status, ascertained that, in each case, the PFT α effects were p53-dependent. The anti-apoptotic effect of PFT α could be demonstrated for appropriate tumorigenic cells (C8) by use of a staining technique, which indicates cellular vitality, and by subsequent quantitative evaluation with a microplate reader. After UV irradiation or application of doxorubicin up to 90% of the cells were protected from cell death by the concomitant addition of PFT α . For the chemotherapeutic agents etoposide, taxol, and Ara C, a maximally twofold protective effect was observed. The PFT α -mediated reduction of p53's ability to induce cell cycle arrest at the G1 stage after treatment with γ -rays was demonstrated by fluorescence-activated cell sorting (FACS) analysis of ConA-fibroblasts. When the cellular DNA is stained with a fluorescent dye, the DNA content in a single cell can be measured cytometrically by this method. Thus, within a certain population the cellular distribution into different stages can be established before and after DNA synthesis.

To clarify the stage at which PFT α acts within the DNA damage-induced signal transduction pathway, wild-type p53 was produced in cells without endogenous p53 (Saos-2) by introduction of an appropriate gene vehicle. As PFT α still suppressed p53-induced apoptosis under these conditions, as compared to the experimental setup when preexisting wild-type p53 becomes damage-induced, the inhibitor must act downstream of p53. From the results obtained by separation of p53 proteins with gel electrophoresis in two dimensions by charge and mass, an alteration of p53 activities by a distinct pattern of modifications could be excluded.^[6] Furthermore, the interaction with certain recognition sequences, underlying the transcriptional transactivation of p53 target genes, was not impaired by PFT α , as the formation of high molecular mass complexes of radioactively labeled DNA fragments and p53 was detected equally well after polyacrylamide gel electrophoresis of proteins from untreated as well as from PFT α -treated cells. The only change which the authors noticed after the addition of PFT α was the decrease of cellular p53 amounts, especially in nuclei, as measured by immunoblot analysis (detection of proteins with specific antibodies after gel electrophoresis and subsequent transfer to a membrane). So far it remained unclear whether this phenomenon can be traced back to diminished de novo synthesis of p53, to increased instability of the protein, or to interference with the nuclear import.

To examine the applicability of PFT α to treatments which protect against the toxic side effects of cancer therapies,

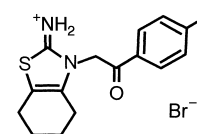


Figure 2. Chemical structure of pifitrin α , 2-(2-imino-4,5,6,7-tetrahydro-benzothiazol-3-yl)-1-p-tolyloethanone hydrobromide.

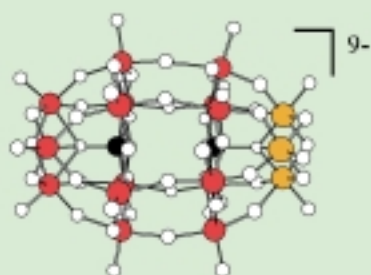
experiments with living animals were performed as the next step. Mice from two different breeding strains were injected intraperitoneally with 2.2 mg kg^{-1} body weight of PFT α and subsequently treated with 8 Gy ionising radiation. The survival rate, body weight, and changes in tissue morphology and apoptosis were monitored for 100 days. Within this time period full protection from irradiation-induced death by PFT α was observed. At the same time the loss of body weight was smaller than without pretreatment. The latter correlated with reduced apoptosis in irradiated tissues, which was shown by a staining technique for the microscopic visualization of chromosome breaks (TUNEL assay). Interestingly, in these mice no tumors were found by the authors up to seven months after irradiation and concomitant drug-mediated inactivation of p53.

It is generally agreed that p53 represents a central factor for the maintenance of genome stability, and, consequently, for the suppression of cancer.^[3] From this, the promising data presented by Komarov and colleagues appear almost unbelievable: Why does the loss of p53 function in mice which were nullizygous for p53 cause the formation of fatal tumors within six months, while the inactivation of p53 by PFT α does not involve unrestrained cell growth, although these animals were additionally exposed to ionising radiation?^[15, 16] Were the tested animals not observed long enough or was the number of mice (30) too small? Long term studies with several thousands of animals seem to be unavoidable, an approach which brings to mind comparable efforts made after the lively discussion about contradictory results for possible side effects of a snowdrop gene in genetically modified potatoes.^[17] However, the apparent discrepancy regarding the PFT α -mediated p53 inactivation without any increase in cancer susceptibilities could be broken up by the multifunctionality of the tumor suppressor. As shown in this work, PFT α suppresses the growth regulatory functions of p53. Nevertheless, the possibility exists that this compound does not influence the surveillance of DNA-repair processes by p53. By analogy, certain p53 mutants still show separate functions in proliferation and DNA-repair.^[10, 18] Indeed, the lack of tumors in mice which were nullizygous for p21, as well as in a fraction of mice which were transgenic for the transcriptionally inactive mutant p53(135Val), already indi-

cated that alternative mechanisms to growth control must contribute to tumor suppression by p53.^[19, 20] If DNA-repair studies and thoroughly executed DNA mutation and rearrangement analyses of tissues after combined PFT α and radiation exposure substantiate the theory that PFT α reduces the p53-dependent suicide program after chemo- or radiotherapeutic treatments without permitting the destabilization of the genome, then indeed, this discovery could help to improve the quality of life for cancer patients in the future.

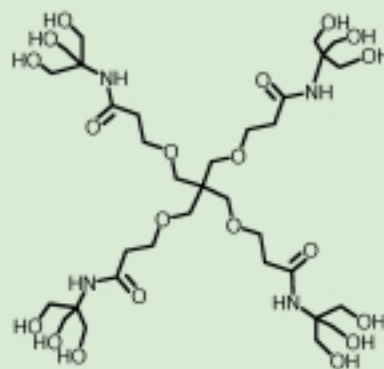
- [1] B. Vogelstein, K. W. Kinzler, *Trends Genet.* **1993**, 9, 138–141.
- [2] M. S. Greenblatt, W. P. Bennett, M. Hollstein, C. C. Harris, *Cancer Res.* **1994**, 54, 4855–4878.
- [3] D. P. Lane, *Nature* **1992**, 358, 15–16.
- [4] G. McGilland, D. E. Fisher, *J. Clin. Invest.* **1999**, 104, 223–225.
- [5] T. Jacks, R. A. Weinberg, *Nature* **1996**, 381, 643–644.
- [6] D. W. Meek, L. E. Campbell, L. J. Jardine, U. Knippschild, L. McKendrick, D. M. Milne, *Biochem. Soc. Trans.* **1997**, 25, 416–419.
- [7] A. Levine, *Cell* **1997**, 88, 323–331.
- [8] F. Janus, N. Albrechtsen, I. Dornreiter, L. Wiesmüller, F. Grosse, W. Deppert, *Cell. Mol. Life Sci.* **1999**, 55, 12–27.
- [9] C. Dudenhöffer, G. Rohaly, K. Will, W. Deppert, L. Wiesmüller, *Mol. Cell. Biol.* **1998**, 18, 5332–5342.
- [10] C. Dudenhöffer, M. Kurth, F. Janus, W. Deppert, L. Wiesmüller, *Oncogene* **1999**, 18, 5773–5784.
- [11] A. Bottger, V. Bottger, A. Sparks, W. L. Liu, S. F. Howard, D. P. Lane, *Curr. Biol.* **1997**, 7, 860–869.
- [12] G. Selivanova, L. Ryabchenko, E. Jansson, V. Iotsova, K. G. Wiman, *Mol. Cell. Biol.* **1999**, 19, 3395–3402.
- [13] M. Favrot, J. L. Coll, N. Louis, A. Negoescu, *Gene Ther.* **1998**, 5, 728–739.
- [14] J. R. Bischoff, D. H. Kirn, A. Williams, C. Heise, S. Horn, M. Muna, L. Ng, J. A. Nye, A. Sampson-Johannes, A. Fattaey, F. McCormick, *Science* **1996**, 274, 373–432.
- [15] P. G. Komarov, E. A. Komarova, R. V. Kondratov, K. Christov-Tselkov, J. S. Coon, M. V. Chernov, A. V. Gudkov, *Science* **1999**, 85, 1733–1737.
- [16] L. A. Donehower, M. Harvey, B. L. Sladge, M. J. McArthur, C. A. Montgomery, J. S. Butel, A. Bradley, *Nature* **1992**, 356, 215–221.
- [17] S. W. Ewen, A. Pusztai, *Lancet* **1999**, 354, 1353–1354.
- [18] Y. Saintigny, D. Rouillard, B. Chaput, T. Soussi, B. S. Lopez, *Oncogene* **1999**, 18, 3553–3565.
- [19] C. Deng, P. Zhang, J. W. Harper, S. J. Elledge, P. Leder, *Cell* **1995**, 82, 675–684.
- [20] D. L. Schaffner, P. Chavez-Barrios, S. L. Huang, R. Barrios, B. F. Dickey, M. R. Shaker, S. Rajagopalan, G. M. Habib, R. M. Lebovitz, M. W. Lieberman, *Lab. Invest.* **1996**, 74, 1005–1011.

Catalytic Polyoxometalate Terminated Dendrimers



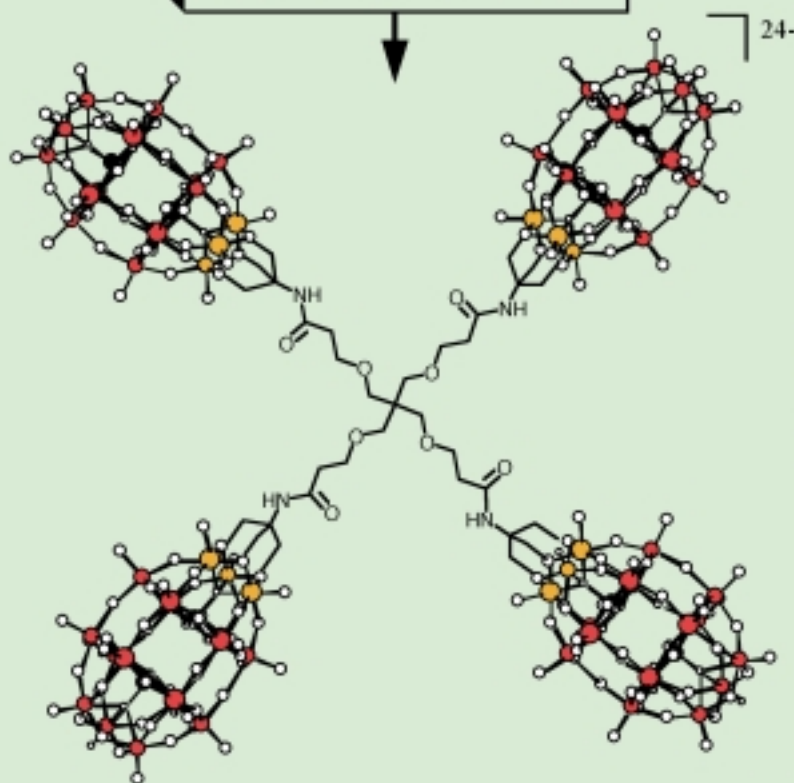
polyoxometalates (POMs) with tunable catalytic properties

+



hydroxylated dendrimers

one-step high-yield
POM esterification...



See next
pages for
details.

● V
● W
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○ O

...yields materials with the advantages of
both POMs and dendrimers

Poly(polyoxometalate) Dendrimers: Molecular Prototypes of New Catalytic Materials**

Huadong Zeng, George R. Newkome,* and Craig L. Hill*

Metallodendrimers composed of tunable catalytic components could offer the selectivity advantages of homogeneous catalysts coupled with the recoverability and increased durability of heterogeneous catalysts. A variety of metal centers have been incorporated into dendritic structures,^[1] but few of the resulting metallodendrimers have been effective catalysts and none have been catalysts for environmentally benign transformations, such as oxidations by peroxides.^[2] The goal of this study was to assess the feasibility of incorporating redox-active polyoxometalates (POMs), a versatile class of homogeneous catalysts for oxidations and other reactions,^[3] into polyhydroxylated polymers that include cotton, other cellulose, and mass-produced polymers, such as polyvinyl alcohol. The few reports of POMs covalently incorporated in polymers do not involve POMs that possess either marked redox properties or demonstrable catalytic chemistry.^[4] Herein we report the esterification of a representative redox and catalytically active POM, $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$, first with MeOH (the sequential formation of the mono-, di-, and trimethyl esters is quantified) and then with two simple dendrimers each with four tris(hydroxymethyl) termini. These triol groups provide a useful handle for NMR spectroscopy, simulate the polyol surfaces of cellulosic materials, and are documented as structural components in POMs.^[5, 6] The resulting tetra(POM) molecules are hydrolytically stable, catalyze peroxide oxidations, and represent the initial examples of a potentially very large class of new POM-containing catalytic materials.

The reaction of MeOH with $(n\text{Bu}_4\text{N})_5[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]$ in dry organic polar aprotic solvents (for example, MeCN, dimethylacetamide (DMA), DMSO) results in replacement of the oxygen atoms bridging the vanadium atoms in the V_3 “cap” of this POM with methoxy groups, which are effectively methyl esters [Eq. (1)]. The hydrogen atoms of the POMs in

this study most likely reside on the oxygen atoms bridging the vanadium atoms.^[7]

Figure 1 illustrates the presence of the terminal V_3 unit and the mixture of mono-, di-, and trimethyl esters that form by the reaction of $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$ with 4.2 equivalents of

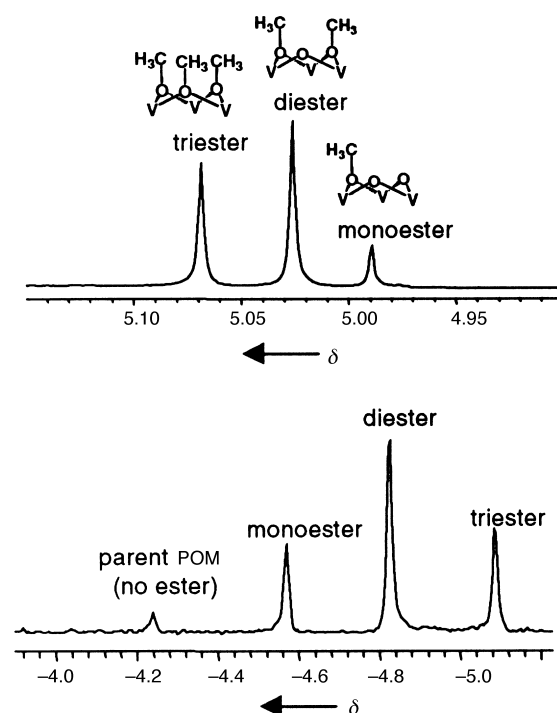
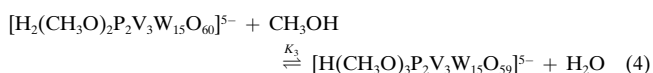
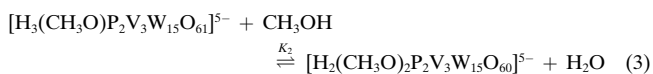
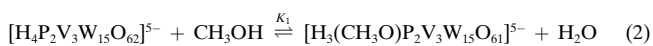


Figure 1. ^1H (top) and ^{31}P (bottom) NMR spectra of an equilibrated CD_3CN solution of MeOH and $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$ (20°C , after 120 h). Only the resonances of the P atom proximal to the V_3 unit in these compounds are shown for clarity.

MeOH. These ester mixtures all exhibit single baseline-resolved peaks in both the ^1H and ^{31}P NMR spectra. Changes in both the ^1H and ^{31}P NMR spectra with time indicate that the monomethyl ester forms first, followed by the diester, and then the triester, all at the expense of the parent $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$ ion, and that the same product distribution results starting with either the parent POM and MeOH or the independently prepared trimethyl POM, $[\text{H}(\text{CH}_3\text{O})_3\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{59}]^{5-}$, and three equivalents of H_2O . The esterification equilibrium is reached after approximately 48 h, and the reactions are highly selective (the only methyl groups detectable are those of the esters and MeOH and the only POM units detectable are those of the parent POM and the three esters). The equilibrium constants for Equations (2)–(4) (K_1 , K_2 , K_3 in CD_3CN , 20°C) evaluated by both ^1H and ^{31}P NMR are 2.0 ± 0.2 , 1.3 ± 0.2 , and 0.37 ± 0.08 , respectively.



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By analogy with Equations (1)–(4), simple representative dendrimers each bearing four tris(hydroxymethyl) groups react with four equivalents of $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$ in dry polar aprotic organic solvents to form the corresponding tetra(POM) molecules (**1** and **2** in Figure 2). A kinetic product mixture of

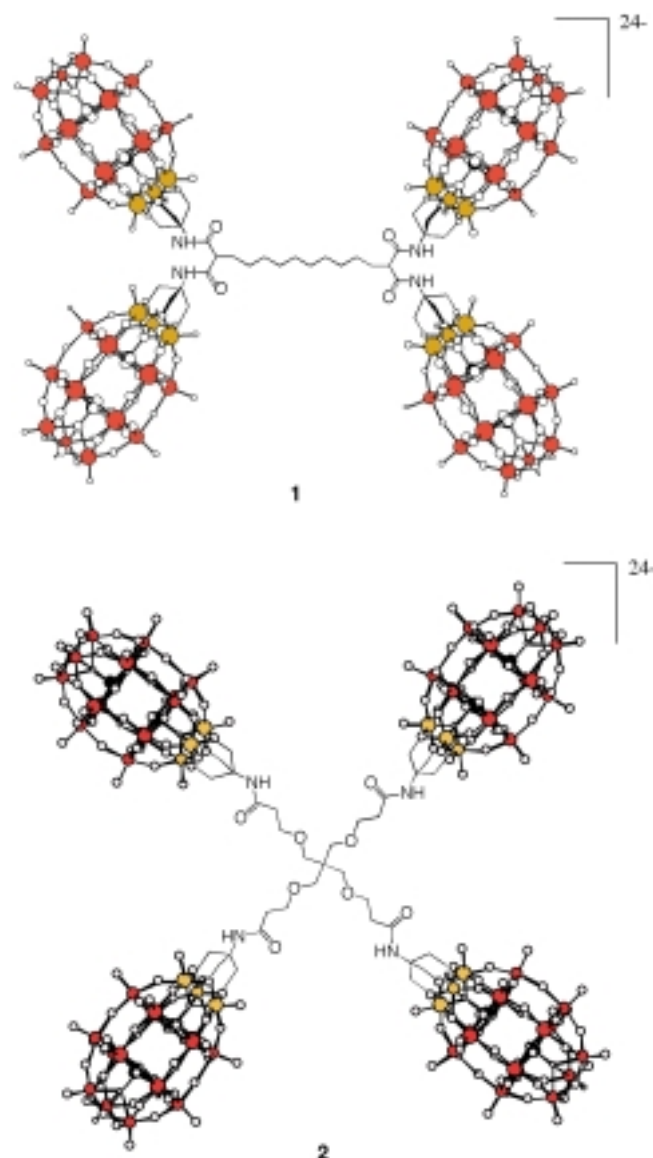
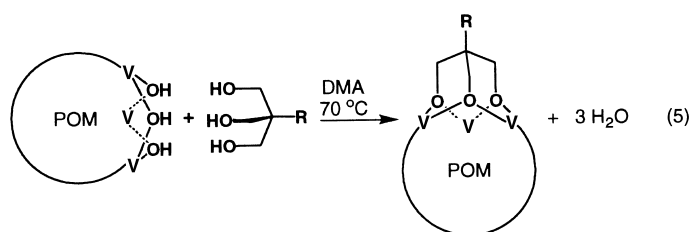


Figure 2. Representative structures of two dendritic tetra(POM) molecules (**1** and **2**). Each has a charge of 24[−] counteracted by 24 cations that are not shown for clarity. The V, W, P, and O atoms are colored orange-yellow, red, black, and white (open circles), respectively.

triester linkages (the product in Equation (5)) plus diester/free-CH₂OH linkages forms initially. The fully H⁺-exchanged forms of **1** and **2** (H-**1** and H-**2**) can be prepared, and both the ion-exchange process (Bu₄N⁺ by H⁺) and heating (<100°C) cause the diester/free-CH₂OH linkages to rearrange to the thermodynamically more stable triester linkages. However, while **1** and **2** are pure by elemental analysis, NMR spectroscopy shows that some diester/free-CH₂OH linkages remain (<5%) even after ion exchange and heating. No monoesters are observed, but this is consistent with the apparent thermodynamic control operable in this system and the clear



order of relative stabilities (triester > diester/free-CH₂OH > monoester). Unlike the nonchelating methyl esters (for example, Equations (2)–(4)), these chelating triesters are so stable to hydrolysis that the corresponding equilibrium constants for hydrolysis could not be assessed experimentally as noted (NMR) by the lack of evidence even after heating in 40/60 D₂O/[D₆]DMSO at 80°C for two days.

Both **1** and H-**1**, a representative mixed (*n*Bu₄N⁺/H⁺) and H⁺ salt, respectively, of these POM ester-bearing molecules, catalyze the oxidation of the thioether tetrahydrothiophene (THT) by both *t*BuOOH and H₂O₂. Figure 3 shows the time

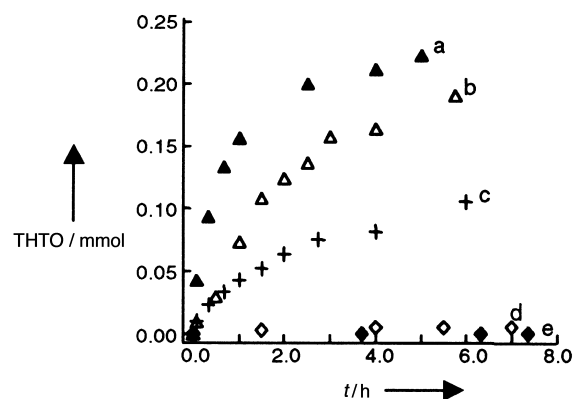


Figure 3. Relative formation rates of tetrahydrothiophene oxide (THTO) in the oxidation of THT by *t*BuOOH catalyzed by a) H-**1**, b) **1** + *p*-C₇H₇SO₃H, c) *p*-C₇H₇SO₃H, d) **1**, and e) nothing (control without catalyst). The conditions for all reactions are otherwise identical (see Experimental Section).

course of exemplary *t*BuOOH oxidations and control reactions. The order of catalytic reactivity is H-**1** > **1** + *p*-C₇H₇SO₃H (a strong MeCN-soluble Brønsted acid) > *p*-C₇H₇SO₃H alone > **1** (Bu₄N⁺ salt) alone ≫ no catalyst. Both calculated and measured pH values indicate that *p*-C₇H₇SO₃H is a stronger acid than H-**1**. Two points are clear: the reaction is catalyzed by strong acids, and the POM imparts considerable activity in addition to its acidity (H-**1** > **1** + *p*-C₇H₇SO₃H and **1** alone ≫ no catalyst). Significantly, these catalysts (**1** and H-**1**) can be easily recovered (Et₂O precipitation and filtration) and reused without loss in catalytic activity. Similar catalysis is seen for THT oxidation by H₂O₂. In this case the catalytic effect (rate with catalyst/rate without catalyst) is lower because the rate without catalyst (H₂O₂ + THT alone) is substantial.

Given the number of redox-active POMs with two or more vanadium atoms and the large number of polyhydroxylated polymers, including cellulose, the prospects for making POM-bound polymer materials with catalytic oxidation activity are considerable.

Experimental Section

Methylation of $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$: MeOH (2.5 μL , 0.061 mmol, 4.2 equiv) was added to $[(n\text{-C}_4\text{H}_9)_4\text{N}]_5[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{18}$ (0.0746 g, 0.0144 mmol) in CD_3CN (0.60 mL), and the reaction was followed by ^1H and ^{31}P NMR spectroscopy. Effective equilibrium at 20 °C was achieved after 48 h. ^1H NMR (400 MHz, 0.024 M, CD_3CN , TMS) for the mono-, di-, and trimethyl esters: $\delta = 4.99$, 5.03, and 5.08, respectively; ^{31}P NMR (162 MHz, 0.024 M, CD_3CN , 85 % H_3PO_4 , only the chemical shifts of the P atom close to the V_3 cap are given) for these isomers: $\delta = -4.56$, -4.81 , and -5.07 , respectively.

Trimethyl ester of $[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]^{5-}$: Excess MeOH (1 mL) and $[(n\text{-C}_4\text{H}_9)_4\text{N}]_5[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]$ (1.347 g, 0.26 mmol) dissolved in MeCN (10 mL) was stirred at 40 °C for 24 h. The solution was then added to diethyl ether (20 mL), and the resulting yellow powder was dried under vacuum for 4 h to give 1.30 g (yield 96 % based on POM). Selected data: ^1H NMR (400 MHz, 0.010 M, CD_3CN , TMS): $\delta = 5.08$ (CH_3O); ^{31}P NMR (162 MHz, 0.010 M, CD_3CN , 85 % H_3PO_4): $\delta = -5.07$, -9.89 . Elemental analysis calcd for $[(n\text{-C}_4\text{H}_9)_4\text{N}]_5[\text{H}(\text{CH}_3\text{O})_3\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{59}]$: C 19.09, H 3.67, N 1.34, P 1.19, V 2.93, W 52.80; found: C 19.07, H 3.52, N 1.52, P 1.18, V 2.94, W 52.61.

1: $[(n\text{-C}_4\text{H}_9)_4\text{N}]_5[\text{H}_4\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]$ (0.743 g) was added to a solution of N,N',N'',N''' -tetrakis[1,3-dihydroxy-2-(hydroxymethyl)prop-2-yl]- $\alpha,\alpha,\omega,\omega$ -dodecane tetracarboxamide⁹¹ (0.99 equiv, 28.3 mg) in DMA (10 mL). After heating the solution to 70 °C for 7 d under argon, it was cooled, added dropwise at 25 °C to stirred Et_2O (100 mL), and the resulting yellow solid collected and redissolved in the minimum volume of MeCN. After filtration, the product was re-precipitated by addition of Et_2O (30 mL). This procedure was repeated twice, and the resulting yellow powder was washed with Et_2O and dried under vacuum at 50 °C for 24 h (yield 90 % based on ligand). The acid form, **H-1**, was obtained by cation exchange using H^+ -loaded Amberlyst 15 resin in DMF solution. **1**: ^1H NMR (500 MHz, 0.015 M, $[\text{D}_6]\text{DMSO}$, TMS): $\delta = 5.52$ and 5.24 (s, 15H, relative ratio 95:5, $\text{CH}_2\text{-O-V}$), 4.78 (s, 4H), 3.59 and 3.82 (s, 9H), 1.26 (broad, 24H); ^{31}P NMR (202 MHz, 0.015 M, $[\text{D}_6]\text{DMSO}$, 85 % H_3PO_4): $\delta = -6.78$, -6.96 , -13.08 , -13.45 , -13.67 ; ^{51}V NMR (132 MHz, 0.015 M, $[\text{D}_6]\text{DMSO}$, neat VOCl_3): $\delta = -490.2$, -516.7 with two small peaks at -459 and -560 ($<5\%$). Elemental analysis calcd for $[(n\text{-C}_4\text{H}_9)_4\text{N}]_{20}\text{H}_4[(\text{C}_{34}\text{H}_{54}\text{O}_{16})_4(\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{59})_4]$: C 19.97, H 3.68, N 1.58, P 1.16, V 2.87, W 51.8; found: C 19.77, H 3.73, N 1.94, P 1.16, V 2.46, W 51.92. **H-1**: ^1H NMR (500 MHz, 0.019 M, $[\text{D}_6]\text{DMSO}$, TMS): $\delta = 5.52$, 1.26; ^{31}P NMR (202 MHz, 0.019 M, $[\text{D}_6]\text{DMSO}$, 85 % H_3PO_4): $\delta = 7.97$, -13.06 and two small peaks at -5.50 and -11.84 ($<5\%$); ^{51}V NMR (132 MHz, 0.019 M, $[\text{D}_6]\text{DMSO}$, neat VOCl_3): $\delta = -542.9$. C,H,N analysis calcd for $\text{H}_{24}[(\text{C}_{34}\text{H}_{54}\text{O}_{16})_4(\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{59})_4] \cdot 41\text{DMF}$: C 9.69, H 1.89, N 3.24; found: C 9.80; H 2.08, N 2.96.

2: The procedure for **1** (above) was used except that $\text{C}[\text{CH}_2\text{OCH}_2\text{CH}_2\text{CONHC}(\text{CH}_2\text{OH})_3]_4$, prepared from 6,6-bis(carboxy-2-oxabutyl)-4,8-dioxaundecane-1,11-dicarboxylic acid,¹⁰⁰ followed by methanolysis and treatment with tris(hydroxymethyl)aminomethane (Tris), was used in place of the arborol precursor. ^1H NMR (500 MHz, 0.012 M, $[\text{D}_6]\text{DMSO}$, TMS): $\delta = 5.70$ and 5.65 (s, 24H in 82:18 ratio), 3.62 (broad, 8H), 3.56 (broad, 8H), 2.49 (s, 8H); ^{31}P NMR (202 MHz, 0.012 M, $[\text{D}_6]\text{DMSO}$, 85 % H_3PO_4): $\delta = -3.94$, -4.07 , -9.81 ; ^{51}V NMR (132 MHz, 0.012 M, $[\text{D}_6]\text{DMSO}$, neat VOCl_3): $\delta = -542.4$. C,H,N analysis calcd for $[(n\text{-C}_4\text{H}_9)_4\text{N}]_{20}\text{H}_4[(\text{C}_{33}\text{H}_{52}\text{O}_{20})_4(\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{59})_4]$: C 19.86, H 3.66, N 1.57; found: C 20.15, H 3.62, N 1.80.

Catalytic oxidation of THT: In a typical reaction the catalyst **1**, or **H-1** (5.0 μmol of the $[\text{P}_2\text{V}_3\text{W}_{15}\text{O}_{62}]$ unit), THT substrate (0.281 mmol), and decane (internal standard, 5.0 μL) were dissolved in solvent (MeCN or $\text{C}_6\text{H}_5\text{CH}_3$, 5.0 mL) containing $[(n\text{-C}_4\text{H}_9)_4\text{N}]\text{HSO}_4$ (50.0 mmol) under argon. For the reactions with $p\text{-C}_6\text{H}_4\text{SO}_3\text{H}$, this acid (30 μmol , equaling the $[\text{H}^+]$ from 5.0 μmol of **H-1**) was added. The reactions were initiated by addition of 90 % aqueous $t\text{BuOOH}$ (0.295 mmol) using a gas-tight syringe. The organic products were identified by GC-MS and quantified by GC using a 5 % methyl siloxane column with a gradient temperature program. Aliquots of the solution (approximately 0.1 mL) were withdrawn at the time intervals shown in Figure 3.

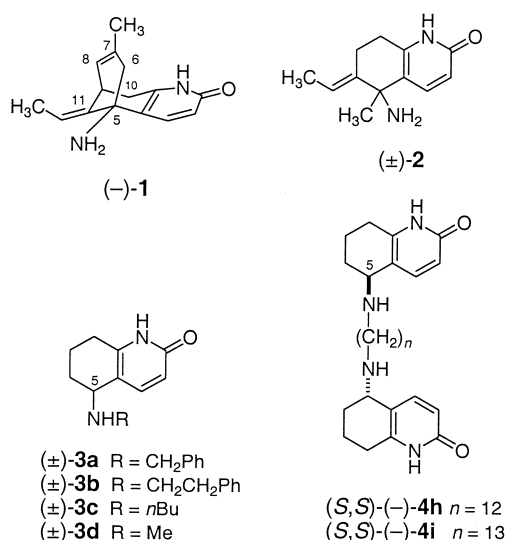
Received: January 4, 2000 [Z14503]

- [1] G. R. Newkome, E. He, C. N. Moorefield, *Chem. Rev.* **1999**, *99*, 1689–1746.
- [2] a) A. Miedaner, C. J. Curtis, R. M. Barkley, D. L. Du Bois, *Inorg. Chem.* **1994**, *33*, 5482–5490; b) G. van Koten, D. M. Grove, *Polym. Mater. Sci. Eng.* **1995**, *73*, 228–229; c) P. Bhyrappa, J. K. Young, J. S. Moore, K. S. Suslick, *J. Am. Chem. Soc.* **1996**, *118*, 5708–5711; d) P. Bhyrappa, J. K. Young, J. S. Moore, K. S. Suslick, *J. Mol. Catal. A* **1996**, *113*, 109–116; e) K. S. Suslick, P. Bhyrappa, *J. Inorg. Biochem.* **1997**, *67*, 234; f) P. B. Rheiner, H. Sellner, D. Seebach, *Helv. Chim. Acta* **1997**, *80*, 2027–2032; g) M. T. Reetz, G. Lohmer, R. Schwickardi, *Angew. Chem.* **1997**, *109*, 1559–1562; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1526–1529; h) H.-F. Chow, C. C. Mak, *J. Org. Chem.* **1997**, *62*, 5116–5127; i) C. C. Mak, H.-F. Chow, *Macromolecules* **1997**, *30*, 1228–1230; j) R. A. Gossage, L. A. Van De Kuil, G. van Koten, *Acc. Chem. Res.* **1998**, *31*, 423–431; k) C. Köllner, B. Pugin, A. Togni, *J. Am. Chem. Soc.* **1998**, *120*, 10274–10275; l) R. A. Gossage, J. Jastrzebski, J. van Ameijde, S. J. E. Mulders, A. J. Brouwer, R. M. J. Liskamp, G. van Koten, *Tetrahedron Lett.* **1999**, *40*, 1413–1416; m) M. Zhao, R. M. Crooks, *Angew. Chem.* **1999**, *111*, 375–377; *Angew. Chem. Int. Ed.* **1999**, *38*, 364–366; n) M. A. Hearshaw, A. T. Hutton, J. R. Moss, K. J. Naidoo in *Advances in Dendritic Macromolecules*, Vol. 4 (Ed.: G. R. Newkome), JAI Press, Stamford, CT, **1999**, pp. 1–60; o) S. C. Bourque, F. Maltais, W.-J. Xiao, O. Tardif, H. Alper, P. Arya, L. E. Manzer, *J. Am. Chem. Soc.* **1999**, *121*, 3035–3038; p) M. Zhao, L. Sun, R. M. Crooks, *Polym. Prepr.* **1999**, *40*, 400–401; q) K. Matyjaszewski, *Chem. Eur. J.* **1999**, *5*, 3095–3102; r) G. E. Oosterom, R. J. van Haaren, J. N. H. Reek, P. C. J. Kamer, P. W. N. M. van Leeuwen, *Chem. Commun.* **1999**, 1119–1120.
- [3] Reviews of POMs and catalytic activity: a) *Polyoxometalates, from Platonic Solids to Anti-Retroviral Activity* (Eds.: M. T. Pope, A. Müller), Kluwer, **1994**; b) C. L. Hill, C. M. Prosser-McCarthy, *Coord. Chem. Rev.* **1995**, *143*, 407–455; c) “Polyoxometalates in Catalysis”: C. L. Hill, *J. Mol. Catal.* **1996**, *114*, 1–371; d) T. Okuhara, N. Mizuno, M. Misono, *Adv. Catal.* **1996**, *41*, 113–252; e) R. Neumann, *Prog. Inorg. Chem.* **1998**, *47*, 317–370.
- [4] Polymers with covalently bound POMs: a) D. E. Katsoulis, J. R. Keryk, *Mater. Res. Soc. Symp. Proc.* **1996**, *435*, 589–594; b) E. M. Ansonii, *Jpn. Kokai Tokkyo Koho* **1996**, 23; c) P. Judeinstein, *J. Sol–Gel Sci. Technol.* **1994**, *2*, 147–151; d) P. Judeinstein, *Chem. Mater.* **1992**, *4*, 4–7.
- [5] Reviews that include such complexes: a) Q. Chen, J. Zubieta, *Coord. Chem. Rev.* **1992**, *114*, 107–167; b) M. I. Khan, J. Zubieta, *Prog. Inorg. Chem.* **1995**, *43*, 1–149; c) J. Zubieta in [3a], pp. 125–156; d) D. Rehder in [3a], pp. 157–166.
- [6] a) M. I. Khan, Q. Chen, D. P. Goshorn, H. Hope, S. Parkin, J. Zubieta, *J. Am. Chem. Soc.* **1992**, *114*, 3341–3346; b) Q. Chen, D. P. Goshorn, C. P. Scholes, X.-I. Tan, J. Zubieta, *J. Am. Chem. Soc.* **1992**, *114*, 4667–4681; c) Y. Hou, C. L. Hill, *J. Am. Chem. Soc.* **1993**, *115*, 11823–11830; d) D. C. Crans, F. Jiang, J. Chen, O. P. Anderson, M. M. Miller, *Inorg. Chem.* **1997**, *36*, 1038–1047; e) D. C. Crans, I. Boukhobza, *J. Am. Chem. Soc.* **1998**, *120*, 8069–8078; the following two references address systems with two rather than three V–O–C bonds: f) C. P. Rao, A. Screedhara, P. V. Rao, M. B. Verghese, K. Rissanen, E. Kolehmainen, N. K. Lokanath, M. A. Sridhar, J. S. Prasad, *J. Chem. Soc. Dalton Trans.* **1998**, 2383–2394; g) E. Rakovsky, L. Zurkova, *Monogr. Ser. Int. Conf. Coord. Chem.* **1999**, *4*, 465–470.
- [7] The hydrogen atoms of the POMs in this study most likely reside on the oxygen atoms bridging the vanadium centers for three reasons: these oxygen atoms are the most basic of the POM anion, they are also the most basic in the entire system (POM anion, cations, and solvent), and the one study involving a mixed $n\text{Bu}_4\text{N}^+/\text{H}^+$ salt of a polyvanadium-containing POM where the protons were structurally located indicated they were on these oxygen atoms in MeCN, see V. W. Day, W. G. Klemperer, D. J. Maltbie, *J. Am. Chem. Soc.* **1987**, *109*, 2991–3002. In consequence, the hydrogen atoms in both formulas and equations of the POMs here have been written to indicate that they are on these POM oxygen atoms.
- [8] R. G. Finke, B. Rapko, R. J. Saxton, P. J. Domaille, *J. Am. Chem. Soc.* **1986**, *108*, 2947–2960.
- [9] G. R. Newkome, G. R. Baker, S. Arai, M. J. Saunders, P. S. Russo, K. J. Theriol, C. N. Moorefield, L. E. Rogers, J. E. Miller, T. R. Licux, M. E. Murray, B. Phillips, L. Pascal, *J. Am. Chem. Soc.* **1990**, *112*, 8458–8465.
- [10] G. R. Newkome, C. D. Weis, *Org. Prep. Proced. Int.* **1996**, *28*, 242–246.

Dimerization of an Inactive Fragment of Huperzine A Produces a Drug with Twice the Potency of the Natural Product**

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Bivalency is an effective strategy for improving drug potency and selectivity.^[1] When multiple recognition sites for the same substrate exist, homodimers can exhibit dramatic performance enhancements relative to the corresponding monomers.^[1a, 2] In this vein, homodimers of structurally complex natural products have recently been investigated.^[3] Herein we demonstrate a new strategy in bivalent drug design by dimerizing an easily synthesized but pharmacologically inactive fragment of Huperzine A (–)-**1**: the optimum dimers (S,S)-(–)-**4h,i** are more than twice as potent as the natural product.



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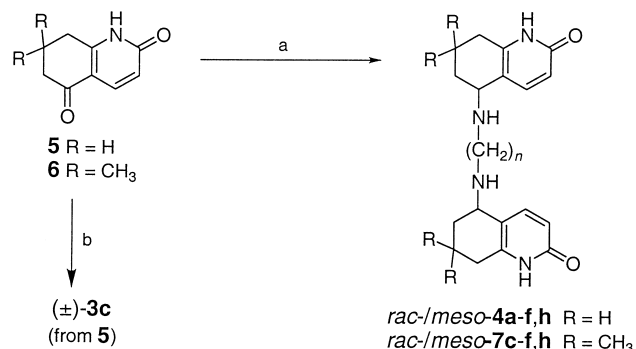
[+] X-ray crystallography

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

Huperzine A (–)-**1**, isolated from the clubmoss *Huperzia serrata*, is a selective and potent reversible acetylcholinesterase (AChE) inhibitor that shows promise for the palliative treatment of Alzheimer's disease.^[4] Asymmetric syntheses of (–)-**1** as short as 12 steps have been reported,^[5] and considerable effort has been directed towards the development of more easily synthesized analogues.^[6] To date 5-amino-2(1*H*)-quinolinones which lack the C6–C8 bridge of **1** have not significantly inhibited AChE (e.g. (±)-**2**, (±)-**3a**, (±)-**3b**, IC₅₀ > 100 000 nM).^[6] Yet, based on the X-ray crystal structure analysis of the *Torpedo* AChE · (–)-**1** complex,^[7] analogue **3** appears to possess much of the requisite functionality. In particular **3** retains the pyridone oxygen atom and NH group of **1**, which form hydrogen bonds to Tyr-130 and to Gly-117/Glu-199 (through a water molecule), respectively, and the 5-amino group of **1**, which undergoes a cation–π-interaction with Trp-84/Phe-330. We conjectured that high affinity of analogues like **3** would be possible, if the loss of hydrophobic contact in the active site could be offset by additional interactions at the AChE peripheral site (12 Å distant from the active site)^[8]. Our studies of alkylene-linked tacrine homodimers^[2a, 9] and heterodimers^[10] have demonstrated that this site binds a variety of protonated amine ligands, including moieties related to **3**.

Thus **5** and **6** (each available in two steps)^[11] were condensed with α,ω-diamines and reduced, affording dimers **4a–f,h** and **7c–f,h** as mixtures of the *rac*- and *meso*-diastereomers (Scheme 1). These compounds were tested



Scheme 1. Synthesis of dimeric inhibitors. a) 0.5 equiv NH₂(CH₂)_nNH₂, cat. CH₃CO₂H, benzene reflux, 24 h; NaBH₄, MeOH, room temperature, 12 h, *rac*-/*meso*-**4a–f,h**: 38–58%, *rac*-/*meso*-**7c–f,h**: 22–35%; b) nBuNH₂, cat. CH₃CO₂H, benzene reflux, 24 h; NaBH₄, MeOH, room temperature, 12 h, 51%. Lettering scheme (see also Scheme 2): **a**, *n* = 5; **b**, *n* = 6; **c**, *n* = 7; **d**, *n* = 8; **e**, *n* = 9; **f**, *n* = 10; **g**, *n* = 11; **h**, *n* = 12; **i**, *n* = 13; **j**, *n* = 14.

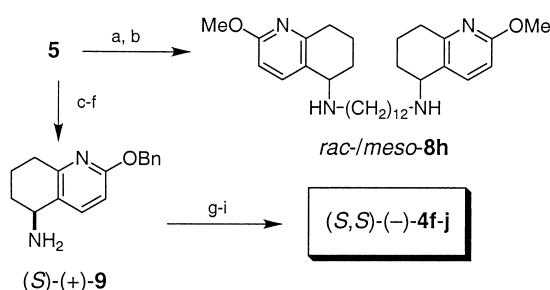
for AChE and butyrylcholinesterase (BChE) inhibition.^[12] As expected, monomer (±)-**3c** proved an extremely weak AChE inhibitor (IC₅₀ ~ 500 000 nM, Table 1, entry 1). However corresponding dimers *rac*-/*meso*-**4a–f,h** showed dramatically enhanced potency, with the highest potency observed at a tether length of 12 methylene groups (*rac*-/*meso*-**4h**, 159 nM, Table 1, entry 2). Dimers *rac*-/*meso*-**7c–f,h** were similarly optimized at *n* = 12, but were less potent than *rac*-/*meso*-**4h** (cf. Table 1, entries 2, 3; data for **4a–f**, **7c–f** not shown).

As a test of the design strategy, 2-methoxypyridine analogue *rac*-/*meso*-**8h** was prepared from **5** (Scheme 2). The 31-

Table 1. Cholinesterase inhibition by huperzine A fragment dimers and controls.

Entry	Drug ^[a]	<i>n</i> ^[b]	AChE IC ₅₀ [nM] ^[c]	BChE IC ₅₀ [nM] ^[d]	Selectivity for AChE ^[e]
1	(±)- 3c	na	500 000 ^[f]	500 000 ^[f]	1
2	<i>rac</i> -/ <i>meso</i> - 4h	12	159 ± 26	24 400 ± 910	153
3	<i>rac</i> -/ <i>meso</i> - 7h	12	3 620 ± 110	60 000 ± 3 230	16.6
4	<i>rac</i> -/ <i>meso</i> - 8h	12	5 000 ± 190	78 900 ± 14 500	15.8
5	(<i>S,S</i>)-(-)- 4f	10	151 ± 36	1 820 ± 70	12.1
6	(<i>S,S</i>)-(-)- 4g	11	84 ± 5	1 160 ± 80	13.8
7	(<i>S,S</i>)-(-)- 4h	12	52 ± 8	9 600 ± 300	185
8	(<i>S,S</i>)-(-)- 4i	13	52 ± 9	16 700 ± 650	321
9	(<i>S,S</i>)-(-)- 4j	14	240 ± 50	59 500 ± 10 100	248
10	(<i>R,R</i>)-(+)- 4h	12	3 130 ± 790	297 000 ± 78 400	94.9
11	(-)- 1 ^[g]	na	115 ± 1	135 000 ± 6 000	1 200
12	tacrine	na	231 ± 16	77.2 ± 5.8	0.3

[a] Drugs **3c**, **4** were assayed as the hydrochloride salts; drugs *rac*-/*meso*-**7** and *rac*-/*meso*-**8** were assayed as the fumaric acid salts. Elemental analyses matched the proposed salt formulations (C, H, N, Cl ± 0.4 %). [b] na = not applicable. [c] Assay performed using rat cortex homogenate, in the presence of ethopropazine as a specific BChE inhibitor. [d] Assay performed using rat serum, in the presence of BW284c51 as a specific AChE inhibitor. [e] Selectivity for AChE is defined as IC₅₀(BChE)/IC₅₀(AChE). [f] Estimated values based on ~50 % inhibition of AChE and BChE at 0.5 mM, the highest drug concentration tested. [g] [α]_D²⁰ = -149° (*c* = 0.17, CHCl₃).



Scheme 2. Synthesis of enantiomerically pure dimers. a) MeI, Ag₂CO₃, CHCl₃, room temperature, 48 h, 81 %; b) 0.5 equiv NH₂(CH₂)₁₂NH₂, cat. CH₃CO₂H, benzene, reflux, 24 h; NaBH₄, MeOH, room temperature, 12 h, 57 %; c) BnBr, Ag₂CO₃, toluene, 48 h, 80 %; d) NH₂OBn·HCl, pyridine, room temperature, 16 h, 95 %; e) BH₃·THF, room temperature, 12 h, reflux 2 h; 20 % KOH, reflux 1.5 h, 66 %; f) (*R*)-(-)-mandelic acid, MeOH, two recrystallizations; CH₂Cl₂/aq. NaOH, 29 %; g) 0.5 equiv ClC(O)-(CH₂)_{*n*-2}C(O)Cl, Et₃N, benzene, reflux 4 h, 80–91 %; h) 6 equiv BH₃·THF, room temperature 12 h, reflux 2 h; 20 % KOH, reflux 1.5 h, 87–92 %; i) H₂, 10 % Pd/C, EtOH, 24 h, 86–91 %. Lettering scheme (see Scheme 1).

fold lower potency relative to *rac*-/*meso*-**4h** (Table 1, entries 2, 4) suggests that, as hoped, hydrogen bonding of the pyridone with the active site contributes to affinity. The synthesis of pure enantiomers of **4f–j** was then undertaken (Scheme 2). Pyridone **5** was converted to (±)-**9** in 50 % yield over three steps, and resolved with (*R*)-(-)-mandelic acid; an X-ray crystal structure analysis of the salt established the *S* configuration for (+)-**9**.^[13] The enantiomeric free bases were transformed to (*S,S*)-(-)-**4f–j** and (*R,R*)-(+)-**4h** in 62–74 % yield over three steps. The 60-fold greater potency of (*S,S*)-(-)-**4h** relative to its enantiomer (Table 1, entries 7 and 10) is suggestive of a multipoint interaction with the AChE active site. The optimization of AChE IC₅₀ in the *S,S* series at a

tether length of 12–13 methylene groups is consistent with simultaneous binding of these drugs to the catalytic and peripheral sites of AChE.^[9, 10] Remarkably the optimum drugs (*S,S*)-(-)-**4h–i** are nearly 10 000-fold more potent than the related monomer (±)-**3c**, and are twice as potent as (-)-**1**, the natural product which inspired their synthesis. Enzyme kinetics confirmed the superior potency of (*S,S*)-(-)-**4h** (*K*_I = 19.6 nM) relative to (-)-**1** (*K*_I = 47.1 nM). Although (*S,S*)-(-)-**4h,i** are not as potent as 10-axial-methyl-huperzine^[4] or tacrine/huperzine hybrids,^[10b, 14] they are easily synthesized and are superior to the latter in terms of selectivity for AChE.

As a prelude to computational and experimental evaluations of the binding of these dimers to AChE, studies of the docking^[15] of monomers (*R*)- and (*S*)-**3d** (protonated at N5) into the active site of AChE were carried out. Both enantiomers were found to bind in a similar way to (-)-**1**, and binding of (*R*)-**3d** was 3 kcal mol⁻¹ more exothermic than binding of (*S*)-**3d**. However, examination of all the computed binding orientations of (*R*)-**3d** reveals that the 5-amino group does not easily accommodate a C₁₂-alkylene tether leading upwards towards the peripheral site (Figure 1, magenta); in contrast (*S*)-**3d** encounters no such problem (Figure 1, cyan). We therefore attribute the observed 60-fold enantio-preference for (*S,S*)-(-)-**4h** to the disposition of the tether leading to the peripheral site.

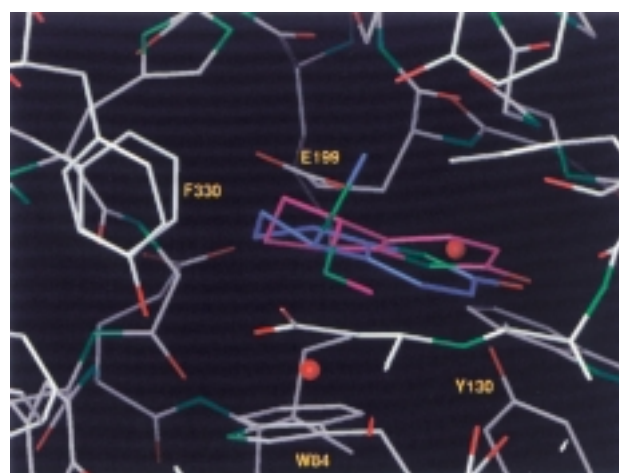


Figure 1. Overlay of the energetically favorable binding orientations of (*R*)-**3d**·H⁺ (magenta) and (*S*)-**3d**·H⁺ (cyan) at the catalytic site of *Torpedo* AChE (white); in each the nitrogen and oxygen atoms are green and red, respectively. F = Phe; E = Glu; Y = Tyr; W = Trp. The perspective shows a cross-section of the catalytic site.

We have demonstrated that dimerization of an easily synthesized but inactive fragment of Huperzine A can produce a drug with greater potency than the natural product itself. Given the importance of structurally complex natural products in drug discovery, we anticipate that the simple approach described here will be useful in developing new leads for other biological targets.

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- [1] a) P. S. Portoghese, *J. Med. Chem.* **1992**, *35*, 1927–1937; b) S. B. Shuker, P. J. Hajduk, R. P. Meadows, S. W. Fesik, *Science* **1996**, *274*, 1531–1534; c) S. Hanessian, P.-P. Lu, J.-Y. Sancéau, P. Chemla, K. Gohda, R. Fonne-Pfister, L. Prade, S. W. Cowan-Jacob, *Angew. Chem.* **1999**, *111*, 3354–3356; *Angew. Chem. Int. Ed.* **1999**, *38*, 3160–3162.
- [2] a) Y.-P. Pang, P. Quiram, T. Jelacic, F. Hong, S. Brimijoin, *J. Biol. Chem.* **1996**, *271*, 23646–23649; b) L. E. Burgess, B. J. Newhouse, P. Ibrahim, J. Rizzi, M. A. Kashem, A. Hartman, B. J. Brandhuber, C. D. Wright, D. S. Thomson, G. P. A. Vigers, K. Koch, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8348–8352.
- [3] a) P. A. Wender, M. F. T. Koehler, D. L. Wright, K. Irie, *Synthesis* **1999**, 1401–1406; b) G. H. Posner, P. Ploypradith, M. H. Parker, H. O'Dowd, S.-H. Woo, J. Northrop, M. Krasavin, P. Dolan, T. W. Kensler, S. Xia, T. A. Shapiro, *J. Med. Chem.* **1999**, *42*, 4275–4280.
- [4] A. P. Kozikowski, W. Tückmantel, *Acc. Chem. Res.* **1999**, *32*, 641–650.
- [5] S. Kaneko, T. Yoshino, T. Katoh, S. Terashima, *Tetrahedron* **1998**, *54*, 5471–5484.
- [6] a) A. P. Kozikowski, C. P. Miller, F. Yamada, Y.-P. Pang, J. H. Miller, M. McKinney, R. G. Ball, *J. Med. Chem.* **1991**, *34*, 3399–3402; b) D. Bai, *Pure Appl. Chem.* **1993**, *65*, 1103–1112; c) D. M. Fink, G. M. Bores, R. C. Efland, F. P. Huger, B. E. Kurys, D. K. Rush, D. E. Selk, *J. Med. Chem.* **1995**, *38*, 3645–3651.
- [7] M. L. Raves, M. Harel, Y.-P. Pang, I. Silman, A. P. Kozikowski, J. L. Sussman, *Nat. Struct. Biol.* **1997**, *4*, 57–63.
- [8] M. Harel, I. Schalk, L. Elhret-Sabatier, F. Bouet, M. Goeldner, C. Hirth, P. H. Axelsen, I. Silman, J. L. Sussman, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9031–9035.
- [9] P. R. Carlier, Y. F. Han, E. S.-H. Chow, C. P.-L. Li, H. Wang, T. X. Lieu, H. S. Wong, Y.-P. Pang, *Bioorg. Med. Chem.* **1999**, *7*, 351–357.
- [10] a) P. R. Carlier, E. S.-H. Chow, Y. Han, J. Liu, J. E. Yazal, Y.-P. Pang, *J. Med. Chem.* **1999**, *42*, 4225–4231; b) P. R. Carlier, D.-M. Du, Y.-F. Han, J. Liu, Y.-P. Pang, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2335–2338.
- [11] a) **5**: M. A. T. Dubas-Sluyter, W. N. Speckamp, H. O. Huisman, *Recueil* **1972**, *91*, 157–160; b) **6**: G. Zacharias, O. S. Wolfbeis, H. Junek, *Monatsh. Chem.* **1974**, *105*, 1283–1291.
- [12] G. L. Ellman, K. D. Courtney, V. J. Andres, R. M. Featherstone, *Biochem. Pharm.* **1961**, *7*, 88–95.
- [13] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137010. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [14] P. Camps, R. E. Achab, D. M. Görbig, J. Morral, D. Muñoz-Torrero, A. Badia, J. E. Baños, N. M. a. Vivas, X. Barril, M. Orozco, F. J. Luque, *J. Med. Chem.* **1999**, *42*, 3227–3242.
- [15] The docking study was performed with the SYSDOC program by employing the AMBER 95 force field and the RESP charge model, as described in the Supporting Information.

Naked-Eye Detection of Anions in Dichloromethane: Colorimetric Anion Sensors Based on Calix[4]pyrrole**

Hidekazu Miyaji, Wataru Sato, and Jonathan L. Sessler*

The development of chemosensors for specific chemical species is emerging as a research area of considerable importance within the generalized field of supramolecular chemistry.^[1] One of the more appealing approaches in this context involves the construction of optical sensors.^[2–6] Such systems generally contain some combination of substrate-recognition functionality (receptor) and optical-signaling capacity (chromophore), either directly linked^[2, 3, 5] or appropriately associated in a noncovalent manner,^[4] and are designed to permit the detection of substrates by binding-induced changes in absorption or emission properties (termed colorimetric and fluorescent sensors, respectively). While the utility of these approaches are becoming increasingly appreciated in terms of both qualitative and quantitative analysis,^[5] the number of colorimetric sensors available at present for anionic substrates remains quite limited.^[6] Indeed, only a few systems are known that undergo color changes of sufficient magnitude that they can be used for the direct “naked-eye” sensing of anions.^[4b, 6b, c] Here we report the synthesis of a new class of covalently linked calix[4]pyrrole–anthraquinone conjugates and show that they act as powerful naked-eye sensors for selected anions (namely, F[−], Cl[−], H₂PO₄[−]) in dichloromethane.

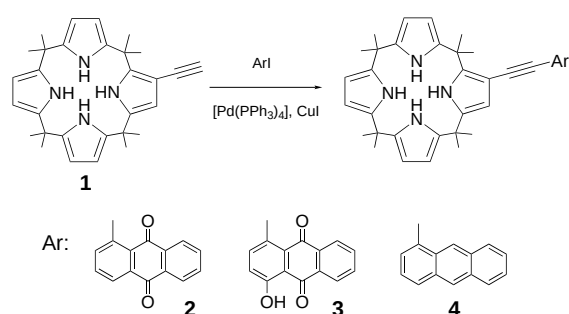
The calix[4]pyrroles,^[7] colorless macrocycles rich in pyrrole NH hydrogen bond donor functionality, are an easy-to-make class of uncharged anion receptors that show considerable promise in the area of anion sensing. In previous work we demonstrated that anion sensors could be generated from calix[4]pyrroles either by attaching a fluorescent reporter group to the basic tetrapyrrolic skeleton^[7d] or through the use of a displacement process involving competition with *para*-nitrophenolate anions.^[4b] Unfortunately, we were unable to produce inherently colored calix[4]pyrrole derivatives that functioned as naked-eye anion sensors. Recently, however, we discovered that calix[4]pyrroles functionalized at the β -pyrrolic positions^[8] may be made using a modified Sonogashira coupling procedure.^[9] By taking advantage of this approach as well as the current availability of the ethynyl-substituted calix[4]pyrrole **1**,^[8] we have now succeeded in preparing (in 73 % yield) an anthraquinone-functionalized system (**2**) that bears an appended chromophore directly

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linked to the calix[4]pyrrole skeleton through a conjugating C–C triple bond (Scheme 1).^[10]



Scheme 1.

The choice of anthraquinone as the chromophore reflected the thinking that it would act as an optically sensitive indicator of anion binding; being electron deficient, it could serve as a “sink” for vectorial charge-transfer processes involving bound anionic guests. The possibility of “color tuning” was also appreciated. Specifically, it was recognized that by using a modified anthraquinone molecule it might be possible to generate naked-eye anion sensors whose spectral characteristics differ from those of **2**. Accordingly, the hydroxyanthraquinone system **3** was also prepared (82% yield), as was the anthracene-containing control compound **4** (69% yield). In all cases, the necessary precursors, 1-iodoanthraquinone,^[11] 1-iodo-4-hydroxyanthraquinone,^[12] and 1-iodoanthracene,^[13] were obtained readily using literature procedures.

Figure 1 shows the changes in the absorption spectrum of receptor **2** (recorded in dichloromethane at a concentration of 5×10^{-5} M) observed upon the addition of tetrabutylammonium fluoride. In the absence of anions, the spectrum of compound **2** is characterized by the presence of two peaks at $\lambda_{\text{max}} = 357$ and 467 nm. Compared to anthraquinone itself ($\lambda_{\text{max}} = 327$ nm^[14]) the position of this latter, longer wavelength absorption feature is red-shifted to the point where it appears in the visible region. This shifting, which has the effect of making compound **2** yellow, is ascribed to the presence of

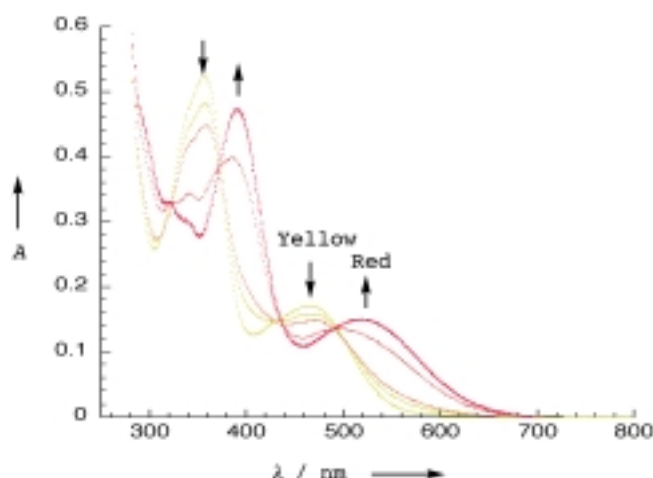


Figure 1. Absorption spectra of **2** recorded in CH_2Cl_2 (5.0×10^{-5} M) after the addition of 0, 1, 2, 4, 6, 8, and 10 equivalents of tetrabutylammonium fluoride.

an extended π -conjugation network involving the anthraquinone moiety, the acetylene linker, and the pyrrole to which it is bound.

As can be seen by inspection of Figure 1, upon the addition of fluoride anion, the peak at 467 nm decreases while a new peak appears at 518 nm. These changes, complete after the addition of only six equivalents of F^- ions, have the effect of changing the color of the solution from yellow to red (Figure 2).^[15] The addition of either chloride or phosphate

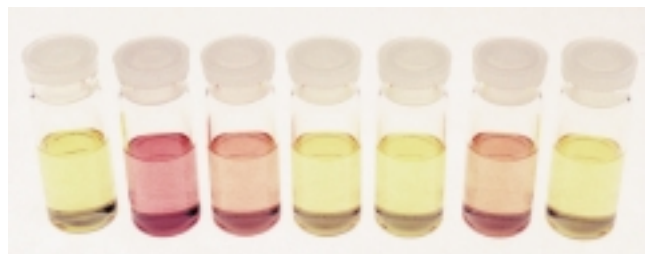


Figure 2. Color changes of **2** in CH_2Cl_2 (5.0×10^{-5} M) before and after the addition of 100 equivalents of representative anions (From left to right: **2**; **2** + F^- ; **2** + Cl^- ; **2** + Br^- ; **2** + I^- ; **2** + H_2PO_4^- ; **2** + HSO_4^-).

ions induced a color change that was less dramatic (that is, from yellow to reddish-orange) and required greater amounts of anion to effect a commensurate change (the absorption peak, originally at 467 nm, shifts to 501 and 497 nm upon the addition of 100 equivalents of tetrabutylammonium chloride and phosphate, respectively).^[15] On the other hand, exposure to bromide, iodide, and sulfate anions, species that do not bind to calix[4]pyrroles appreciably,^[7a] did not lead to any noticeable change in color (Figure 2).^[15] This dramatic combination of anion-specific response/nonresponse makes this system an effective naked-eye-detectable anion sensor under these solution-phase conditions.

Figure 3, which shows the color changes (if any) induced upon the addition of 100 equivalents of various anions to dilute (5×10^{-5} M) solutions of **3** in dichloromethane, serves to

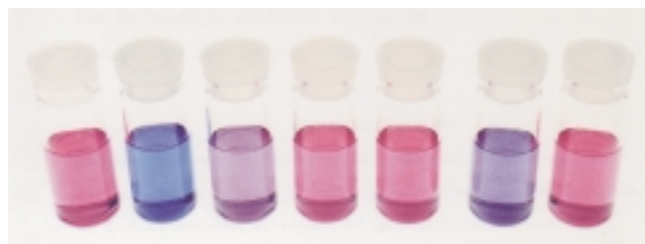


Figure 3. Color changes of **3** in CH_2Cl_2 (5.0×10^{-5} M) before and after the addition of 100 equivalents of representative anions (From left to right: **3**; **3** + F^- ; **3** + Cl^- ; **3** + Br^- ; **3** + I^- ; **3** + H_2PO_4^- ; **3** + HSO_4^-).

illustrate that the “color tuning” proposed for this system was indeed observed by experiment. Specifically, this particular calix[4]pyrrole derivative changes color from red to blue or purple upon the addition of anions, again with the effect being most dramatic and most pronounced in the case of fluoride ions.

The above qualitative changes are reflected in more quantifiable terms in the corresponding absorption spectra

(Figure 4). Whereas 4-hydroxyanthraquinone itself is pale yellow and displays an absorption maximum at 405 nm in dichloromethane,^[14] conjugation to calix[4]pyrrole through an alkyne spacer shifts the absorption maximum to the red

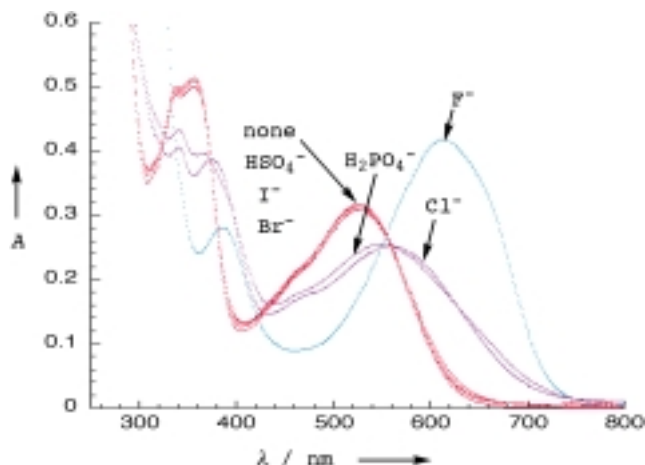


Figure 4. Absorption spectra of **3** recorded in CH_2Cl_2 ($5.0 \times 10^{-5} \text{ M}$) after the addition of 100 equivalents of representative anions.

region of the spectrum and produces a species with a lowest energy absorption maximum at 526 nm. Upon the addition of fluoride ions, the absorption maximum is shifted even further (to 613 nm) with the net result that the solution turns blue (approximately 20 equivalents are generally sufficient to effect near-complete color conversion). Similar, but less pronounced bathochromic shifts are seen upon the addition of excess chloride and dihydrogen phosphate ions, with the lowest energy maxima being observed at 567 and 549 nm, respectively. In these cases the colors of the solution change from red to purple and from red to dark purple, respectively. On the other hand, no significant color changes were observed upon the addition of bromide, iodide, or sulfate ions. These spectral results, as well as the corresponding visual changes, are thus completely consistent with the association constants recorded for **3**, namely $\text{F}^- > \text{Cl}^- > \text{H}_2\text{PO}_4^- \gg \text{Br}^- \sim \text{I}^- \sim \text{HSO}_4^-$.^[15]

While the origins of the dramatic color changes observed with chemosensors **2** and **3**, tentatively ascribed to charge-transfer interactions between the electron-rich, calix[4]pyrrole-bound anions and the electron-deficient anthraquinone moieties, remain the subject of current study, it is important to note that the anthracene-containing control compound **4**, a calix[4]pyrrole system bearing a more electron-rich appendage, does not act as a true naked-eye-detectable anion sensor. In this instance, addition of 100 equivalents of tetrabutylammonium fluoride to a $5 \times 10^{-5} \text{ M}$ solution in CH_2Cl_2 engendered only a slight bathochromic shift in the absorption maximum (λ_{max} shifts from 408 to 416 nm) along with a modest reduction in absorption intensity. Detailed ^1H NMR spectroscopic and fluorescence analyses revealed, however, that **4** nonetheless acts as an efficient receptor for F^- , Cl^- , and H_2PO_4^- ions under these conditions.

The need for effective anion binding (as well as an appended chromophore) was supported by control studies

carried out using unsubstituted anthraquinone. Even in the presence of a vast excess of tetrabutylammonium fluoride, no change in either the position or intensity of the absorption maximum at 327 nm was seen in dichloromethane. This finding is thus consistent with the earlier observations that even such electron-rich anions as bromide and iodide do not modify the color of sensors **2** or **3**; they are simply not bound to any appreciable extent in dichloromethane.

In conclusion, we have succeeded in preparing a new class of anion sensors that not only allow for the facile colorimetric detection of F^- , Cl^- , and H_2PO_4^- ions but which are also amenable to “color tuning”. To the best of our knowledge these systems are the first naked-eye-detectable chemosensors that permit discrimination between different anionic substrates as the result of disparate changes in color. Such systems are likely to be of particular benefit in situations where sophisticated instrumentation might be lacking (for example, in field tests). They could also prove useful in the development of multianalyte sensor arrays,^[16] wherein a nonbinary signaling response is advantageous and apolar binding conditions often pertain.

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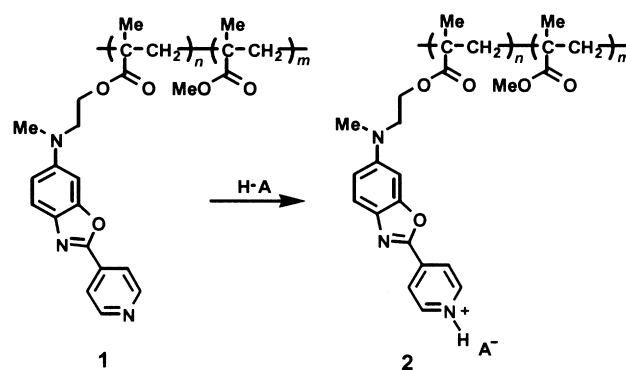
- [1] a) Z. Brzózka in *Comprehensive Supramolecular Chemistry*, Vol. 10 (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, K. S. Suslick), Pergamon, Oxford, **1996**, pp. 187–212; b) M. Pietraszkiewicz in *Comprehensive Supramolecular Chemistry*, Vol. 10 (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, K. S. Suslick), Pergamon, Oxford, **1996**, pp. 225–266.
- [2] a) H.-G. Löhr, F. Vögtle, *Acc. Chem. Res.* **1985**, *18*, 65–72; b) M. Takagi in *Cation Binding by Macrocycles* (Eds.: Y. Inoue, G. W. Gokel), Marcel Dekker, New York, **1990**, pp. 465–496; c) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566, and references therein; d) A. W. Czarnik in *Frontiers in Supramolecular Organic Chemistry and Photochemistry* (Eds.: H.-J. Schneider, H. Dürr), VCH, Weinheim, **1991**, pp. 109–122; e) L. Fabbri, M. Licchelli, P. Pallavicini, L. Parodi, A. Taglietti in *Transition Metals in Supramolecular Chemistry* (Ed.: J.-P. Sauvage), Wiley, Chichester, **1999**, pp. 93–134.
- [3] a) L. Fabbri, A. Poggi, *Chem. Soc. Rev.* **1995**, *24*, 197–202; b) Y. Kubo, S. Maeda, S. Tokita, M. Kubo, *Nature* **1996**, *382*, 522–523; c) K. Koumoto, M. Takeuchi, S. Shinkai, *Supramol. Chem.* **1998**, *9*, 203–210; d) K. Fuji, K. Tsubaki, K. Tanaka, N. Hayashi, T. Otsubo, T. Kinoshita, *J. Am. Chem. Soc.* **1999**, *121*, 3807–3808; e) Y. Kubo, S. Obara, S. Tokita, *Chem. Commun.* **1999**, 2399–2400.
- [4] a) A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 862–865; *Angew. Chem. Int. Ed.* **1998**, *37*, 649–652; b) P. A. Gale, L. J. Twyman, C. I. Handlin, J. L. Sessler, *Chem. Commun.* **1999**, 1851–1852; c) J. O. Smith, D. A. Olson, B. A. Armitage, *J. Am. Chem. Soc.* **1999**, *121*, 2686–2695.
- [5] *Chemosensors of Ion and Molecular Recognition* (Eds.: J.-P. Desvergne, A. W. Czarnik), Kluwer, Dordrecht, **1997**, Vol. 492.
- [6] a) F. P. Schmidtchen, M. Berger, *Chem. Rev.* **1997**, *97*, 1609–1646, and references therein; b) J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903–3906; *Angew. Chem. Int. Ed.* **1999**, *38*, 3666–3669; c) C. B. Black, B. Andrioletti, A. C. Try, C. Ruiperez, J. L. Sessler, *J. Am. Chem. Soc.* **1999**, *121*, 10438–10439.
- [7] a) P. A. Gale, J. L. Sessler, V. Král, *Chem. Commun.* **1998**, 1–8; b) J. L. Sessler, P. Anzenbacher, Jr., K. Jursíková, H. Miyaji, J. W. Genge, N. A. Tvermoes, W. E. Allen, J. A. Shriver, P. A. Gale, V. Král, *Pure Appl. Chem.* **1998**, *70*, 2401–2408; c) J. L. Sessler, P. A. Gale in *The Porphyrin Handbook*, Vol. 6 (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, San Diego, **1999**, pp. 257–278; d) H. Miyaji, P. Anzenbacher Jr., J. L. Sessler, E. R. Bleasdale, P. A. Gale, *Chem. Commun.* **1999**, 1723–1724.

- [8] H. Miyaji, W. Sato, J. L. Sessler, V. Lynch, *Tetrahedron Lett.* in press.
- [9] a) K. Sonogashira in *Metal-catalyzed Cross Coupling Reactions* (Eds.: F. Diederich, P. J. Stang), WILEY-VCH, Weinheim, **1998**, pp. 203–230; b) K. Sonogashira in *Comprehensive Organic Synthesis*, Vol. 3 (Ed.: B. M. Trost), Pergamon, Oxford, **1990**, pp. 521–549.
- [10] All compounds gave spectroscopic consistent with the proposed structures; see the Supporting Information.
- [11] H. O. House, D. Koepsell, W. Jaeger, *J. Org. Chem.* **1973**, *38*, 1167–1173.
- [12] A. E. Goldstein, *J. Am. Chem. Soc.* **1939**, *61*, 1600–1601.
- [13] T. P. Galevska, A. A. Moroz, R. N. Myasnikova, M. S. Shvartsberg, *J. Org. Chem. USSR* **1988**, *24*, 358–362.
- [14] H. Labhart, *Helv. Chim. Acta* **1957**, *152*, 1410–1420.
- [15] $\log K_a$ values (from UV/Vis titration experiments for the association of **2**, **3**, and **4** with fluoride, chloride, and phosphate in CH_2Cl_2) were 3.71 (**2** with F^-), 3.16 (**2** with Cl^-), 3.04 (**2** with H_2PO_4^-), 3.43 (**3** with F^-), 3.13 (**3** with Cl^-), 2.76 (**3** with H_2PO_4^-), 3.66 (**4** with F^-), 3.16 (**4** with Cl^-), and 2.86 (**4** with H_2PO_4^-); see the Supporting Information. The $\log K_a$ values for bromide, iodide, and sulfate ions were too low to measure accurately using either UV/Vis or ^1H NMR spectroscopic titrations (that is, $\log K_a < 1.3$).
- [16] J. J. Lavigne, S. Savoy, M. B. Clevenger, J. E. Ritchie, B. McDoniel, S.-J. Yoo, E. V. Anslyn, J. T. McDevitt, J. B. Shear, D. Neikirk, *J. Am. Chem. Soc.* **1998**, *120*, 6429–6430.

Photoacid-Induced Fluorescence Quenching: A New Strategy for Fluorescent Imaging in Polymer Films**

Jong-Man Kim,* Tae-Eun Chang, Jong-Hee Kang, Ki Hong Park, Dong-Keun Han, and Kwang-Duk Ahn*

The generation of patterned images in polymer films has gained much attention in fundamental and applied research areas. The development of patterned functional images is also of great importance in the electronics industry. Recently, the generation of functional images by selective immobilization of organic dyes in polymer films has been reported.^[1–3] The selective removal of labile acidic protecting groups by photo-induced chemical transformation followed by chemisorption of organic dyes from solution into the patterned polymer film afforded μm -scale functional images. Recently, we described finely resolved patterned fluorescent images with these transiently protected precursor molecules.^[4, 5] As part of our efforts to produce functional images in polymer films we now report the synthesis of the novel copolymer **1**, which has pendant pyridylbenzoxazole groups, and its use in the generation of fluorescent images by photolithographic methods.



The strategy for the design of a molecule for fluorescent imaging is based on the following facts: in a conjugated aromatic system, the nature of fluorescence is often greatly affected by substituents. A strong fluorescence is, in general, observed when an aromatic compound has extended π conjugation and contains electron-donor substituents, such as hydroxy or amino groups, at both ends of this conjugated system. The fluorescence of the molecule, however, drastically decreases if one of the electron-donating substituents is replaced by an electron-withdrawing substituent, such as a nitro group. An electron-withdrawing effect, leading to fluorescence quenching, can also be induced when the fluorescent chromophore has a functional group which can interact with an acid. We have now designed the copolymer **1**, which has fluorescent pyridylbenzoxazole moieties as pendant groups. The benzoxazole chromophore of **1** has an electron-donating amino group at one end and an electron-rich pyridyl group at the other. However, the strongly fluorescent nature of **1** is expected to be affected when the electronic state of the benzoxazole chromophore is disturbed by interaction with an acid, as in **2**. If the acid-induced fluorescence quenching is significant, and occurs only in selected areas, then patterned fluorescent images will be obtained.

The preparation of the pyridylbenzoxazole monomer **8** is shown in Scheme 1. In general, benzoxazole derivatives are prepared by a prior formation of an *o*-hydroxy amide through the condensation of *o*-aminophenol with an acid chloride at low temperature, followed by thermal cycloaddition at high temperature.^[6] In this cyclization step unwanted side reactions often occur. To avoid these we have developed a reaction with mild conditions for the formation of the benzoxazole ring. Thus, protection of the hydroxy group of 5-fluoro-2-nitrophenol (**3**) followed by aromatic substitution with (2-methylamino)ethanol provided the alcohol **5** in good yield, which was converted to pyridylbenzoxazole **7** in three steps. The removal of the protecting group and reduction of the nitro group by hydrogenation followed by treatment with 4-pyridinecarboxaldehyde gave the imine intermediate **6**. The oxidative cyclization of **6** with lead(IV) acetate, at room temperature, provided the benzoxazole chromophore **7** in high yield (80% from **5**). Purification after the deprotection, imine formation, and cyclization steps was not necessary. The pyridylbenzoxazole **7** was converted into the desired monomer **8** (4PBZMA) by treatment with methacryloyl chloride.

Copolymerization of the monomer **8** with methyl methacrylate (MMA) was carried out with a 1:1 molar feed ratio in

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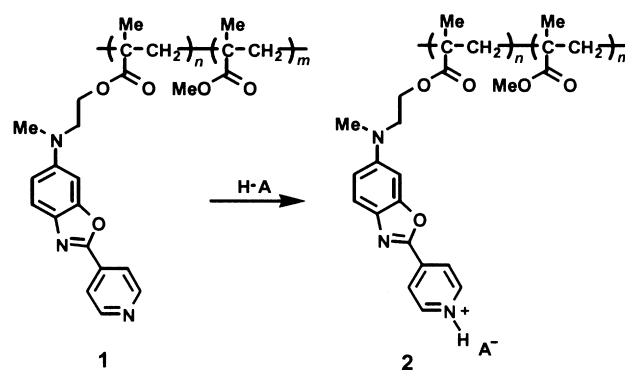
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- [8] H. Miyaji, W. Sato, J. L. Sessler, V. Lynch, *Tetrahedron Lett.* in press.
- [9] a) K. Sonogashira in *Metal-catalyzed Cross Coupling Reactions* (Eds.: F. Diederich, P. J. Stang), WILEY-VCH, Weinheim, **1998**, pp. 203–230; b) K. Sonogashira in *Comprehensive Organic Synthesis*, Vol. 3 (Ed.: B. M. Trost), Pergamon, Oxford, **1990**, pp. 521–549.
- [10] All compounds gave spectroscopic consistent with the proposed structures; see the Supporting Information.
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- [13] T. P. Galevskaya, A. A. Moroz, R. N. Myasnikova, M. S. Shvartsberg, *J. Org. Chem. USSR* **1988**, *24*, 358–362.
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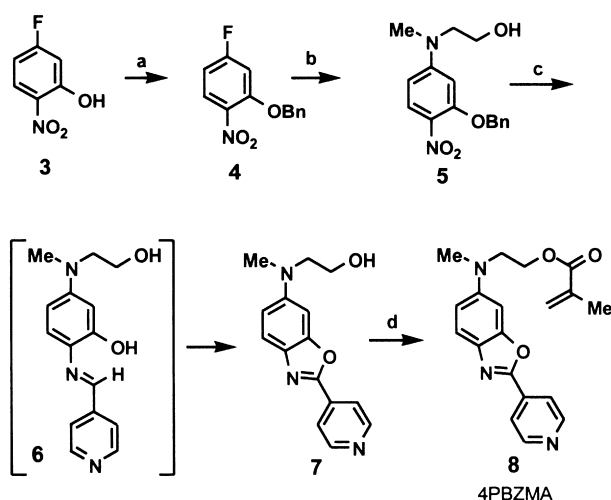
The strategy for the design of a molecule for fluorescent imaging is based on the following facts: in a conjugated aromatic system, the nature of fluorescence is often greatly affected by substituents. A strong fluorescence is, in general, observed when an aromatic compound has extended π conjugation and contains electron-donor substituents, such as hydroxy or amino groups, at both ends of this conjugated system. The fluorescence of the molecule, however, drastically decreases if one of the electron-donating substituents is replaced by an electron-withdrawing substituent, such as a nitro group. An electron-withdrawing effect, leading to fluorescence quenching, can also be induced when the fluorescent chromophore has a functional group which can interact with an acid. We have now designed the copolymer **1**, which has fluorescent pyridylbenzoxazole moieties as pendant groups. The benzoxazole chromophore of **1** has an electron-donating amino group at one end and an electron-rich pyridyl group at the other. However, the strongly fluorescent nature of **1** is expected to be affected when the electronic state of the benzoxazole chromophore is disturbed by interaction with an acid, as in **2**. If the acid-induced fluorescence quenching is significant, and occurs only in selected areas, then patterned fluorescent images will be obtained.

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Scheme 1. Synthesis of the pyridylbenzoxazole monomer **8**. a) Benzyl bromide, K_2CO_3 , DMF, $100^\circ C$, 3 h, 83%; b) 2-(methylamino)ethanol, K_2CO_3 , DMF, $100^\circ C$, 3 h, 85%; c) 1. H_2 , 10% Pd/C, THF, $25^\circ C$, 12 h; 2. 4-pyridinecarboxaldehyde, THF, $25^\circ C$, 3 h; 3. $Pb(OAc)_4$, THF, $25^\circ C$, 3 h, 80% from **5**; d) methacryloyl chloride, TEA (triethylamine), CH_2Cl_2 , $25^\circ C$, 12 h, 74%.

1-methyl-2-pyrrolidinone (NMP) using 2,2'-azobis(isobutyronitrile) (AIBN) as a radical initiator. The copolymer **1**, after precipitation into methanol, was obtained as a yellow powder in 59% yield. The polymer has a weight-average molecular weight (M_w) of 61 000 with a polydispersity of 2.07. The 4:6 ratio of pyridylbenzoxazole monomer to MMA in **1** was confirmed by NMR and UV spectroscopic analyses.

The UV/Vis spectrum of **1** has a maximum absorption wavelength at 378 nm which is attributed to the pyridylbenzoxazole groups. To investigate the possible formation of **2** and the fluorescence quenching by acid-induced chemical transformation, a thin polymer film containing **1** (77 wt %) and a photoacid generator (triphenylsulfonium hexafluoroantimonate (TPSHFA),^[7–10] 23 wt %) was prepared by spin-coating a chloroform solution on a quartz plate. This film was irradiated with UV light (360 nm) and the progress was monitored by both UV/Vis and fluorescence spectroscopy. As displayed in Figure 1a, absorption at the wavelength maximum of the benzoxazole chromophore (378 nm) decreased upon irradiation and a new band appeared at 486 nm. The position of this new wavelength maximum (486 nm) is in the region expected for the protonated benzoxazole chromophore found in **2**. The formation of the protonated species **2** in the polymer film was also deduced from comparison of the color of the film before and after irradiation. When the lightly yellow, transparent polymer film was exposed to UV light for 30 s, a highly transparent red film was obtained. These results demonstrate that the strong acid (presumably hydrogen fluoride (HF) and/or hydrogen hexafluoroantimonate ($HSbF_6$)), generated by irradiation of the photoacid generator, protonates the nitrogen atoms of the pyridyl groups. The two isosbestic points (331, 422 nm) demonstrate that the process is an A- to B-type conversion without intermediate species. More interesting results became apparent from a comparison of the fluorescence spectra before and after irradiation. Irradiation of the polymer film for 10 s resulted in

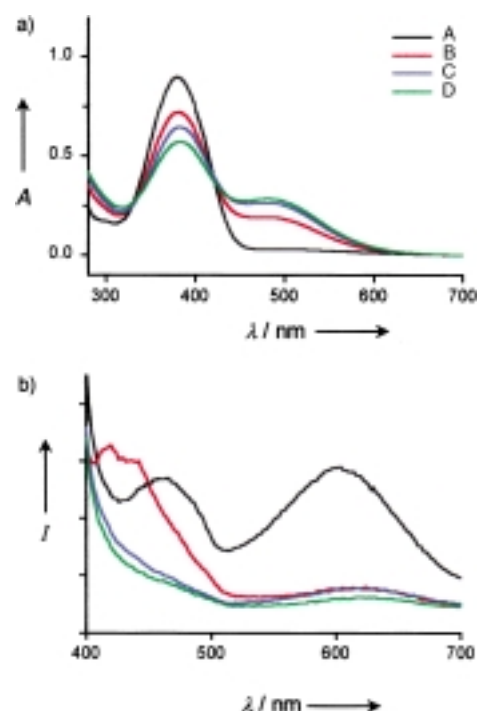


Figure 1. Time course revealed by a) UV absorption and b) fluorescence emission spectroscopy. Monitoring of a 0.2- μm thick film containing polymer **1** (77 wt %) and the photoacid generator TPSHFA (23 wt %) on a quartz plate after UV irradiation (360 nm) followed by heating at $120^\circ C$ for 1 min (irradiation time: (A) 0, (B) 10, (C) 30, (D) 180 s, respectively).

the disappearance of the fluorescence at 600 nm (Figure 1b). Further irradiation had no substantial effect on the fluorescence quenching.

Formation of patterned fluorescent images with **1** requires generation of acids in the specific areas. For this purpose, a polymer film containing **1** and TPSHFA on a silicon wafer was exposed through a photomask. As shown in Figure 2, fluorescent image patterns appeared under fluorescent microscopy, the dark areas are the portions exposed through the photomask.

We have prepared a polymer having pyridylbenzoxazole pendants for fluorescent imaging. Synthesis of the monomer was straightforward and the copolymer **1** was readily prepared from an AIBN-initiated radical polymerization with methyl



Figure 2. Fluorescent image patterns obtained with a 1.0- μm thick film containing copolymer **1** (77 wt %) and TPSHFA (23 wt %) on a silicon wafer after UV irradiation (360 nm) for 10 s (200 mJ cm^{-2}) through a photomask followed by heating at $120^\circ C$ for 1 min.

methacrylate as a comonomer. The copolymer **1** was shown to have good film-forming properties on a solid substrate. The fluorescence of the polymer was effectively quenched by photochemically generated acid and afforded finely resolved fluorescent images on a solid substrate.

Experimental Section

4: Potassium carbonate (82.9 g, 0.60 mol) and benzyl bromide (136.8 g, 0.80 mol) were added to a solution of 5-fluoro-2-nitrophenol (**3**) (94.3 g, 0.60 mol) in DMF (200 mL). The resulting mixture was stirred at 100 °C for 3 h, filtered, and the filtrate diluted with diethyl ether. The organic layer was washed with water and dried with anhydrous magnesium sulfate. Concentration, followed by vacuum distillation (142–146 °C, 0.1 Torr) of the residue gave **4** (123.1 g, 83 %) as a yellow solid. M.p. 55–56 °C; ¹H NMR (200 MHz, CDCl₃): δ = 5.18 (s, 2H), 6.66–6.87 (m, 2H), 7.32–7.49 (m, 5H), 7.94 (d, 1H); elemental analysis calcd (%) for C₁₃H₁₀N₂O₃F₁ (247.23): C 63.16, H 4.08, N 5.67; found: C 63.50, H 4.09, N 5.21.

5: Potassium carbonate (51.83 g, 0.38 mol) was added to a solution of **4** (123.1 g, 0.50 mol) and 2-(methylamino)ethanol (56.3 g, 0.75 mol) in DMF (200 mL). The resulting mixture was heated at 100 °C for 3 h and poured into water (1 L). The yellow precipitate was collected, dried, and dissolved in THF. Precipitation into hexane provided **5** (128.4 g, 85.3 %). M.p. 104–106 °C; ¹H NMR (200 MHz, CDCl₃): δ = 2.41 (s, 1H), 3.03 (s, 3H), 3.50 (t, 2H), 3.77 (t, 2H), 5.13 (s, 2H), 6.10–6.22 (m, 2H), 7.27–7.50 (m, 5H), 7.88 (d, 1H); elemental analysis calcd (%) for C₁₆H₁₈N₂O₄: (302.33): C 63.57, H 6.00, N 9.27; found: C 63.50, H 6.09, N 9.21.

7: A mixture of **5** (24.2 g, 0.08 mol) and 10 % Pd/C (3.9 g) in THF (200 mL) was stirred under hydrogen at 25 °C for 12 h. The catalyst was removed by filtration, and 4-pyridinecarboxaldehyde (8.57 g, 0.08 mmol) was added to the filtrate. The resulting solution was stirred at 25 °C for 3 h. Lead(IV) acetate (44.38 g, 0.10 mol) was slowly added to the solution and the mixture stirred vigorously at 25 °C for 3 h. Removal of lead diacetate followed by flash column chromatography (silica gel, THF) gave **7** (17.14 g, 80.0 %) as a yellow solid. M.p. 178–180 °C; ¹H NMR (200 MHz, [D₆]DMSO): δ = 3.03 (s, 3H), 3.34–3.63 (m, 4H), 4.72 (t, 1H), 6.88 (d, 1H), 7.01 (s, 1H), 7.51 (d, 1H), 7.97 (d, 2H), 8.78 (d, 2H); elemental analysis calcd (%) for C₁₅H₁₅N₃O₂ (269.30): C 66.9, H 5.61, N 15.60; found: C 66.1, H 5.69, N 15.41.

8: The benzoxazole intermediate **7** (4.80 g, 17.84 mmol) was dissolved in dichloromethane (200 mL) and methacryloyl chloride (3.73 g, 35.68 mmol) and TEA (6.0 mL, 43.11 mmol) were added. The mixture was stirred at 25 °C for 12 h, poured into water, and extracted with dichloromethane. The organic layer was concentrated in vacuo, and silica gel column chromatography (25 % EtOAc/hexane) afforded **8** (4.47 g, 74.4 %). M.p. 60–62 °C; λ_{max} (CHCl₃) = 378 nm; ¹H NMR (200 MHz, CDCl₃): δ = 1.90 (s, 3H), 3.07 (s, 3H), 3.73 (t, 2H), 4.36 (t, 2H), 5.53 (s, 1H), 6.05 (s, 1H), 6.82–6.87 (m, 2H), 7.60 (d, 1H), 7.99 (d, 2H), 8.74 (d, 2H); elemental analysis calcd (%) for C₁₉H₁₉N₃O₃ (327.38): C 67.64, H 5.68, N 12.45; found: C 67.9, H 5.74, N 12.2.

Copolymer 1 P(4PBZMA/MMA): A solution containing monomer **8** (0.50 g, 1.48 mmol), MMA (0.15 g, 1.49 mmol), AIBN (9.7 mg) and NMP (2.5 mL) in an ampoule was subjected to repeated freeze–thaw cycles before the ampoule was sealed under vacuum. The sealed ampoule was heated at 65 °C for 40 h. After polymerization, the product was precipitated into methanol and dried to give **1** (0.385 g, 59.2 %) as a yellow powder.

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- [1] A. M. Vekselman, C. Zhang, G. D. Darling, *Chem. Mater.* **1997**, *9*, 1942.
- [2] C. Zhang, A. M. Vekselman, G. D. Darling, *Chem. Mater.* **1995**, *7*, 850.
- [3] M. Schilling, H. E. Katz, F. M. Houlihan, J. M. Kometani, S. M. Stein, O. Nalamasu, *Macromolecules* **1995**, *28*, 110.
- [4] J.-M. Kim, J.-H. Kang, D. K. Han, C.-W. Lee, K.-D. Ahn, *Chem. Mater.* **1998**, *10*, 2332.
- [5] J.-M. Kim, T.-E. Chang, J.-H. Kang, D. K. Han, K.-D. Ahn, *Adv. Mater.* **1999**, *11*, 1499.

- [6] K. H. Park, K. M. Yean, M. Y. Lee, S.-D. Lee, D.-H. Shin, C. J. Lee, N. Kim, *Polymer* **1998**, *39*, 7061.
- [7] E. Reichmanis, F. M. Houlihan, O. Nalamasu, T. X. Neenan, *Chem. Mater.* **1991**, *3*, 394.
- [8] S. A. Macdonald, C. G. Willson, J. M. J. Fréchet, *Acc. Chem. Res.* **1994**, *27*, 151.
- [9] Q. J. Niu, J. M. J. Fréchet, *Angew. Chem.* **1998**, *110*, 685; *Angew. Chem. Int. Ed.* **1998**, *37*, 667.
- [10] C.-M. Chung, K.-D. Ahn, *React. Func. Polym.* **1999**, *40*, 1.

Halogen Bonding versus Hydrogen Bonding in Driving Self-Assembly Processes**

Eleonora Corradi, Stefano V. Meille, Maria T. Messina, Pierangelo Metrangolo,* and Giuseppe Resnati*

It is well known^[1] that carbon-bound halogen atoms (frequently iodine and bromine, but also chlorine and even fluorine) can act as electron acceptors and form short contacts with different species (both neutral and negatively charged) that possess an ability to act as an electron donor.^[2] These noncovalent interactions can be strong enough to control the aggregation of organic molecules in solid,^[3] liquid,^[4] and gas^[5, 6] phases and the term “halogen bonding”^[2, 6] has been suggested in order to emphasize the similarity with hydrogen bonding. While metal-ion coordination^[7] is frequently the key feature in inorganic supramolecular systems, hydrogen bonding^[8] is by far the most frequently used tool to assemble organic molecules in solid, liquid, or gas phases, and it plays an important role in stabilizing supramolecular aggregates even in water.

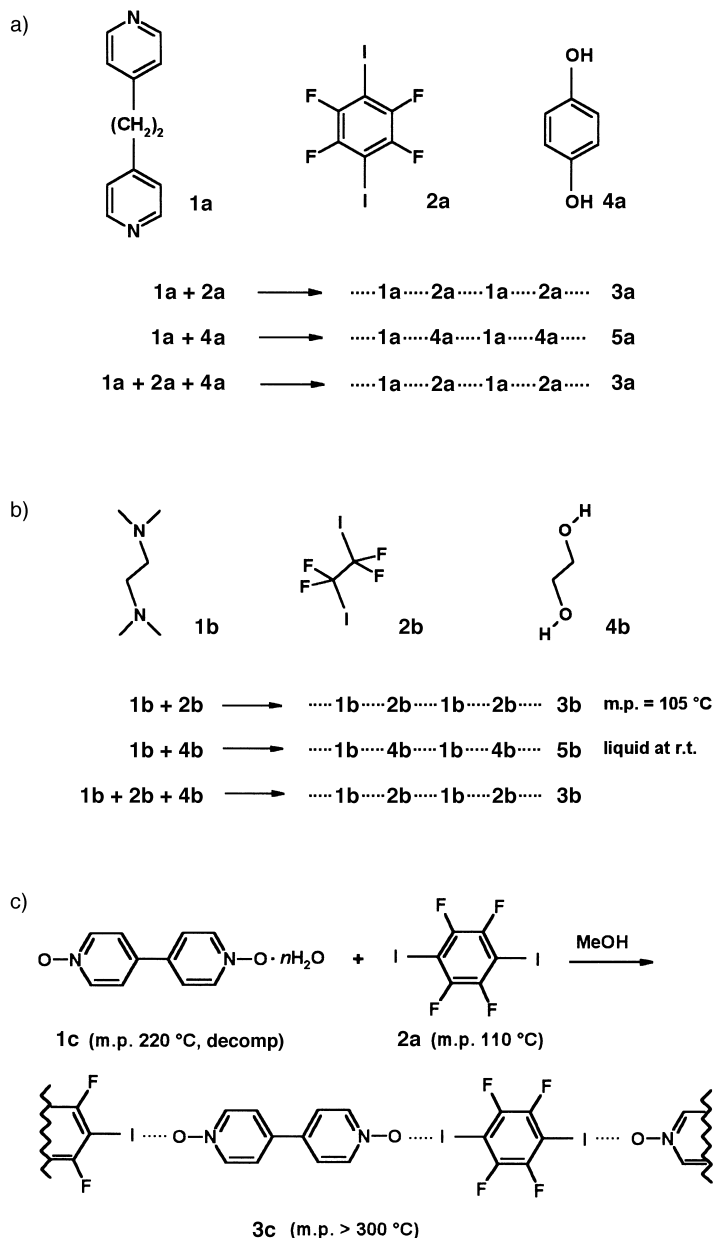
Here we show that when the recognition pattern controlling the self-assembly process can be based on either hydrogen or halogen bonding, the latter can dominate over the former and, under appropriate conditions, will single out the molecules that will be involved in the construction of supramolecular architectures. We have already described specific I⋯N intermolecular interactions occurring between α,ω-diiodoperfluoroalkanes and dinitrogen hydrocarbons.^[9–11] These interactions are strong enough to overcome the low affinity^[12] that exists between perfluorocarbons and hydrocarbons to assemble them into oligomeric structures in the liquid phase^[4] and into crystalline 1D networks in the solid phase.^[9–11]

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the authors.

The ability of halogen bonding to force perfluorocarbons and hydrocarbons to cocrystallize is shown here to apply not only to aliphatic but also to aromatic systems. Specifically, crystallization of equimolar amounts of 1,2-bis(4-pyridyl)ethane (**1a**) and 1,4-diiodotetrafluorobenzene (**2a**) from acetone gives the halogen-bonded infinite chains **3a** (Scheme 1a). Slow evaporation of equimolar solutions of **1a** and



Scheme 1. Schematic diagrams representing the formation of noncovalent copolymers **3** and **5**.

hydroquinone (**4a**) in the same solvent affords the hydrogen-bonded^[13] 1D network **5a**. Both **3a** and **5a** precipitate as white crystals whose structures were determined by single-crystal X-ray diffraction^[14] (Figure 1). The geometric parameters of the hydrogen and halogen bonds conform with values observed in similar systems.^[2, 8, 10] The conformation of **1a** is different in the two cocrystals as a result of the different

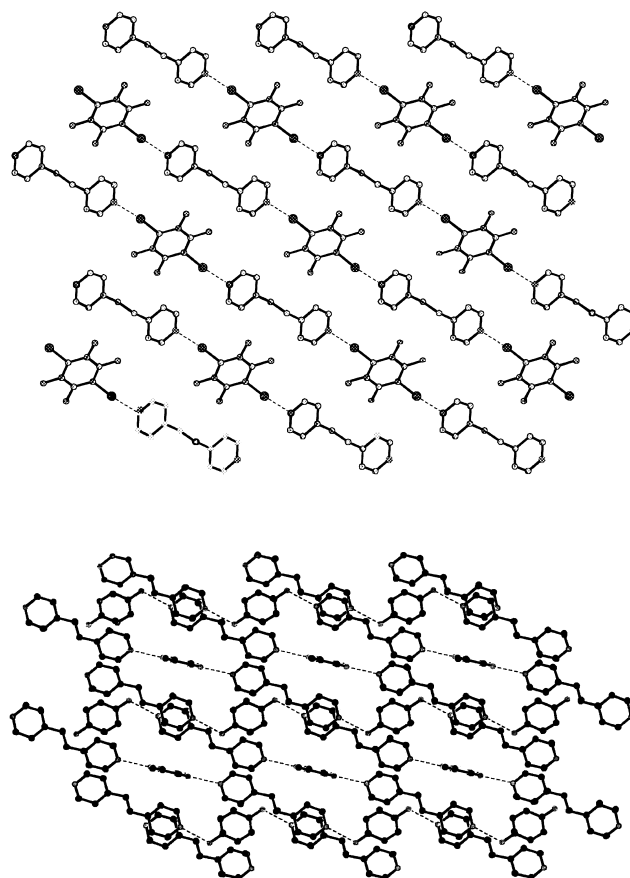


Figure 1. Packing of cocrystal **3a** (top) and **5a** (bottom) viewed down the crystallographic *a* axis. Hydrogen atoms are omitted for clarity. Dotted lines represent halogen bonds ($N \cdots I$: 2.79(5) Å; $N \cdots I \cdots C$: 175.9(1)°) and hydrogen bonds ($N_1 \cdots H_1$: 1.75(3) Å; $N_1 \cdots H_1 \cdots O_1$: 169.3(1)°; $N_2 \cdots H_2$: 1.67(4) Å; $N_2 \cdots H_2 \cdots O_2$: 167.0(1)°) in cocrystal **3a** and **5a**, respectively.

requirements of the intermolecular interactions acting in the two architectures, namely, the halogen and hydrogen bonds, respectively.

In order to directly contrast hydrogen and halogen bonds, a competitive experiment was devised. Equimolar amounts of **1a**, **2a**, and **4a** were dissolved in excess acetone in a vial of clear borosilicate glass and the opened vial was closed in a cylindrical wide-mouth bottle containing vaseline oil. After a few days at room temperature cocrystal **3a**, composed of **1a** and **2a**, separated in pure form. Microanalyses, thin-layer chromatography, and NMR spectroscopic studies (¹H and ¹⁹F nuclei) confirmed that **4a** remained in the liquid phase. Halogen bonding proves more effective than hydrogen bonding in this system in controlling the construction of programmed supramolecular architectures.

While the correlation among crystal packing, intermolecular interaction strength, and melting point cannot be quantified, the thermal characterization of compounds **1–5** by differential scanning calorimetry (Figure 2) nevertheless gives some useful qualitative information. The endotherms corresponding to the melting of pure components **1a** and **2a** are missing from the heating curve of cocrystal **3a**, which shows a single endotherm at higher temperature than the melting points of **1a** and **2a**. This observation indicates the formation of a new crystal species containing the two

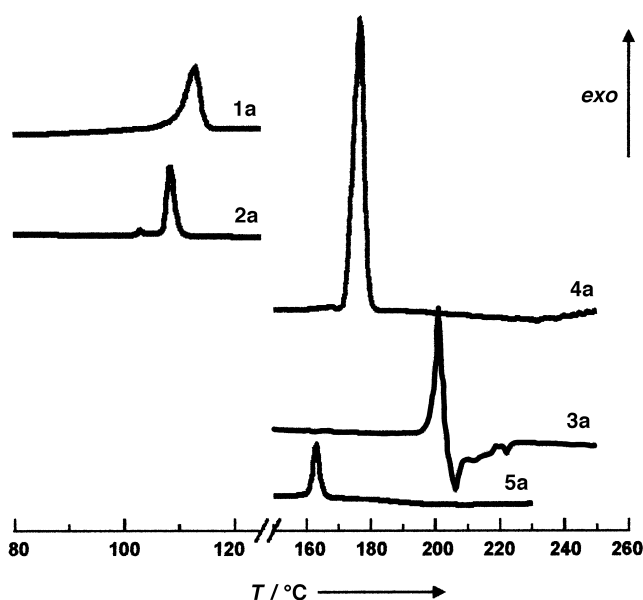


Figure 2. Heating curves (heating rate $10^{\circ}\text{Cmin}^{-1}$) of **1a**, **2a**, **4a**, **3a**, and **5a**. The heating curve of infinite 1D network **3a** suggests the halogen bonding as a pre-reactive state^[6] since the endotherm of the melting process is rapidly followed by an exothermic process.

components. Hydrogen bonding plays a major role in controlling the packing of both cocrystal **5a** and homocrystal **4a**,^[15] and cocrystal **5a** melts at a lower temperature than homocrystal **4a**. In contrast, cocrystal **3a**, where the self-assembly of simple modules is mainly driven by halogen bonding, melts at a higher temperature than pure starting units **1a** ($\Delta T = m.p._{3a} - m.p._{1a} = 89^{\circ}\text{C}$) and **2a** ($\Delta T = 93^{\circ}\text{C}$). Cocrystal **3a** also melts at a higher temperature than cocrystal **5a** ($\Delta T = 38^{\circ}\text{C}$). The measurement of the enthalpies of fusion confirms the results shown by the melting temperatures and qualitatively indicates that **3a**, the assembly of which is driven by halogen bonding, is more effectively stabilized than solids held together by both weak (van der Waals forces) and strong (hydrogen-bonding) intermolecular interactions. The enthalpy of fusion of **3a** (probably underestimated as an exothermic decomposition follows the melting) is 56 kJ mol^{-1} , which is about 50 % higher than the sum of the energies of fusion of its components $[\Delta H_{\text{fus}}(\mathbf{1a}) + \Delta H_{\text{fus}}(\mathbf{2a})]$, while $\Delta H_{\text{fus}}(\mathbf{5a})$ is 41 kJ mol^{-1} , only 10 % higher than $[\Delta H_{\text{fus}}(\mathbf{1a}) + \Delta H_{\text{fus}}(\mathbf{4a})]$.

Additional competitive experiments performed with aliphatic molecular units further proved the ability of halogen bonding to prevail over hydrogen bonding in identifying the complementary motifs to be involved in the supramolecular self-assembly process. We already reported how *N,N,N',N'*-tetramethylethylenediamine (TMEDA, **1b**) and 1,2-diiodotetrafluoroethane (**2b**), which melt at -55 and -27°C , respectively, cocrystallize to give halogen-bonded chain **3b**, which melts at 105°C .^[10] In contrast, neat equimolar amounts of **1b** and ethylene glycol (**4b**, m.p. -13°C) remain liquid at room temperature and solidification is observed only on cooling the mixture to -60°C . When a stoichiometric amount of **1b** was added neat to a solution of equimolar quantities of **2b** and **4b** in chloroform, the rapid precipitation of the pure cocrystal **3b** occurred (Scheme 1b). In aqueous solution the

lone pair of electrons on the nitrogen atom of **1b** is involved in the formation of hydrogen bonds with the solvent. Even under these conditions the halogen-bonding interaction is able to induce the self-assembly of single units **1b** and **2b** into infinite chain **3b**. Indeed, the cocrystal **3b** rapidly precipitated in a pure form on stirring the two-phase system of pure 1,2-diiodotetrafluoroethane (**2b**) and a 1M solution of TMEDA in water at room temperature. *N,N,N',N'*-Tetramethyl-1,4-butanediamine as well as its -1,6-hexane- analogue and 4,4'-dipyridine behaved similarly. In all these cases halogen bonding is strong enough to drive the phasing out of different diamines from the aqueous solution, thus proving the ability of halogen bonding to also overpower hydrogen bonding in a cooperative hydrogen-bonded network.^[8]

The oxygen atoms of pyridine *N*-oxide derivatives form strong hydrogen bonds as shown in the IR spectra of methanol solutions, where ν_{OH} shifts to low frequencies in the presence of pyridine-1-oxide (from 3650 to 3360 cm^{-1}). 4,4'-Dipyridyl-1,1'-dioxide is hygroscopic and usually handled in the air as the polyhydrated form 4,4'-dipyridyl-1,1'-dioxide $\cdot n\text{H}_2\text{O}$ (**1c**, m.p. 220°C). When equimolar amounts of **1c** and 1,4-diiodotetrafluorobenzene (**2a**, m.p. 110°C) are crystallized from methanol, the halogen-bonded infinite chain **3c** (m.p. $> 300^{\circ}\text{C}$) precipitates (Scheme 1c, see Supporting Information). Not only nitrogen atoms but also oxygen atoms can therefore form strong and selective halogen bonds that successfully rival the hydrogen bonds in controlling the self-assembly of organic compounds.

Solution calorimetry, as well as IR and NMR spectroscopy are simple and convenient tools for studying weak intermolecular interactions. Some similarities between hydrogen and halogen bonding were evident by the two spectroscopic techniques and additional information on the relative strength of the interactions was obtained by solution calorimetry. The position of ν_{OH} in IR spectra is a simple and sensitive probe to rank the strength of hydrogen bonding. Analogously, ν_{CH} of donor units **1a** and **1b** are shifted to higher frequencies in cocrystals **3a** and **3b**. Larger shifts correspond to stronger halogen bonds^[16] and this is consistent with the $n \rightarrow \sigma^*$ character of the halogen bonding.^[17] The difference in the chemical shift in the ^{19}F NMR spectra of *p*-fluorophenol ($\Delta\delta_{\text{F}}$) has been used as a simple, effective, and sensitive parameter to grade the involvement of the phenol oxygen atom in hydrogen-bond formation^[18] and similarly the $\Delta\delta_{\text{F}}$ value in ^{19}F NMR spectra of perfluorocarbon iodides in the presence of electron donors is a sensitive parameter for probing the strength of the interaction.^[4, 10, 16]

Monofunctional donor and acceptor modules were selected for solution calorimetry experiments. The 1:1 complexes formed by these units remain liquid at room temperature and by neglecting solvation effects, expected to be minor in our case,^[12] the enthalpy of halogen-bond formation can be evaluated free from interferences of the enthalpy of crystallization. Specifically, when a small quantity of 1-iodoperfluorohexane was injected into a large excess of 2,2,6,6-tetramethylpiperidine (100 equiv), an exothermic effect of 31.4 kJ mol^{-1} was measured with an adiabatic accelerating rate calorimeter (ARC). This thermal effect corresponds to the enthalpy of formation of the complex (ΔH_{f}), its value is in

remarkable agreement with literature data for similar interactions^[19] and with quantum mechanical calculations, which yielded an interaction energy of 26 kJ mol⁻¹ for CF₃I...NH₃ in the gas phase.^[20] The value for a prototype hydrogen bonding (triethylamine/*n*-butanol) was determined as $\Delta H_f = 23$ kJ mol⁻¹ by using the same calorimetric technique.^[21] The difference between the halogen and hydrogen bonding for other couples can be even larger than we report since the amine used by us is known to be a medium-strength electron donor.^[4]

Fluorine and perfluorinated residues are strongly electron withdrawing, which promotes^[22] the tendency of the iodine atoms of **2a** and **2b** to behave as electron acceptors in halogen-bonded networks. Nevertheless, halogen bonding also succeeds in driving the self-assembly of organic compounds in the absence of this promoting effect^[1, 2] as formation of an electron donor–acceptor complex has been reported for several non-fluorinated iodoorganic derivatives having quite different structures.^[23] Bromine atoms are less effective than iodine atoms as electron-acceptor sites, nevertheless halogen-bonded cocrystals involving bromoorganic compounds have been described.^[24] Also chlorine, an even less-effective electron-acceptor atom than bromine, can be involved in halogen-bonded aggregates in the solid,^[25] liquid, and gas phases.^[6] Indeed, the relevance of halogen bonding in the self-assembly of structurally different molecules extends to quite different fields, spanning from biopharmacology,^[26] to material science, and crystal engineering.^[27] Although in the examples described here either the halogen or the hydrogen bonding drives the crystal packing and selects the molecular units assembled in the supramolecular architecture, the two interactions could also co-exist in the same crystal and cooperate in building more complex and hopefully functional^[29] intermolecular structures.

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- [1] O. Hassel, *Science* **1970**, 170, 497; G. R. Desiraju, *Angew. Chem.* **1995**, 107, 2397; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 2311.
- [2] J. M. Dumas, L. Gomel, M. Guerin, *The Chemistry of Functional Groups, Supplement D* (Eds.: S. Patai, Z. Rappoport), Wiley, New York, **1983**, p. 985; J. P. M. Lommerse, A. J. Stone, R. Taylor, F. H. Allen, *J. Am. Chem. Soc.* **1996**, 118, 3108; H. A. Bent, *Chem. Rev.* **1968**, 68, 587.
- [3] A. Farina, S. V. Meille, M. T. Messina, P. Metrangolo, G. Resnati, G. Vecchio, *Angew. Chem.* **1999**, 111, 2585; *Angew. Chem. Int. Ed.* **1999**, 38, 2433.
- [4] M. T. Messina, P. Metrangolo, W. Panzeri, E. Ragg, G. Resnati, *Tetrahedron Lett.* **1998**, 39, 9069.
- [5] N. F. Cheetham, A. D. E. Pullin, *Chem. Commun.* **1967**, 233.
- [6] A. C. Legon, *Angew. Chem.* **1999**, 111, 2850; *Angew. Chem. Int. Ed.* **1999**, 38, 2687; A. C. Legon, *Chem. Eur. J.* **1998**, 4, 1890.
- [7] *Transition Metals in Supramolecular Chemistry* (Ed.: J.-P. Sauvage), Wiley, Chichester, **1999**.
- [8] G. A. Jeffrey, *An Introduction to Hydrogen Bonding*, Oxford University Press, Oxford, **1997**; O. Ermer, A. Eling, *J. Chem. Soc. Perkin Trans. 2* **1994**, 925; C. B. Acheröy, K. R. Seddon, *Chem. Soc. Rev.* **1993**, 22, 397.
- [9] A. Manfredi, M. T. Messina, P. Metrangolo, T. Pilati, S. Quici, G. Resnati, *Supramol. Chem.*, submitted.

- [10] V. Amico, S. V. Meille, E. Corradi, M. T. Messina, G. Resnati, *J. Am. Chem. Soc.* **1998**, 120, 8261.
- [11] E. Corradi, S. V. Meille, M. T. Messina, P. Metrangolo, G. Resnati, *Tetrahedron Lett.* **1999**, 40, 7519; A. Lunghi, P. Cardillo, M. T. Messina, P. Metrangolo, W. Panzeri, G. Resnati, *J. Fluorine Chem.* **1998**, 91, 191.
- [12] Perfluorocarbon (PFC) derivatives show a unique combination of physical properties that induce a low affinity for their analogous hydrocarbon (HC) parents. Heats of solution and cohesive pressures of PFCs are quite different from those of HCs. The enthalpies of interaction between PFCs and HCs are smaller than those between HCs. Perfluorinated alkanes, ethers, and tertiary amines are practically insoluble in water and their miscibility with many organic solvents is limited. B. E. Smart in *Organofluorine Chemistry: Principles and Commercial Applications* (Eds.: R. E. Banks, B. E. Smart, J. C. Tatlow), Plenum, New York, **1994**; J. Hildebrand, D. R. F. Cochran, *J. Am. Chem. Soc.* **1949**, 71, 22; D. L. Dorset, *Macromolecules* **1990**, 23, 894.
- [13] T. Koyama, A. Wakisaka, *J. Chem. Soc. Faraday Trans.* **1997**, 93, 3813.
- [14] Precipitation of cocrystals **3a–c** and **5a** occurs from solutions that are quite far from saturation in the single components **1a–c** and **2a**. This observation further proves the ability of halogen bonding to overcome hydrogen bonding and other intermolecular interactions to drive the formation of a crystalline solid. In cocrystal **3a**, the aromatic rings stack parallel to each other, about 5 Å apart, forming distinct columns of perfluorinated and hydrocarbon compounds. This arrangement is probably imposed by van der Waals interactions involving iodine atoms and the 1,2-bis(4-pyridyl)ethane molecules. On the contrary, in cocrystal **5a**, the columnar structure is absent and the hydroquinone units are arranged in a herringbone way, where the aromatic rings are tilted by about 60° with respect to each other. Data were collected for both **3a** and **5a** using $\lambda_{\text{CuK}\alpha} = 1.54178$ Å, $\theta/2\theta$ scan mode ($3.5 \leq \theta \leq 70.0^\circ$, at 293(2) K and corrected for Lorentz, polarization and absorption; both structures were solved by direct methods using SIR97 and refined using SHELX97 against F^2 , with hydrogen atoms at calculated positions in the riding mode. Crystal data are as follows: cocrystal **3a**: $0.65 \times 0.50 \times 0.35$ mm³, triclinic, space group $P\bar{1}$, $a = 5.032(2)$, $b = 9.902(4)$, $c = 10.598(5)$ Å, $\alpha = 64.70(3)$, $\beta = 82.24(4)$, $\gamma = 87.94(4)^\circ$; $V = 472.9(5)$ Å³; $Z = 1$; $\rho_{\text{calc}} = 2.058$ g cm⁻³, $\mu = 26.5$ mm⁻¹; 2151 collected reflections, 1588 independent, 119 refined parameters, $R_1 = 0.067$ (for 1573 data with $I \geq 2\sigma(I)$). Cocrystal **5a**: $0.55 \times 0.40 \times 0.35$ mm³, triclinic, space group $P\bar{1}$, $a = 7.458(1)$, $b = 9.424(1)$, $c = 11.804(1)$ Å, $\alpha = 95.234(6)$, $\beta = 93.023(2)$, $\gamma = 108.338(8)^\circ$; $V = 781.3(1)$ Å³; $Z = 2$; $\rho_{\text{calc}} = 1.251$ g cm⁻³, $\mu = 0.66$ mm⁻¹; 5168 collected reflections, 2737 independent, 208 refined parameters $R_1 = 0.060$ (for 1604 data with $I \geq 2\sigma(I)$). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-137462 **3a** and -137463 for **5a**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [15] K. Maartmen-Moe, *Acta Crystallogr.* **1966**, 21, 979.
- [16] "Asymmetric Fluoroorganic Chemistry: Synthesis, Applications and Future Directions": M. T. Messina, P. Metrangolo, G. Resnati, *ACS Symp. Ser.* **1999**, 746.
- [17] R. Foster, *Organic Charge-Transfer Complexes*, Academic Press, London, **1969**, p. 100.
- [18] D. Gurka, R. W. Taft, *J. Am. Chem. Soc.* **1969**, 91, 4794.
- [19] D. W. Larsen, A. L. Allred, *J. Phys. Chem.* **1965**, 69, 2400.
- [20] S. V. Meille, P. Metrangolo, G. Raos, G. Resnati, G. Valerio, *J. Phys. Chem. A*, in press.
- [21] E. M. Arnett, L. Joris, E. Mitchell, T. S. S. R. Murty, T. M. Gorrie, P. Von R. Schleyer, *J. Am. Chem. Soc.* **1970**, 92, 2365.
- [22] R. Weiss, O. Schwab, F. Hampel, *Chem. Eur. J.* **1999**, 5, 968.
- [23] A. Ranganathan, V. R. Pediredi, *Tetrahedron Lett.* **1998**, 39, 1803; R. Weiss, M. Rechinger, F. Hampel, A. Wolski, *Angew. Chem.* **1995**, 107, 483; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 441; J. A. R. P. Sarma, F. H. Allen, V. J. Hoy, J. A. K. Howard, R. Thaimattam, K. Biradha, G. R. Desiraju, *Chem. Commun.* **1997**, 101.
- [24] J. P. Lorand, *Tetrahedron Lett.* **1971**, 2511.
- [25] K. Xu, M. Ho, R. A. Pascal, Jr., *J. Am. Chem. Soc.* **1994**, 116, 105.

- [26] L. K. Steinrauf, J. A. Hamilton, B. C. Braden, J. R. Murrel, M. D. Benson, *J. Biol. Chem.* **1993**, 268, 2425.
 [27] G. R. Desiraju, *Crystal Engineering: The Design of Organic Solids*, Elsevier, Amsterdam, **1989**.
 [28] J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, Weinheim, **1995**.

Spin-Density Map of the Triplet Ground State of a Titanium(IV) Complex with Schiff-Base Diquinone Radical Ligands: An Investigation Using Polarized-Neutron Diffraction and Density-Functional Theory**

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During the last decade, the design of new molecular ferromagnets has been a field of growing interest.^[1] Ferromagnetic interactions between organic radicals, although rare, are now recognized as real.^[2–5] The final goal of obtaining molecular-based ferromagnets may be achieved if, for example, the individual building blocks carrying unpaired electrons are arranged such that the magnetic orbitals remain orthogonal to each other.^[6] With this in mind, attempts have been made to use organic radicals bound to diamagnetic metal ions and some elegant examples of moderate ferromagnetic coupling have been reported.^[7] In particular, a titanium(IV) complex TiL_2 , where L is the dianion of the radical ligand 3,5-di-*tert*-butyl-1,2-semiquinonato-1-(2-hydroxy-3,5-di-*tert*-butyl-phenyl)imine, has been found to be characterized by a triplet ground state arising from a ferromagnetic interaction ($J = 56 \text{ cm}^{-1}$) between the two $\frac{1}{2}$ -spin coordinated radical ligands.^[8] This compound crystallizes in the triclinic $P\bar{1}$ space group. The metal ion is octahedrally coordinated by the two ligands, which occupy the meridional sites of the coordination polyhedron (Figure 1). The two ligands are orthogonal to each

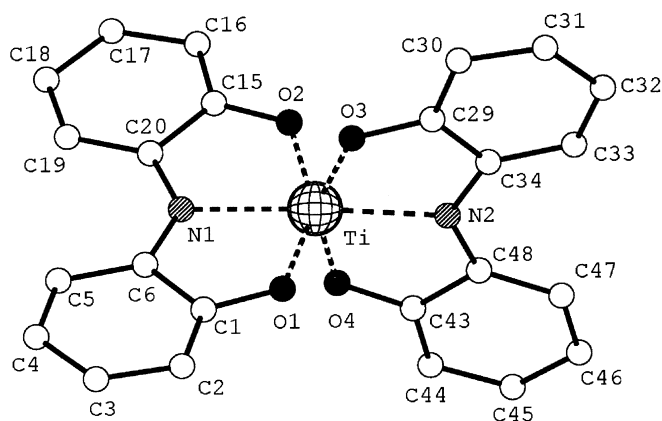


Figure 1. View of the TiL_2 molecule showing the numbering of the atoms. The *tert*-butyl groups are omitted for clarity.

other and the dihedral angles between the aromatic rings in the two ligands are 9.7° and 5.4° , respectively.

It has been proposed^[8] that the triplet ground state is stabilized by the topological degeneracy of the two magnetic orbitals of the ligands. Assuming an overall C_{2v} symmetry for the complex, the two π^* magnetic orbitals span the b_1 and b_2 irreducible representations and are, therefore, orthogonal to each other and this provides a rationalization of the observed ferromagnetic coupling.

The exchange interactions between the two radicals may arise from both direct overlap between the ligand magnetic orbitals (the shortest interligand oxygen–oxygen distances are about $2.7 \text{ \AA}$) and superexchange as mediated by metal orbitals.^[8, 9] In the former case, the unpaired spin density (SD) is expected to be located onto the ligands, whereas in the latter a sizeable SD must be observed also on the metal ion, which, according to the formal Ti^{4+} charge, has a d^0 configuration.

In this communication, we report the preliminary results concerning a polarized neutron diffraction (PND) study of the TiL_2 compound. The PND technique is well adapted for measuring the spin-density distribution in magnetic crystalline molecular compounds.^[10, 11] It is applicable to single crystals in which the spin density has been partly or totally aligned by an external magnetic field.

The PND experiment was performed on the polarized-neutron lifting-counter diffractometer 5c1 at the Orphee reactor (CEA-Saclay, France). A small TiL_2 crystal, $2 \times 2 \times 1 \text{ mm}^3$, was mounted with the a axis parallel to the 7 T applied magnetic field. The flipping ratios (F_N) of the more intense reflections were measured: $F_N = 39:1$ at $T = 1.5 \text{ K}$. By measuring the integrated nuclear intensities of the same set of reflections in a zero field,^[12] it was found that the room temperature X-ray crystal structure^[8] could be used to model and normalize the F_N and to determine their phase. The magnetic structure factors (F_M) were obtained through standard techniques.^[12]

There are different possible approaches to analyze the PND experimental data.^[11] A versatile method is based on the modeling of the spin-density distribution $s(r)$ followed by a direct expansion of the spin density around the nuclei. This

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method incorporates a superposition of aspherical densities, expressed at any point r as atomic contributions developed in series of real spherical harmonics [Eq. (1)], where l and n are the principal and magnetic quantum numbers, and $R_l(r)$ and $y_{lm}(\theta, \varphi)$ the radial and angular part respectively. Consequently, the spin distribution depends on parameters—the population coefficients P_{lm} and the Slater-type radial function of the spin density $R_l(r)$ —which are determined by the best fit of the experimental data.

$$s(r) = \sum_{\text{atoms}} \sum_l R_l(r) \sum_m P_{lm} y_{lm}(\theta, \varphi) \quad (1)$$

For the present case, since the number of the experimental F_M values are low due to the small size of the crystal, the following constraints were used: 1) the carbon atoms of the *tert*-butyl groups were not taken into account; 2) only spherical contributions were refined; and 3) the refinement was performed in two stages.^[12] For the refinement, first the carbon atoms of each phenyl ring were successively refined, together with nitrogen, oxygen, and titanium atoms. Only contributions from the C1, C6, C15, C20, C29, C34, C43, C48, N1, N2, and Ti atoms were found to be significant. Consequently, only contributions from these atoms were kept in the second refinement stage^[13]. Table 1 displays the resulting

Table 1. Atomic spin populations obtained by multipole refinement and standard deviations in parentheses, together with the results of DFT calculations.

Atoms	Experimental ^[a] (μ_B)	DFT Calculations (μ_B)	
		VWN-Stoll ^[b]	BLYP ^[c]
C1	0.24(4)	0.09	0.10
C6	0.12(6)	0.01	−0.01
C15	0.22(5)	0.11	0.12
C20	0.10(7)	0.01	−0.01
C29	0.24(6)	0.10	0.12
C34	−0.05(4)	0.00	−0.01
C43	0.20(5)	0.10	0.11
C48	0.04(4)	0.01	−0.01
N1	0.21(7)	0.25	0.27
N2	0.19(7)	0.23	0.26
Ti	0.17(3)	0.09	0.08
O1	−0.03(6)	0.06	0.06
O2	0.03(6)	0.07	0.07
O3	0.01(6)	0.06	0.06
O4	0.04(6)	0.07	0.07

[a] Refinement statistics: $wR(F) = 16.3\%$, $\chi^2 = 1.31$. [b] Local spin-density approximation. [c] Generalized gradient approximation.

experimental spin populations, together with the statistics of refinement and the oxygen spin populations obtained in the first stage. Figure 2 shows the projection of the spin density onto the mean plane of the two ligands.

The main features of the obtained spin-density distribution are the following: 1) no significant spin population is observed on the oxygen atoms; 2) strong, positive spin populations are found on each carbon atom corresponding to the four C–O bonds of the complex (C1, C15, C29, C43); 3) strong, positive spin populations are obtained on the N1 and N2 atoms with a small asymmetry favoring N1; and 4) a significant degree of the spin density is found on the titanium center.

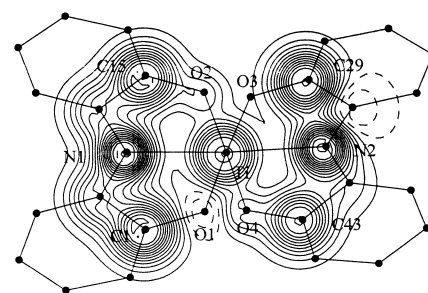


Figure 2. Spin-population density projected onto the mean plane generated by the coordinated ligands L. Contour steps are $0.008 \mu_B \text{ Å}^{-2}$; positive contours (—), negative contours (---).

Although the experimental results are less accurate than those generally obtained from the PND technique (due to the limitation to 39 reflections), the observed effects are significant. The experimental determination of SD in this complex provides primarily clear answers concerning questions of the pathway of ferromagnetic interactions. Titanium(IV) is a d^0 ion, so the spin population observed on that atom is due to a “transfer” from the ligands. Consequently, the metal ion is involved in the exchange pathway. The very low spin density on the oxygen atoms suggests that these atoms do not play any major role in determining interligand magnetic interactions. Therefore, an exchange pathway due to the direct overlap of the ligand magnetic orbitals can be ruled out.

These results are in agreement with the exchange mechanism suggested, on the basis of density functional theory (DFT) calculations, for the exchange coupling constants in the TiL_2 and SnL_2 complexes.^[9] Here, we have applied the same computational model to calculate the spin-density distribution on the TiL_2 complex. This calculation^[14] was performed on a model molecule in which the *tert*-butyl groups were replaced by hydrogen atoms based on the room temperature X-ray crystal structure. The same model molecule, under C_{2v} symmetry, was used in ref. [9]. The spin populations, computed using the Mulliken population analysis.^[15] Calculations were performed using the local spin-density approximation (VWN-Stoll)^[16] and the generalized gradient approximation (BLYP)^[17] for the exchange and correlation functionals. The results of the calculations are reported and compared with the experiment (Table 1). In agreement with the experimental results, a larger spin density is computed on the nitrogen atoms than on the oxygen atoms and a sizeable spin density, although smaller than the measured value, is found on the titanium atom. The sources of the spin density are 74% from the Ti 3d and 26% from the 3p orbitals. The values of the computed density are almost independent of the functional used.

A second series of PND experiments, utilizing a larger crystal, is planned in order to increase the precision of the results and to permit a description of the spin density beyond the spherical contributions. The latter point is of utmost importance in order to extract from the results which of the metal orbitals are involved in the exchange mechanism and to determine the geometries of the 2p orbitals in the C1–C6–N1–C20–C15 and C29–C34–N2–C48–C43 fragments.

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- [1] *Magnetic Molecular Materials* (Eds.: D. Gatteschi, O. Kahn, J. S. Miller, F. Palacio), Kluwer Academic Publishers, Dordrecht, **1991**.
- [2] A. Caneschi, F. Ferraro, D. Gatteschi, A. Le Lirzin, M. Novak, E. Rentschler, R. Sessoli, *Adv. Mater.* **1995**, *7*, 476.
- [3] M. Kinoshita, P. Turek, H. Tamura, K. Nozana, D. Shiomi, Y. Nakazama, M. Ishikawa, M. Takahashi, K. Awaga, T. Inabe, Y. Muruyama, *Chem. Lett.* **1991**, 1225.
- [4] H. Iwamura, K. Inoue, N. Koga, T. Hayamizu in *Magnetism: A Supramolecular Function* (Ed: O. Kahn), NATO ASI Series C484, Kluwer Academic Publishers, Dordrecht, **1996**, pp. 157–180.
- [5] J. Cirujeda, M. Mas, E. Molins, F. L. de Panthou, J. Laugier, J. G. Park, C. Paulsen, P. Rey, C. Rovira, J. Veciana, *J. Chem. Soc. Chem. Commun.* **1995**, 709.
- [6] a) O. Kahn, J. Larionova, L. Ouahab, *Chem. Commun.* **1999**, 945; b) J. S. Miller, A. J. Epstein, *Chem. Commun.* **1998**, 1319.
- [7] a) A. Cogne, A. Grand, P. Rey, R. Subra, *J. Am. Chem. Soc.* **1989**, *111*, 3230; b) D. M. Adam, A. L. Rheingold, A. Dei, D. N. Hendrickson, *Angew. Chem.* **1993**, *105*, 434; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 391; c) H. Oshio, M. Yamamoto, T. Ito, *J. Chem. Soc. Dalton Trans.* **1999**, 2641.
- [8] a) A. Caneschi, A. Dei, D. Gatteschi, *J. Chem. Soc. Chem. Commun.* **1992**, 630; b) S. Bruni, A. Caneschi, F. Cariati, C. Delfs, A. Dei, D. Gatteschi, *J. Am. Chem. Soc.* **1994**, *116*, 1388.
- [9] A. Bencini, I. Ciofini, E. Giannasi, C. A. Daul, K. Doclo, *Inorg. Chem.* **1998**, *37*, 3719.
- [10] a) Y. Pontillon, T. Akita, A. Grand, K. Kobayashi, E. Lelievre-Berna, J. Pecaut, E. Ressouche, J. Schweizer, *J. Am. Chem. Soc.* **1999**, *121*, 10126; b) Y. Pontillon, A. Caneschi, D. Gatteschi, A. Grand, E. Ressouche, R. Sessoli, J. Schweizer, *Chem. Eur. J.* **1999**, *12*, 3616; c) Y. Pontillon, E. Lelievre-Berna, A. Caneschi, D. Gatteschi, R. Sessoli, E. Ressouche, J. Schweizer, *J. Am. Chem. Soc.* **1999**, *121*, 5342; d) M. A. Aebersold, B. Gillon, O. Plantevin, L. Pardi, O. Kahn, P. Bergerat, I. von Seggern, F. Tuzek, L. Ohrstrom, A. Grand, E. Lelievre-Berna, *J. Am. Chem. Soc.* **1998**, *120*, 5238; e) E. Ressouche, J. X. Boucherle, B. Gillon, P. Rey, J. Schweizer, *J. Am. Chem. Soc.* **1993**, *115*, 3610.
- [11] For more details, see: B. Gillon, J. Schweizer in *The Interest in Polarized Neutron Diffraction. In Molecules in Physics, Chemistry, and Biology, Vol. II* (Ed: Jean Maruani), Kluwer Academic Publisher, Dordrecht, **1989**, p. 111.
- [12] The refinement was done using a modification of the least-squares refinement program MOLLY: N. K. Hansen, P. Coppens, *Acta Crystallogr. A* **1978**, *34*, 909.
- [13] A model-free spin density reconstruction (the Maximum of Entropy method; see ref. [10]) has also been performed. The corresponding map shows that the main part of the spin density is carried by the two C-C-N-C-C fragments and on the Ti atom, as obtained in the refinement.
- [14] *Amsterdam Density Functional (ADF) Scientific Computing and Modelling, Revision 02 1999*, Vrije Universiteit Amsterdam.
- [15] R. S. Mulliken, *J. Chem. Phys.* **1955**, *23*, 1833.
- [16] a) J. C. Slater, *Quantum Theory of Molecules and Solids. Vol. 4: Self-Consistent Field for Molecules and Solids*, McGraw-Hill, New York, **1974**; b) S. H. Vosko, L. Wilk, M. Nusair, *Can. J. Phys.* **1980**, *58*, 1200; c) H. Stoll, C. M. E. Pavlidou, H. Preuss, *Theor. Chim. Acta* **1978**, *49*, 143.
- [17] a) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785; b) A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098.

A Novel Solid-Phase Assembly for Identifying Potent and Selective RNA Ligands**

Nathan W. Luedtke and Yitzhak Tor*

Replication of the human immunodeficiency virus (HIV-1) requires an ordered pattern of viral gene expression.^[1] This process is dependent upon the association of Rev, an essential viral regulatory protein, with its respective RNA binding site, the Rev-response-element (RRE).^[2] The RRE is necessary for the active export of unspliced genomic viral RNA from the nucleus and serves as part of the envelope–protein coding sequence.^[2] This dual function makes the Rev–RRE binding event an attractive target for therapeutic intervention because the evolution of resistant variants may be prevented or impeded. Small molecules that specifically bind the RRE and preclude or competitively displace the Rev protein are therefore promising antiviral candidates.^[3–5]

Aminoglycoside antibiotics have been shown to competitively block the binding of the Rev protein to the RRE, thus providing an important precedent for the use of low molecular weight ligands to target viral RNA sites.^[3, 6] These natural antibiotics, however, bind the RRE with relatively low affinity and specificity.^[7] Since aminoglycosides and most other RNA ligands appear to recognize certain RNA folds, rather than specific RNA sequences, the design and discovery of RNA ligands is a challenging and empirical process.^[7b] Commonly employed RNA-binding assays are limited in their ability to probe both the affinity and specificity of potential binders.^[7a, 8, 9] New approaches that allow the rapid determination of both the RNA affinity and specificity of small molecules will assist in the discovery of new lead compounds and advance the understanding of RNA recognition. To this end, we report the assembly of an immobilized RNA–protein complex and demonstrate its application to the discovery and characterization of new RNA binders.

The high-affinity Rev binding site within the RRE is the purine-rich bulge shown in Figure 1.^[10] The arginine-rich segment, Rev_{34–50}, binds the RRE with a dissociation constant similar to that of the full-length Rev protein.^[11] We have developed an assay based on the competition between potential RNA binders and a fluorescent Rev peptide (“Rev-Fl”) for binding to an immobilized RRE fragment. The assay identifies small molecules that specifically interfere with Rev–RRE binding (Figure 1). Ligands that bind to the

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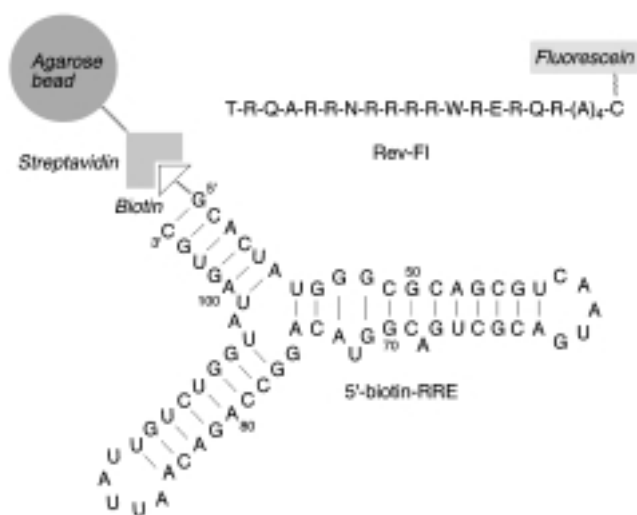


Figure 1. Schematic representation of the immobilized RNA-peptide assembly, including the proposed secondary structure of the biotinylated RRE and the fluorescently tagged arginine-rich RNA binding domain of the Rev protein ("Rev-Fl").

RRE will effectively compete with Rev-Fl, thus releasing the peptide into solution. The amount of fluorescent peptide in solution or remaining on the solid support is then quantified. The assay's stringency level can be tuned by adding nucleic acids, proteins, or any other competing species. Nonselective ligands will be scavenged by competitor molecules present in solution. Only when a highly selective RRE binder is present will a positive fluorescence signal be elicited in solution. The global selectivity of the inhibitor can then be assessed based upon the identities of the competing species.

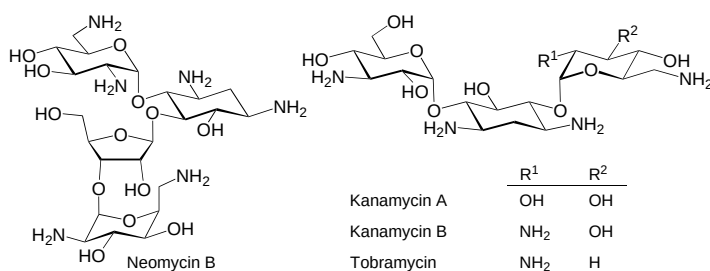
The components of the assembly are shown schematically in Figure 1 and include: a) insoluble agarose beads covalently modified with streptavidin, b) a biotinylated RRE fragment, and c) a fluorescein-labeled Rev fragment.^[12, 13] Assembly of these three components forms an immobilized ternary complex, in which the biotinylated RRE binds to streptavidin, and the fluorescein-tagged Rev specifically binds to the RRE.^[14] The Rev-Fl-RRE dissociation constant ($K_d = 2.5$ nM) has been found to be the same in solution as on the solid support.^[15]

To demonstrate the effectiveness and dependability of the solid-phase assay, aminoglycoside antibiotics, a thoroughly investigated family of RRE binders, have been examined.^[7b] Table 1 summarizes the IC_{50} values obtained by the solid-phase assay in comparison with those obtained by fluorescence anisotropy measurements.^[16] An excellent correlation is observed between the two methods for the aminoglycoside family. The assay faithfully reproduces the known trend in RRE affinity of these natural products (neomycin B > tobramycin > kanamycin B > kanamycin A).^[3, 7a] Repeating the experiments in the presence of nucleic acid competitors confirmed the selectivity typically exhibited by aminoglycosides.^[7b] A low affinity to double-stranded DNA is evident from essentially identical IC_{50} values in the presence and absence of plasmid DNA, and promiscuous RNA binding is manifested as higher IC_{50} values in the presence of transfer RNAs.^[17]

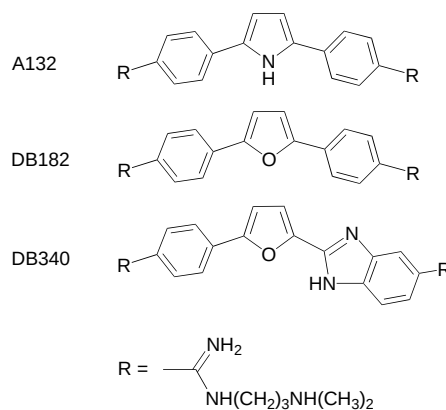
Table 1. IC_{50} values [μ M] as determined by fluorescence anisotropy and the novel solid-phase assembly.^[a]

Compound	Anisotropy	Solid phase	Solid phase + DNA	Solid phase + tRNA
neomycin B	7	7	8	20
tobramycin	47	45	50	100
kanamycin B	80	90	90	170
kanamycin A	780	750	750	1200
DB340	0.3	0.1	1.3	0.7
DB182	1.5	0.4	4	0.8
A132	10 ^[b]	1.2	10	3
Δ -[Ru(bpy) ₂ (eilat)] ²⁺	1.0	2.0	10	2.0
Δ -[Ru(bpy) ₂ (eilat)] ²⁺	1.0	2.0	50	2.0
[Ru(bpy) ₃] ²⁺	170 ^[b]	> 10 000	n.d. ^[c]	n.d. ^[c]

[a] See Supporting Information for experimental details and conditions. The standard deviations of all results are within $\pm 25\%$ of the reported value. [b] Significant fluorescence quenching occurred during the course of titration. See ref. [20]. [c] n.d. = not determined.



To examine the versatility of the assembly-based assay the competitive binding of polycyclic aromatic amidines to the RRE has also been examined.^[18] This family of ligands is more potent than aminoglycosides (see Table 1). Their previously reported trend in RRE affinity is again accurately reproduced

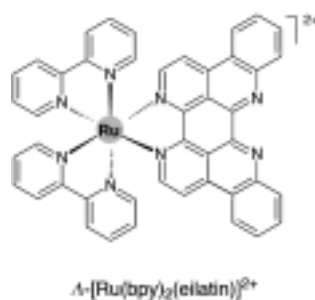
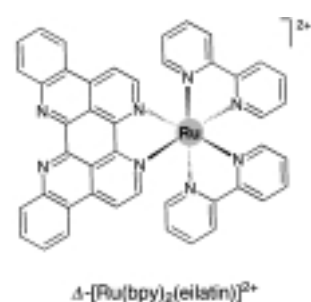


(DB340 > DB182 > A132).^[18] A tenfold increase in IC_{50} values in the presence of competing DNA indicates that these compounds also have a relatively high affinity for double-stranded DNA.^[17] This result clearly illustrates how the observed potency of an inhibitor is influenced by its selectivity.

Discrepancies between IC_{50} values determined from the solid-phase assay and the values obtained by fluorescence anisotropy measurements reflect a general problem associated with applying fluorescence anisotropy to binders that are either fluorescent or fluorescence quenchers (Table 1).^[19] The

solid-phase assay easily adapts to this problem: if an inhibitor interferes with Rev-Fl emission, the supernatant (containing excess inhibitor and displaced Rev-Fl) is simply washed away, and the fluorescence remaining on the solid support is quantified.^[13] Gel mobility shift assays confirm that, for these inhibitors, IC₅₀ values obtained from the solid-phase assembly are more accurate than the IC₅₀ values derived from fluorescence anisotropy measurements.^[13, 20]

The novel solid-support assembly has also been used to identify and characterize new Rev-RRE inhibitors. The enantiomeric, octahedral metal complexes Δ - and Λ -[Ru(bpy)₂(eilatrin)]²⁺ are potent inhibitors of the Rev-RRE complex and exhibit intriguing selectivities.^[21] The fluorescence anisotropy and the solid-phase assays indicate that both enantiomers have the same affinity for the RRE (Table 1). In the presence of DNA, however, the Λ enantiomer loses its



potency by a factor of 25, while that of the Δ enantiomer is reduced only by a factor of 5. This result suggests that the Λ enantiomer has a significantly higher affinity for double-stranded DNA than the Δ enantiomer. The potency of neither is affected by the presence of tRNA. This observation illustrates that these metal complexes have the opposite nucleic acid selectivity as compared to the aminoglycoside antibiotics family.

Both selectivity and affinity are crucial for the design and evaluation of new RNA-protein inhibitors.^[5, 7b] Assembly of the immobilized Rev-RRE complex has generated a

simple, rapid, and quantitative assay that facilitates the discovery and characterization of new RNA binders. Since competitive binding studies can be conducted in complex mixtures of biomolecules, this assembly provides a powerful tool for the characterization of the inhibitor's affinity and selectivity. Critical evaluation of the assay has shown that the expected inhibitory activities of diverse and potentially problematic families of molecules are consistently and faithfully reproduced. This prototypical approach is amenable to automation and high-throughput screening and is applicable to other RNA-protein complexes.

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- [1] Y. N. Vaishnav, F. Wong-Staal, *Annu. Rev. Biochem.* **1991**, 60, 577–630.
- [2] A. D. Frankel, J. A. T. Young, *Annu. Rev. Biochem.* **1998**, 67, 1–25; V. W. Pollard, M. H. Malim, *Annu. Rev. Microbiol.* **1998**, 52, 491–532; T. J. Hope, *Arch. Biochem. Biophys.* **1999**, 365, 186–191.
- [3] M. L. Zapp, S. Stern, M. R. Green, *Cell* **1993**, 74, 969–978.
- [4] S. R. Kirk, N. W. Luedtke, Y. Tor, *J. Am. Chem. Soc.* **2000**, 122, 980–981.

- [5] Y. Tor, *Angew. Chem.* **1999**, 111, 1681–1685; *Angew. Chem. Int. Ed.* **1999**, 38, 1579–1582.
- [6] For other modulators of the Rev-RRE interaction, see W. K. C. Park, M. Auer, H. Jaksche, C.-H. Wong, *J. Am. Chem. Soc.* **1996**, 118, 10150–10155; M. L. Zapp, D. W. Young, A. Kumar, R. Singh, D. W. Boykin, W. D. Wilson, M. R. Green, *Bioorg. Med. Chem.* **1997**, 5, 1149–1155.
- [7] a) M. Hendrix, E. S. Priestley, G. F. Joyce, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, 119, 3641–3648; b) K. Michael, Y. Tor, *Chem. Eur. J.* **1998**, 4, 2091–2098.
- [8] Many biophysical techniques, including fluorescence anisotropy and surface plasmon resonance, are not amenable to the unambiguous analysis of complex mixtures, which makes the evaluation of selectivity an arduous process.^[7a]
- [9] See also: R. H. Griffey, M. J. Greig, H. An, H. Sasmor, S. Manalili, *J. Am. Chem. Soc.* **1999**, 121, 474–475; K. Hamasaki, R. R. Rando, *Anal. Biochem.* **1998**, 261, 183–190.
- [10] S. M. Holland, M. Chavez, S. Gerstberger, S. Venkatesan, *J. Virol.* **1992**, 66, 3699–3706; L. S. Tilley, M. H. Malim, H. K. Tewary, P. G. Stockley, B. R. Cullen, *Proc. Natl. Acad. Sci. USA* **1992**, 89, 758–762.
- [11] J. Kjems, B. J. Canlan, A. D. Frankel, P. A. Sharp, *EMBO J.* **1992**, 11, 1119–1129.
- [12] Modified Rev_{34–50} peptides of the sequence ³⁴_{suc}TRQARRN-RRRRWRERQR⁵⁰AAAAC_{am} have been employed for all experiments. The conjugation of fluorescein to the cysteine residue results in “Rev-Fl”, which has approximately the same affinity to the RRE as the unlabeled Rev peptide ($K_d = 1.2$ nM).^[13]
- [13] See Supporting Information for experimental details.
- [14] Neither Rev-Fl nor the RRE bind to the streptavidin-agarose assembly nonspecifically.^[13] Following assembly of the immobilized Rev-RRE complex, addition of excess nucleic acid does not significantly displace Rev-Fl off of the immobilized RRE.^[17] In contrast, very low concentrations of the unlabeled Rev peptide or non-biotinylated RRE (40 and 90 nM, respectively) are needed to displace 50% of Rev-Fl. These results taken together suggest a high degree of binding specificity for the immobilized Rev-RRE complex.
- [15] Fluorescence anisotropy was used to determine a K_d of 2.3 ± 0.5 nM for Rev-Fl-RRE in solution. A K_d of 2.5 ± 2 nM is measured for the solid-phase-immobilized complex.^[13] This K_d value is found to be independent of the “effective loading” of the RRE on the agarose (from 0.1–2.0 pmol mL⁻¹ gel), which suggests that the immobilized RRE complexes are noninteracting over this loading range.
- [16] Since the binding stoichiometry of these compounds is unknown, their IC₅₀ values are reported.
- [17] Experiments that include competing nucleic acids contain 15 μg mL⁻¹ of plasmid DNA (pGEM) or 65 μg mL⁻¹ of a complex mixture of yeast pre-tRNAs and mature tRNAs (Sigma type X). This is approximately a 50-fold molar excess of DNA nucleotides and a 200-fold molar excess of a tRNA nucleotides (relative to the RRE). These concentrations lead to a minimal (5–10%) displacement of the Rev-Fl from the solid support.
- [18] K. Li, G. Xiao, T. Rigl, A. Kumar, D. W. Boykin, W. D. Wilson, *Structure, Motion, Interaction and Expression of Biological Macromolecules* (Eds.: R. H. Sarma, M. H. Sarma), Adenine Press, **1998**, pp. 137–145.
- [19] For a review on fluorescence anisotropy and its limitations, see R. F. Steiner, *Topics in Fluorescence Spectroscopy, Vol. 2: Principles* (Ed.: J. R. Lakowicz), Plenum, New York, **1991**, pp. 1–52; see also: A. J. Pope, U. M. Haupts, K. J. Moore, *Drug Discovery Today* **1999**, 4, 350–362.
- [20] In accordance with the solid phase assay results, gel-shift experiments indicate that all “polycyclic aromatic derivatives” were more effective than neomycin B.^[13] [Ru(bpy)₃]²⁺ was also tested by the solid-phase assay, fluorescence anisotropy, and gel shift mobility. Due to fluorescence quenching of Rev-Fl by [Ru(bpy)₃]²⁺, fluorescence anisotropy titrations give a false dissociation profile and an IC₅₀ value of 170 μM. Both the solid-phase assay and gel shift mobility experiments, however, indicate that [Ru(bpy)₃]²⁺ does not inhibit the Rev-RRE complex at all (up to 10 mM).^[13]
- [21] For the synthesis and photophysical properties of these complexes, see A. Rudi, Y. Kashman, Y. D. Gut, F. Lellouche, M. Kol, *Chem. Commun.* **1997**, 17–18.

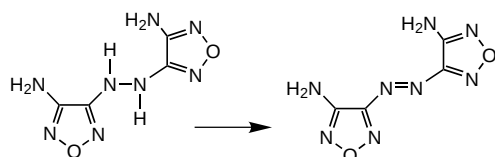
3,3'-Azobis(6-amino-1,2,4,5-tetrazine): A Novel High-Nitrogen Energetic Material**

David E. Chavez, Michael A. Hiskey,* and
Richard D. Gilardi

The synthesis of compounds known as high-nitrogen energetic materials has been the focus of our group for the past decade. High-nitrogen compounds form a unique class of energetic materials deriving most of their energy from their very high positive heats of formation rather than from oxidation of the carbon backbone, as with traditional energetic materials.^[1] The high nitrogen content typically leads to high densities, and the low amount of hydrogen and carbon also allows for a good oxygen balance to be achieved more easily; oxygen balance is a measure of the oxygen/fuel ratio in a compound. We have demonstrated that high-nitrogen materials can show remarkable insensitivity to electrostatic discharge, friction, and impact.

One high-nitrogen system extensively studied by our group is the 1,2,4,5-tetrazine ring. We have synthesized several energetic 1,2,4,5-tetrazines having applications as propellants, explosives, and pyrotechnic ingredients.^[2] In the pursuit of new high-nitrogen materials with novel properties, we have recently been studying azo-1,2,4,5-tetrazines.

We became interested in the synthesis of azo-1,2,4,5-tetrazines from our previous studies on azo-1,2,5-oxadiazoles. We found that 327 kJ mol⁻¹ of energy is gained in the transformation of 4,4'-hydrazobis(1,2,5-oxadiazol-3-amine) to 4,4'-azobis(1,2,5-oxadiazol-3-amine) (Scheme 1). The latter material is a thermally stable, insensitive explosive.^[3] Extrapolating from these data, 3,3'-azobis(6-amino-1,2,4,5-tetrazine)



$$\Delta H_f = 209 \text{ kJ mol}^{-1}$$

$$\Delta H_f = 536 \text{ kJ mol}^{-1}$$

Scheme 1. Energy gained in the transformation of a hydrazo group to an azo group in the case of amino-1,2,5-oxadiazoles.

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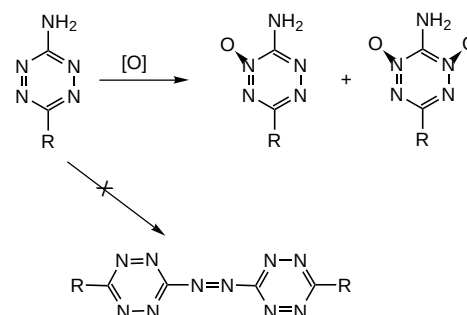
[**] This work was supported at Los Alamos by the joint program of the Department of Defense and the Department of Energy for the preparation and characterization of new energetic materials, and at the Naval Research Laboratory by the Office of Naval Research, Mechanics Division.



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(1; see Scheme 3) would give an even higher heat of formation due to the intrinsically large heat of formation of the 1,2,4,5-tetrazine ring. Tetrazine rings linked by an azo group are practically nonexistent. The only synthesis of azo-1,2,4,5-tetrazines in the literature was reported by Russian scientists in 1971 and 1990.^[4] Although they describe the preparation of 3,3'-azobis(6-phenyl-1,2,4,5-tetrazine) and 3,3'-azobis[6-(4-chlorophenyl)-1,2,4,5-tetrazine], no physical properties or proof of structure were given for the compounds.

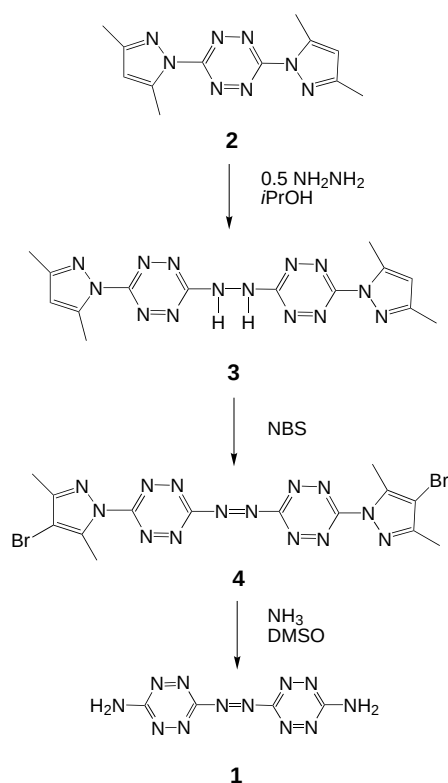
We have previously shown that oxidation of 3-amino-1,2,4,5-tetrazines leads to the formation of *N*-oxides in which the oxide moiety is α to the amino group (Scheme 2).^[2b,c] In the case of 3,6-diamino-1,2,4,5-tetrazine ($R = \text{NH}_2$), *N*-oxide groups are formed at the 1- and 4-positions, whereas with



Scheme 2. Oxidation of 3-amino-1,2,4,5-tetrazines. $R = \text{NH}_2$, H; see text for details.

3-amino-1,2,4,5-tetrazines ($R = \text{H}$), *N*-oxidation occurs at the two ring nitrogen atoms α to the amino group. Stronger oxidizers (hypofluorous acid, peroxytrifluoroacetic acid) can also oxidize an amino group to a nitro group when 3,6-diamino-1,2,4,5-tetrazine is the substrate. Under no circumstances have we observed the formation of an azo or azoxy linkage. Thus the formation of the azo group must be accomplished by a different synthetic approach. Here we describe our synthesis of **1**, as well as some of the properties of this novel high-nitrogen material.

The preparation of **1** is shown in Scheme 3. The 3,5-dimethylpyrazol-1-yl moieties of 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine (**2**) have been shown to be good leaving groups in nucleophilic displacements on 1,2,4,5-tetrazines.^[5] With readily available **2** as a starting material,^[5a] the hydrazo compound **3** was prepared by treatment of **2** with 0.5 equiv of hydrazine. A variety of oxidizing reagents typically used to oxidize a hydrazo group to an azo moiety (Br_2 , NO_2 , MnO_2 , HgO , HONO) did not lead to the formation of the azo group. Oxidation was only achieved with *N*-bromosuccinimide (NBS), which also brominated the 3,5-dimethylpyrazol-1-yl rings to give the azo compound **4**. The formation of the azo group was confirmed by the absence of signals and stretches for NH in the ¹H NMR and IR spectra as well as elemental analysis. Treatment of **4** with ammonia in acetonitrile yielded a precipitate, which upon analysis showed that complete displacement of the 4-bromo-3,5-dimethylpyrazol-1-yl groups did not occur. However, when the reaction was conducted in dimethyl sulfoxide (DMSO) followed by treatment of the



Scheme 3. Synthesis of **1**.

reaction mixture with 2-propanol, a red-brown precipitate was isolated.

According to NMR spectroscopy this material was the bis-DMSO solvate of **1**; however, elemental analysis was inconsistent with the proposed structure, as is typical of compounds high in nitrogen. An X-ray crystal structure analysis^[6] of this material confirmed the structure to be the bis-DMSO solvate of **1** (Figure 1), thus providing the first evidence for the synthesis of an azo-1,2,4,5-tetrazine. A density of 1.526 g cm^{-3} was determined from the X-ray crystal structure. The molecules are in an *E* configuration and form planar sheets despite the presence of DMSO molecules in the crystal. We believed that this graphite-like structure would lead to a high

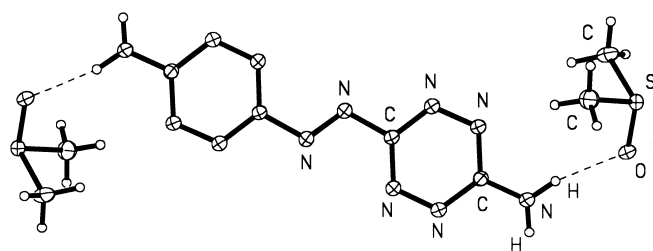


Figure 1. A drawing of one formula unit of the bis(DMSO) solvate of **1** (half of this is the asymmetric unit of the crystal, that is, the labeled atoms). There are strong linear hydrogen bonds linking every amino hydrogen atom with a neighboring DMSO; two are shown as dashed lines. Each DMSO oxygen atom also accepts two hydrogen bonds (not shown), thus linking molecules of **1** into long planar ribbons. Side-by-side ribbons are parallel and coplanar, so they form extended planar (average deviation 0.08 Å) sheets. The perpendicular distance between adjacent sheets is only 3.22 Å , which is even shorter than the interplanar spacing in graphite (3.4 Å).

density of the neat material. Indeed, a gas pycnometry density of 1.84 g cm^{-3} was determined for pure **1**, which is likely to be the most dense C,H,N molecule known.^[7]

The DMSO solvate was easily broken by treatment with boiling water to give pure **1**. The pure material is thermally stable to 252°C (differential scanning calorimetry), and the heat of formation was measured to be $+862\text{ kJ mol}^{-1}$ by combustion calorimetry. This is a very high heat of formation and when normalized, a value of 43.1 kJ per atom is realized. Some sensitivity properties include a drop weight impact value of 70 cm (the value for the popular high-explosive HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) is 25 cm), despite the fact that there are no oxygen atoms in the molecule. The compound is insensitive to initiation by spark ($>0.36\text{ J}$) or friction (BAM, $>36\text{ kg}$).^[8] We are currently evaluating the oxidation of **1** with oxygen transfer reagents.^[9]

Experimental Section

3: To **2** (270.0 g , 1.0 mol) in 2-propanol (2 L) in a mechanically stirred 5-L, 3-necked flask was added anhydrous hydrazine (16.0 g , 0.5 mol) all at once with efficient stirring. The mixture was refluxed for 2 h and allowed to cool and stir overnight. The orange product was filtered and washed with 2-propanol to give 143.0 g of pure **3**. The mother liquor was allowed to stand for a few weeks and refiltered to provide an additional 7.3 g of product identical to the first crop (total yield 79%); m.p. $215\text{--}217^\circ\text{C}$. IR (KBr): $\tilde{\nu} = 3194, 1575, 1490, 1415\text{ cm}^{-1}$; $^1\text{H NMR}$ (270 MHz , $[\text{D}_6]\text{DMSO}$, 25°C , TMS): $\delta = 2.21\text{ (s, 6H)}$, 2.49 (s, 6H) , 6.22 (s, 2H) , 11.2 (brs, 2H) ; $^{13}\text{C NMR}$ (270 MHz , $[\text{D}_6]\text{DMSO}$, 25°C , TMS): $\delta = 12.3, 13.0, 109.5, 142.3, 151.3, 158.9, 162.7$; elemental analysis calcd for $\text{C}_{14}\text{H}_{16}\text{N}_{14}$: C 44.21 , H 4.24 , N 51.55 ; found: C 43.95 , H 4.40 , N 51.39 .

4: *N*-Bromosuccinimide (267.4 g , 1.5 mol) was dissolved in acetonitrile (2 L) in a 5-L, 3-necked flask equipped with a mechanical stirrer. Compound **3** (143.0 g , 0.38 mol) was added portionwise over 15 min , and the mixture was allowed to stir for 1 h . The purple precipitated product was filtered and washed with *tert*-butyl methyl ether and air-dried to yield 197.0 g of **4** (98%); m.p. $195\text{--}197^\circ\text{C}$. IR (KBr): $\tilde{\nu} = 1501, 1450\text{ cm}^{-1}$; $^1\text{H NMR}$ (270 MHz , CDCl_3 , 25°C , TMS): $\delta = 2.44\text{ (s, 6H)}$, 2.84 (s, 6H) ; $^{13}\text{C NMR}$ (270 MHz , CDCl_3 , 25°C , TMS): $\delta = 13.0, 14.4, 104.4, 142.2, 155.3, 159.3, 167.9$; elemental analysis calcd for $\text{C}_{14}\text{H}_{12}\text{N}_{14}\text{Br}_2$: C 31.36 , H 2.26 , N 36.57 ; found: C 30.99 , H 2.21 , N 36.51 .

1: Ammonia was bubbled rapidly through DMSO (1 L) in a 4-L beaker until 7.0 g (0.41 mol) were absorbed. To this was added **4** (110.4 g , 0.206 mol) with stirring within 1 min . The reaction mixture was allowed to stir for 15 min and then diluted with 2-propanol (1 L). The red solid was filtered, washed with 2-propanol, and air-dried to yield 34.3 g of **1**·2DMSO (44%). This material was boiled in water (250 mL) for a few minutes to break the DMSO solvate, filtered, washed with water, and air-dried to give pure **1**; m.p. 252°C (decomp.). IR (KBr): $\tilde{\nu} = 3372, 3283, 3194, 1629, 1506\text{ cm}^{-1}$; $^1\text{H NMR}$ (270 MHz , $[\text{D}_7]\text{DMF}$, 25°C , TMS): $\delta = 8.93\text{ (brs, 4H)}$; $^{13}\text{C NMR}$ (270 MHz , $[\text{D}_7]\text{DMF}$, 25°C , TMS): $\delta = 163.6, 167.9$; elemental analysis calcd for $\text{C}_4\text{H}_4\text{N}_{12}$: C 21.82 , H 1.83 , N 76.35 ; found: C 21.46 , H 2.11 , N 67.70 .

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- [1] M.-X. Zhang, P. E. Eaton, R. D. Gilardi, *Angew. Chem.* **2000**, *112*, 422–426; *Angew. Chem. Int. Ed.* **2000**, *39*, 401–404.
- [2] a) D. E. Chavez, M. A. Hiskey, *J. Pyrotech.* **1998**, *7*, 11; b) D. E. Chavez, M. A. Hiskey, *J. Energ. Mater.* **1999**, *17*, 357; c) M. D. Coburn, M. A. Hiskey, K.-Y. Lee, D. G. Ott, M. M. Stinecipher, *J. Heterocycl. Chem.* **1993**, *30*, 1593.
- [3] D. E. Chavez, L. Hill, M. A. Hiskey, S. A. Kinkad, *J. Energ. Mater.*, in press.

- [4] a) V. A. Ershov, I. Y. Postovskii, *Khim. Geterosikl. Soedin.* **1971**, 571; *Chem. Heterocycl. Compd. (Engl. Transl.)* **1971**, 537; b) E. G. Kovalev, V. A. Anufriev, G. L. Rusinov, *Khim. Geterosikl. Soedin.* **1990**, 1691; *Chem. Heterocycl. Compd. (Engl. Transl.)* **1990**, 1408.
- [5] a) M. D. Coburn, G. A. Buntain, B. W. Harris, M. A. Hiskey, K.-Y. Lee, D. G. Ott, *J. Heterocycl. Chem.* **1991**, 28, 2049; b) C. Glidewell, P. Lightfoot, B. J. L. Royles, D. M. Smith, *J. Chem. Soc. Perkin Trans. 2* **1997**, 1167.
- [6] Crystal data for **4** ($\text{C}_8\text{H}_{16}\text{N}_{12}\text{O}_2\text{S}_2$): $M_r = 376.45$, triclinic, space group $P\bar{1}$; $a = 5.3757(4)$, $b = 9.197(1)$, $c = 9.3683(8)$ Å, $\alpha = 67.564(7)$, $\beta = 75.634(7)$, $\gamma = 76.442(7)^\circ$, $V = 409.65(6)$ Å³, $Z = 1$, $\rho_{\text{calcd}} = 1.526$ mg mm⁻³; 1392 data measured to $2\theta_{\text{max}} = 115^\circ$; $R = 0.0279$, $wR2 = 0.0745$ for all 1106 unique reflections. X-ray intensity data were measured on a Bruker diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.54178$ Å) at $T = 294$ K. The structure were solved with the aid of program XS, and refined with full-matrix least-squares program XL, from SHELXTL software (SHELXTL96 G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, 46, 467). The XL program minimizes F^2 differences, and uses all data; that is, weak data are not considered "unobserved", and are included in the R factors reported. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-138299. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [7] The highest density C,H,N compound reported in the Cambridge Crystallographic Data Centre data base is 5,5'-bi-1H-tetrazole (density 1.738 g cm⁻³): P. J. Steel, *J. Chem. Crystallogr.* **1996**, 26, 399.
- [8] For an explanation of methods for characterizing explosive sensitivity, see R. T. Paine, W. Koestle, T. T. Borek, G. L. Wood, E. A. Pruss, E. N. Duesler, M. A. Hiskey, *Inorg. Chem.* **1999**, 38, 3738.
- [9] S. Rozen, S. Dayan, *Angew. Chem.* **1999**, 111, 3680–3682; *Angew. Chem. Int. Ed.* **1999**, 38, 3471–3473.

Synthesis of Prussian Blue Nanoparticles and Nanocrystal Superlattices in Reverse Microemulsions**

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Among known molecular magnets, Prussian blue (*catena*-[$\text{MFe}^{\text{III}}\{\text{Fe}^{\text{II}}(\text{CN})_6\}$] ($\text{M} = \text{Li}, \text{Na}, \text{K}, \text{NH}_4$)) and related cyanometallate-based coordination polymers offer a range of compounds with unique versatility. The variety of structures and magnetic properties of this family of compounds has been extensively investigated,^[1] and recently reviewed.^[2] Although much work has focused on the relationship between the unit-cell structure and magnetic properties, relatively few attempts have been made towards understanding and controlling the growth mechanism of these magnetic coordination polymers.

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This is an important aspect in the study of molecular magnets because compounds with appropriate magnetic properties require further fabrication and processing if functional devices and materials are to be produced.

Recently, organic supramolecular templates and organized reaction media have been used for the construction of higher-order assembly of traditional inorganic solids, such as silica,^[3] calcium carbonate,^[4] and iron oxides.^[5] These processes allow the control of properties such as particle size, particle shape, surface texture, and organization to be integrated directly into the synthesis method. It seems feasible that similar strategies could also be developed for the coupled synthesis and construction of both molecular materials and coordination polymers to produce complex functional materials that are organized beyond the length scale of the unit cell. As a first step towards this objective, we address the possibility of coupling the synthesis of Prussian blue crystals with its emergent properties such as size, shape, and higher-order assembly.

Periodically banded arrangements (Liesegang bands) have been produced previously for the precipitation of Prussian blue on perfluorinated membranes.^[6] Similarly, a dissipative structure has been briefly reported.^[7] More recently, the growth of Prussian blue under Langmuir monolayers^[8] and in the interlayer spaces of lamellar vesicles has been described.^[9] These studies have focused on the spatial confinement of Prussian blue rather than the colloidal and mesoscale properties of the constituent crystals. Herein, we show that by confining the synthesis to nanoscale water droplets formed in reverse microemulsions prepared from the anionic surfactant sodium bis(2-ethylhexyl)sulfosuccinate (AOT), hydrophobic Prussian blue nanoparticles with a uniform shape and size can be routinely prepared. A related material, $[\text{Cu}_2\{\text{Fe}(\text{CN})_6\}]$, has been recently synthesized in microemulsion media, although the resulting nanoparticles were highly disorganized.^[10] In our experiments, the growth of the nanoparticles within the restricted reaction field is controlled by a multistep process involving the slow photoreduction of $[\text{Fe}(\text{C}_2\text{O}_4)_3]^{3-}$ to produce Fe^{II} ions that subsequently react with $[\text{Fe}(\text{CN})_6]^{3-}$ ions to generate nuclei and clusters of Prussian blue encapsulated within the water droplets. Growth of the molecular magnet occurs by further exchange and fusion between microemulsion droplets to produce nanoparticles encapsulated in a shell of surfactant molecules. Moreover, the highly hydrophobic surface properties of the Prussian blue nanoparticles enable the facile preparation of self assembled nanoparticle arrays with 2-D and 3-D superlattice ordering.

AOT reverse microemulsions containing 0.3 M ammonium iron(III) oxalate and 0.3 M ammonium ferricyanide aqueous nanodroplets at $w = 5-20$ ($w = [\text{H}_2\text{O}]/[\text{AOT}]$) were transparent yellow fluids that were stable in the dark at room temperature. Exposure of the microemulsions to daylight transformed them to a transparent blue solution in two days and no precipitate was observed for two weeks. UV/Vis absorption spectra showed a broad band at 680 nm consistent with the Prussian blue $\{\text{Fe}^{\text{III}}[(t_2g)^3(e_g)^2]\text{Fe}^{\text{II}}[(t_2g)^6]\} \rightarrow \{\text{Fe}^{\text{II}}[(t_2g)^4(e_g)^2]\text{Fe}^{\text{III}}[(t_2g)^5]\}$ electronic transition.^[11] FT-IR spectra contained a major band at 2069 cm⁻¹ that corresponds to the Fe–CN stretching mode in the cyanometallate lattice.

Transmission electron microscopy (TEM) images of samples taken from the blue microemulsions ($w = 15$) after four days showed the presence of cubic Prussian blue nanoparticles with a uniform shape and size that self assembled into 2-D (or 3-D) highly ordered square (cubic) superlattices (Figure 1 a). The crystals have a characteristic length of 16 nm on average,

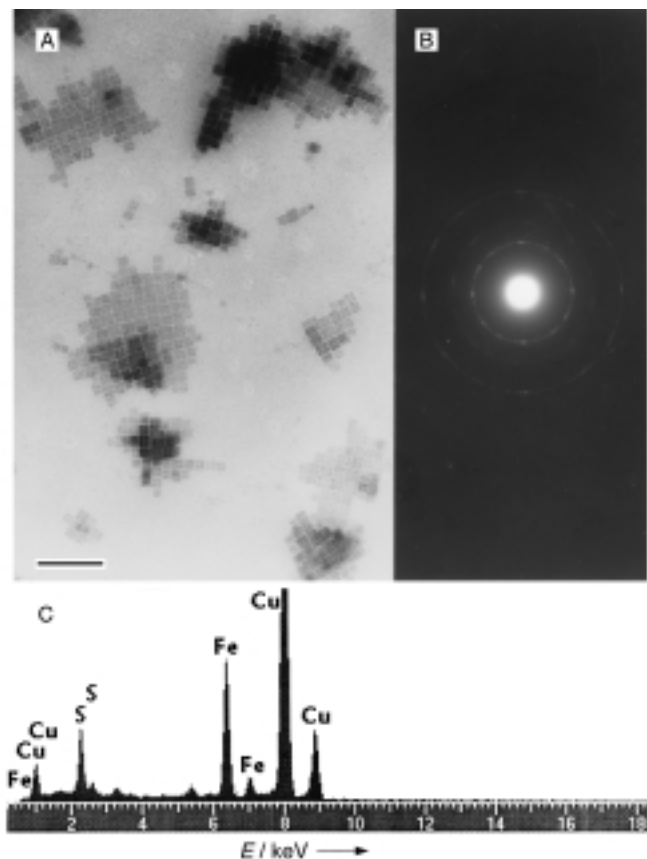


Figure 1. A) TEM image of cubic Prussian blue nanoparticles formed in AOT microemulsions at $w = 15$ showing self assembled 2-D and 3-D superlattices (scale bar 100 nm). B) Electron-diffraction pattern from the superlattice structures showing rings corresponding to the Prussian blue cubic structure; unit cell parameters $a = 1.013$ nm. C) EDXA spectrum of the superlattice structure showing the presence of iron and sulfur, corresponding to Prussian blue crystals and AOT surfactant, respectively. Copper peaks are due to the sample grid.

a narrow particle size distribution σ of 2.7 nm (Figure 2 a), and a regular cubic morphology consisting of smooth, well defined faces. Although the nanoparticles were, to some extent, sensitive to the electron beam, electron-diffraction patterns showed d spacings of 2.3, 2.5, 3.6, and 5.1 Å (Figure 1 b), which is consistent with a well ordered Prussian blue crystal structure.^[12]

Typically, the superlattices were organized in domains up to 200 nm in length (60–100 nanoparticles) while the individual nanoparticles were separated by a regular 2 to 3 nm spacing, which is consistent with an interdigitated bilayer of AOT molecules.^[13] The energy-dispersive X-ray microanalysis (EDXA) results show the presence of iron and sulfur (6.4 and 2.3 keV, respectively; Figure 1 c), to confirm the presence

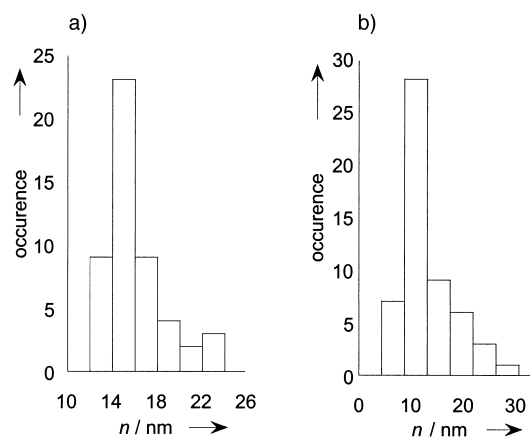


Figure 2. Particle size distributions for Prussian blue nanoparticles formed in AOT microemulsions ($w = 15$, 0.3 M reactants). a) After four days; b) after two days.

of AOT in the Prussian blue nanoparticle array. The self-assembly of the superlattice is therefore a facile process in which the hydrophobic interactions between the surface-adsorbed AOT molecules dominate the interparticle forces during solvent evaporation from the TEM grid. Moreover, the large degree of order in the superlattice domain is a direct consequence of the high concentration of regularly shaped, narrowly distributed Prussian blue nanoparticles produced by undertaking the reaction in organized media. Indeed, the regular cubic morphology indicates minimal interaction between the crystal surfaces and negatively charged AOT headgroups during crystal growth.

These results were in marked contrast to the control experiments. Synthesis in water alone gave a turbid green solution and a blue bulk precipitate that consisted of large (100–250 nm) discrete cubic particles with a broad size distribution. Reactions in isooctane without AOT produced a phase-separated aqueous blue phase containing crystals similar to those formed in pure water. No product was associated with the isooctane phase.

Formation of the 2-D square superlattice structure from the microemulsion reaction media was time dependent. For example, at $w = 15$ and from 0.3 M reaction solution, samples taken for TEM analysis after two days showed discrete cubic nanoparticles but no long-range ordering (Figure 3 a). These particles were smaller and more heterogeneous in size distribution (mean 14 nm, $\sigma = 4.9$ nm) than those collected after four days (Figure 2 b) which showed well ordered superlattice structures (Figure 3 b). Prussian blue nanoparticles collected from the same microemulsion after two weeks were slightly larger (mean 18 nm, $\sigma = 2.1$) but the domain length of the superlattices was reduced due to the increased number of nanoparticles in the solution (Figure 3 c).

The size of the individual nanoparticles was dependent on the concentrations of both the ammonium iron(III) oxalate and ammonium ferricyanide solutions. No significant changes in size (mean 16 nm) were observed at $w = 15$ between concentration values of 0.1–0.3 M. However, at lower concentrations (0.05 M), pale blue, transparent microemulsions were observed after two days in daylight, which transformed

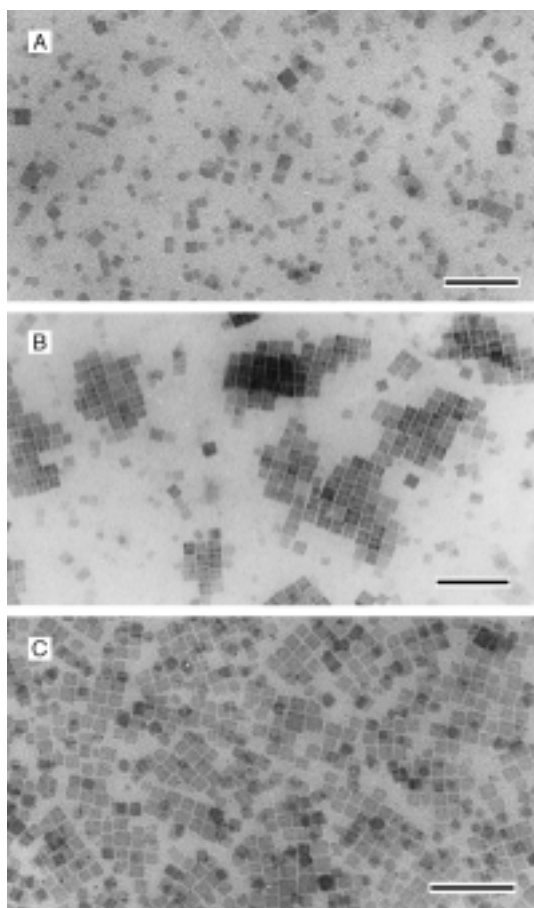


Figure 3. TEM images of discrete Prussian blue nanocrystals formed A) after two days from AOT microemulsions at $w = 15$; B) after four days showing superlattice structures; C) after two weeks, showing well defined crystals but a smaller superlattice domain sizes. Scale bars 100 nm.

into a transparent yellow solution and blue precipitate after three days. The TEM images showed the presence of regular, cubic Prussian blue nanoparticles in the precipitate. The particles had a mean size of 39 nm ($\sigma = 6.1$ nm) and self assembled into superlattice domains (Figure 4a). No blue color developed in the microemulsions at reactant concentrations of 0.01M, although a small yield of superlattice structures, consisting of cubic particles with an average size of 54 nm ($\sigma = 4.2$ nm), were observed (Figure 4b). These observations indicate that below a threshold value of 0.1M, lower reactant concentrations result in fewer crystals of significantly larger dimensions. This is consistent with a nucleation-controlled process of Prussian blue deposition in the microemulsion droplets.

The size of the nanoparticles was reduced when the water content (equivalently, droplet size) of the microemulsions was decreased. For example, discrete cubic particles were synthesized at $w = 10$. These nanoparticles were homogeneous in size (mean 12 nm, $\sigma = 1.4$ nm) and self assembled into small superlattice domains, often consisting of only four to twenty nanoparticles. No nanoparticles were obtained at $w = 5$, most probably because there is only a sufficient amount of water for headgroup hydration (reverse micelles) under these conditions.

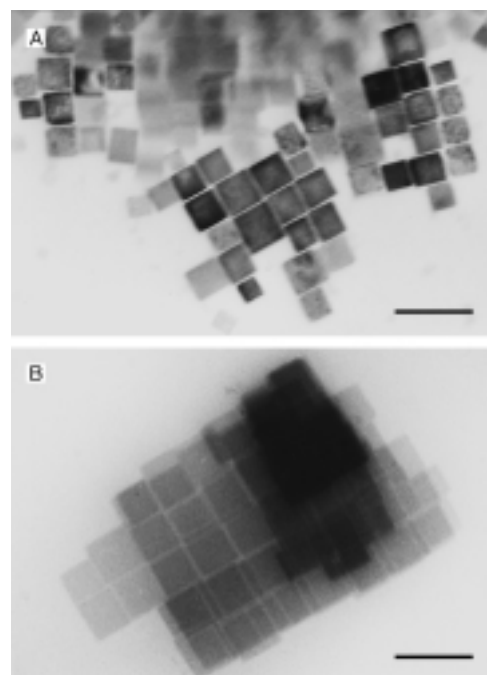


Figure 4. TEM images of Prussian blue nanoparticles and superlattices at $w = 15$ and reactant concentrations of A) 0.05M and B) 0.01M. Scale bars 100 nm.

Control over the size of the Prussian blue crystals clearly depends on various factors such as the rate of nucleation (photoreduction rates and ionic concentrations) and the number of ions of individual water droplets (w values and concentrations). However, at $w > 5$, the nanocrystals are much larger than would be predicted on considering the statistical distribution of reactants among the nanometer-size water droplets. For example, at 0.3M and $w = 15$, we estimate from the known diameter (5.4 nm) of NaAOT microemulsion droplets that there are approximately 15 ions in each water-filled cage.^[13] A 16 nm nanoparticle contains approximately 4000 unit cells, that is 16000 $[\text{Fe}(\text{CN})_6]^{4-}$ and 16000 Fe^{III} ions. This means that at least 1000 complete transfers would have to take place to produce crystals of the size observed. It remains unclear why the particle size is so uniform. One possibility is that in a given concentration range, the nanoparticle growth is self-limiting. This limitation is imposed by thermodynamic constraints arising from the droplet membrane curvature due to the limited number of water molecules that can be distributed around the crystal surfaces.

The use of in situ photoreduction to prepare nanoparticles and superlattices of molecular magnets from microemulsion fluids could have general applications in photo-patterning processes. For example, photo-formation of Prussian blue has been investigated as an alternative photographic-printing method,^[11] and is particularly important when localized reactions and homogenous seeding are required. Studies of photo-patterning of microemulsion thin films is the subject of further investigations.

Experimental Section

Prussian blue was prepared as the mixed valence ammonium cyanometallate $[(\text{NH}_4)_x\text{Fe}_y\{\text{Fe}(\text{CN})_6\}]$; $x + 3y = 4$ according to a reported method.^[14]

Ammonium iron(III) oxalate ($(\text{NH}_4)_3[\text{Fe}(\text{C}_2\text{O}_4)_3] \cdot 3 \text{H}_2\text{O}$, $M_w = 428$; 2.568 g, 6 mmol) was dissolved in hot distilled water (3 mL, 50 °C) contained in a foil-covered flask. Finely powdered potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$, $M_w = 329$; 0.987 g, 3 mmol) was added to the hot solution with vigorous stirring for 20 min and then sonicated for 2 min. The solution was left to cool in the dark for several hours. Green crystals of $\text{K}_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ were removed by filtration in the dark from the mixture of $(\text{NH}_4)_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ and $(\text{NH}_4)_3[\text{Fe}(\text{CN})_6]$ solutions. We used this procedure specifically to remove potassium ions from the reaction mixture as ammonium cyanometallate is significantly more stable than the potassium salt of Prussian blue. The solution was then diluted to 10 mL with distilled water to give an equimolar mixture of $(\text{NH}_4)_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ and $(\text{NH}_4)_3[\text{Fe}(\text{CN})_6]$ at a concentration of 0.3 M.

A small amount (0.09–0.36 mL) of the $(\text{NH}_4)_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ and $(\text{NH}_4)_3[\text{Fe}(\text{CN})_6]$ mixture (0.01–0.3 M in water) was added to an isooctane solution of NaAOT (10 mL, 0.1 M in isooctane) at room temperature in the dark to produce an AOT water-in-oil microemulsions at $w = 5$ –20. Higher water levels ($w = 15$ –20) resulted in a slight phase separation of the water in the microemulsion due to the high ionic strength of the reaction solution. This separation could be prevented by using lower concentrations of the reactants. The microemulsions were exposed to daylight for various periods to slowly photoreduce the $[\text{Fe}(\text{C}_2\text{O}_4)_3]^{3-}$ ions to $[\text{Fe}(\text{C}_2\text{O}_4)_3]^{4-}$, which serve as a source of Fe^{II} ions for treatment with aqueous $[\text{Fe}(\text{CN})_6]^{3-}$. This latter process is analogous to the Turnbull method for producing Prussian blue.

Samples for analytical TEM and UV/Vis spectroscopy were taken over two weeks. For TEM, droplets of the microemulsion were air-dried onto Formvar-coated, carbon-reinforced, 3 mm diameter, copper electron microscope grids.

Control experiments used equivalent amounts of the $(\text{NH}_4)_3[\text{Fe}(\text{C}_2\text{O}_4)_3]$ and $(\text{NH}_4)_3[\text{Fe}(\text{CN})_6]$ aqueous mixture (0.3 M) added to 10 mL of isooctane or water in the absence of NaAOT.

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- [1] a) S. Ferlay, T. Mallah, R. Ouahès, P. Veillet, M. Verdaguer, *Nature* **1995**, 378, 701; b) S. Ohkoshi, Y. Abe, A. Fujishima, K. Hashimoto, *Phys. Rev. Lett.* **1999**, 82, 1285.
- [2] M. Verdaguer, A. Bleutzen, V. Marvaud, J. Vaissermann, M. Seuleiman, C. Desplanches, A. Scullier, C. Train, R. Garde, G. Gelly, C. Lomenech, I. Rosenman, P. Veillet, C. Cartier, F. Villain, *Coord. Chem. Rev.* **1999**, 190–192, 1023.
- [3] a) C. T. Kresge, M. E. Leonowicz, W. E. Roth, J. C. Vartuli, J. S. Beck, *Nature* **1992**, 359, 710; b) S. Mann, S. L. Burkett, S. A. Davis, C. E. Fowler, N. H. Mendelson, S. D. Sims, D. Walsh, N. T. Whilton, *Chem. Mater.* **1998**, 9, 2300; c) C. G. Göltner, S. Henke, M. C. Weisenberger, M. Antonietti, *Angew. Chem.* **1998**, 110, 633; *Angew. Chem. Int. Ed.* **1998**, 37, 613.
- [4] a) A. L. Litvin, S. Valiyaveetil, J. Tingle, D. L. Kaplan, S. Mann, *Adv. Mater.* **1997**, 9, 124; b) D. Walsh, B. Lebeau, S. Mann, *Adv. Mater.* **1999**, 11, 324.
- [5] a) D. D. Archibald, S. Mann, *Nature* **1993**, 364, 430; b) D. Walsh, S. Mann, *Adv. Mater.* **1997**, 9, 658.
- [6] K. Honda, H. Hayashi, K. Chiba, *Chem. Lett.* **1988**, 191.
- [7] D. Avnir, M. L. Kagan, W. Ross, *Chem. Phys. Lett.* **1987**, 135, 177.
- [8] a) S. Ravaine, C. Lafuente, C. Mingotaud, *Langmuir* **1998**, 14, 6347; b) R. Saliba, B. Agricole, C. Mingotaud, S. Ravaine, *J. Chem. Phys. B* **1999**, 103, 9712.
- [9] Y. Einagam, O. Sato, A. Fujishima, K. Hashimoto, *J. Am. Chem. Soc.* **1999**, 121, 3745.
- [10] S. P. Moulik, G. C. De, A. K. Panda, B. B. Bhowmik, A. R. Das, *Langmuir* **1999**, 15, 8361.
- [11] M. Ware, *Cyanotype: The History, Science and Art of Photographic Printing in Prussian Blue*, The Science Museum and the National Museum of Photography, Film and Television, London, **1999**, p. 142, and references therein.
- [12] F. Herren, P. Fischer, A. Ludi, W. Haelg, *Inorg. Chem.* **1980**, 19, 956.
- [13] M. Li, H. Schnablegger, S. Mann, *Nature* **1999**, 402, 393.
- [14] M. Ware, *Ag⁺ Photogr.* **1995**, 7, 74.

Synthesis and Characterization of Ph_4TeI_4 , Containing a Te_4 Square, and $\text{Ph}_3\text{PTe}(\text{Ph})\text{I}$

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The interaction of organoelement compounds of Group 16 with dihalogens is a subject of considerable current interest. For example, the dihalogen adducts of organoselenium compounds^[1, 2] and selenoamides^[3–5] have all been intensively studied by a variety of research groups in the last few years. In addition, we have recently reported the structure of $\text{Ph}_3\text{PSe}(\text{Ph})\text{I}$, formed from the reaction of Ph_3P with $\text{Ph}_2\text{Se}_2\text{I}_2$.^[6] Many of the dihalogen compounds formed lie close to the ionic/covalent borderline, and several structural types have been identified which are dependent on the substituents R, the donor atom, the identity of the halogen, and, in some cases, the dielectric constant of the solvent in which such compounds are prepared. For example, dimethylselenide diiodine adopts a three-coordinate charge-transfer (CT) structure, $\text{Me}_2\text{SeI}-\text{I}$,^[1] whereas the corresponding dibromide adopts a disphenoidal “see-saw” structure, Me_2SeBr_2 . On the other hand, Me_2SBr_2 is a CT complex, $\text{Me}_2\text{SBr}-\text{Br}$, thus illustrating the importance of the donor atom on the nature of the dihalogen adduct produced.^[7] Also of relevance to the present study is the isolation of the first stable alkanetellurenyl pseudohalides by Fimml and Sladky, who reported on compounds of formula $(\text{Me}_3\text{Si})_3\text{CTeX}$ ($\text{X} = \text{CN}, \text{SCN}, \text{SeCN}, \text{NCO}, \text{N}_3$).^[8]

The interaction of Ph_2Se_2 with diiodine has been described by du Mont and co-workers, and a very interesting CT complex was observed in the solid state.^[9] The X-ray crystal structure of this molecule revealed a dimeric centrosymmetric moiety containing short and long iodine–iodine distances (2.775(1) and 3.588(1) Å, Figure 1). In view of this interesting (and unexpected) structure, we were interested in synthesizing the analogous tellurium compound from the stoichiometric reaction of Ph_2Te_2 and molecular iodine in diethyl ether in order to determine if the compound produced is isomorphous with $\text{Ph}_2\text{Se}_2\text{I}_2$ or whether a new structural motif would be revealed.

Diphenyl ditelluride reacts rapidly with diiodine to produce an intensely violet-black product. Elemental analysis of this material revealed the stoichiometry “ PhTeI ”, suggesting a product analogous to $\text{Ph}_2\text{Se}_2\text{I}_2$ from du Mont et al.^[9] We were nevertheless interested in crystallographically characterizing the compound for comparative purposes. The X-ray crystal

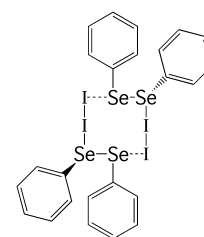


Figure 1. The structure of $\text{Ph}_2\text{Se}_2\text{I}_2$ as described by du Mont et al.^[13]

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structure^[10] revealed a novel structural modification not previously observed for organoelement–halogen compounds of Group 16: Individual PhTeI units are weakly linked into a tetramer through weak tellurium–tellurium bonds, the structure being best represented as Ph₄Te₄I₄ (**1**, Figure 2). This compound is quite different from Ph₂Se₂I₂ and illustrates the sensitivity of the structure of such systems on the Group 15 or 16 atom, a phenomenon previously described by other workers and us.^[1, 7, 9]

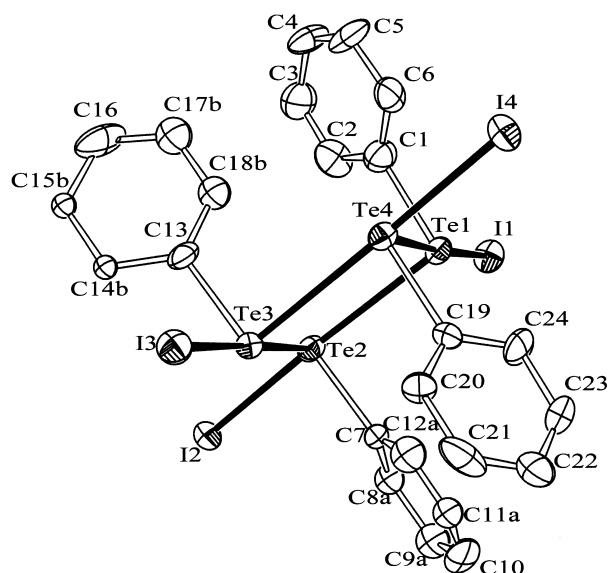


Figure 2. The X-ray crystal structure of **1**. Selected bond lengths [Å] and angles [°]: Te1–I1 2.799(2), Te2–I2 2.8181(14), Te3–I3 2.822(2), Te4–I4 2.830(2), Te1–Te2 3.1474(14), Te2–Te3 3.125(2), Te3–Te4 3.1627(14), Te4–Te1 3.175(2), I1–I4^a 3.841(3), I1–I2^b 3.852(3), I2–I3^c 3.898(3), I3–I4^d 3.856(3)—where ^a denotes $x + 1/2, y - 1/2, z$; ^b denotes $x + 1/2, y + 1/2, z$; ^c denotes $x + 1/2, y - 1/2, z$; and ^d denotes $1/2 - x, y - 1/2, z$; I1–Te1–Te(2) 94.09(4), I1–Te1–Te4 176.12(4), Te2–Te1–Te4 89.78(3), I2–Te2–Te1 175.56(5), I2–Te2–Te3 93.75(4), Te1–Te2–Te3 90.49(3), I3–Te3–Te2 175.77(4), I3–Te3–Te4 93.70(4), Te2–Te3–Te4 90.40(3), I4–Te4–Te1 93.92(4), I4–Te4–Te3 176.77(5), Te1–Te4–Te3 89.31(3).

The Te–Te bonds in **1** range from 3.125(2) to 3.175(2) Å, and are thus considerably longer than $d(\text{Te–Te})$ in diphenyl ditelluride (2.705(1) Å).^[11] The coordination around tellurium may be interpreted by postulating the presence of a stereochemically active lone pair on the tellurium atom, giving rise to a pseudo-trigonal-bipyramidal geometry. The existence of a compound of formula PhTeI was first reported by Schulz and Klar in 1974, who proposed a halide-bridged structure for the compound to explain its low solubility.^[12] Similarly, Alcock and Harrison reported the formation of PhTeI in chloroform; however, attempted recrystallization of this product resulted in the isolation of the decomposition products Ph₂TeI₂ and PhTeI₃ from hot toluene and toluene at room temperature, respectively.^[13] Both structures exhibit halide bridges.

Also of interest in the structure of **1** is the presence of weak secondary I...I interactions ranging from 3.841(3) to 3.898(3) Å, which is shorter than the sum of the van der Waals radii for two iodine atoms (4.3 Å). These very weak, but significant, contacts help to stabilize **1** and link the structure as a whole into a network of small Te₄ squares and larger I₄

square (Figure 3). Although no organotelluride–halogen compound that adopts this structure has been previously described, two compounds that contain the Te₄ unit have been reported quite recently. du Mont and co-workers have described the structure of Ph₃TeTe(*p*-tol) (tol = tolyl) in which cyclic

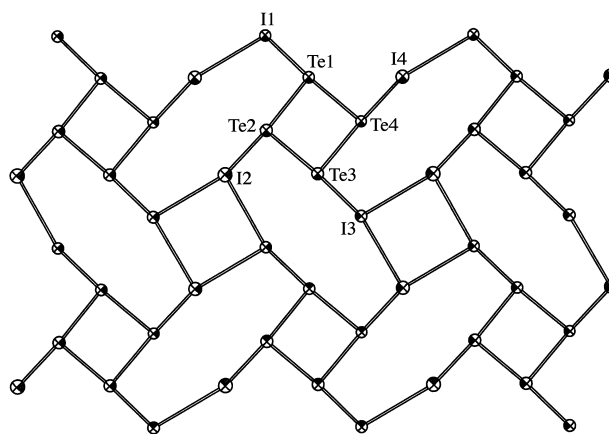
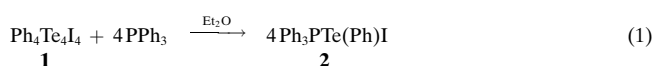


Figure 3. The crystal packing of **1** illustrating the long-range I...I contacts linking the structure into small (Te₄) and large squares (I₄), $d(\text{I–I}) = 3.841(3)–3.898(3)$ Å; phenyl rings are omitted for clarity.

units of composition R₈Te₄ are revealed, linked through weak Te–Te interactions.^[14] The compound as a whole may be thought of as a tetramer of diaryltellurium. The Te–Te distances for this compound range from 3.3233(13) to 3.5958(13) Å, and are considerably longer than those observed in **1** (3.125(2)–3.175(2) Å) but may still be regarded as Te–Te bonds when compared to the sum of the van der Waals radii for two tellurium atoms (4.40 Å). One other compound that also contains the Te₄ unit is the organometallic complex (μ -4-cyclotetratellurido) icosacarbonyltetrachromium, in which the tellurium atoms form a Te₄ square, each of which is also bonded to a Cr(CO)₅ unit.^[15] The values for $d(\text{Te–Te})$ for this complex range from 2.818(1) to 2.895(1) Å, and are thus considerably shorter than those observed in **1**, as expected.

As well as elucidating the structure of **1**, we were interested in examining whether it would react with a tertiary phosphine at ambient temperatures. Consequently, **1** was treated with PPh₃ in a 1:4 stoichiometric ratio [Eq. (1)].



Compound **1** reacts very rapidly with PPh₃ to produce the deep yellow Ph₃PTe(Ph)I (**2**). The X-ray crystal structure^[16] of this material reveals a CT complex (Figure 4). The Te–I distances (3.0930(9) Å) clearly represents a CT interaction when compared to the sum of the van der Waals radii of the two atoms and is similar to the I–I distance in Ph₃PI₂ (3.142(2) Å).^[17] The CT nature of **2** is confirmed by the geometry at the tellurium center, which is T-shaped (C1–Te1–P1 91.0(2)°, C1–Te1–I1 89.2(2)°) and not bent, as would be expected for an ionic compound. Additionally, angle P1–Te1–I1 is essentially linear (179.45(5)°).

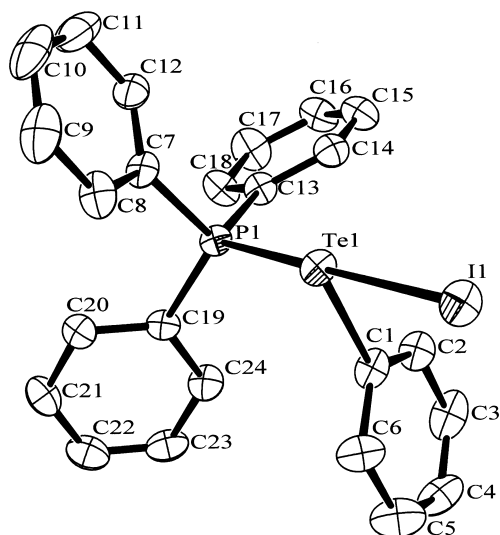


Figure 4. The X-ray crystal structure of **2**. Selected bond lengths [Å] and angles [°]: I1–Te1 3.0930(9), Te1–C1 2.109(7), Te1–P1 2.568(2); C1–Te1–P1 91.0(2), C1–Te1–I1 89.2(2), P1–Te1–I1 179.45(5), C19–P1–C7 109.2(3), C19–P1–Te1 118.3(2), C2–C1–Te1 121.3(6).

Only one compound of formula $R_3PTe(R)I$ has previously been described, $Ph_3PTe(2\text{-Naphthyl})I$, by Petragnani and Moura Campos in 1965.^[18] However, no single-crystal X-ray structure determination was attempted on this material. A wider class of compounds of general formula $LTe(R)X$ (where L is a neutral donor ligand) has been long recognized, having first been reported in the pioneering work of Foss and Husebye,^[19] who investigated the structures of $PhTe(tu)X$ (tu = thiourea; X = Br, Cl). Both structures contain divalent tellurium atoms bonded to a phenyl group, a thiourea molecule, and, in *trans* position to the latter, a halogen atom. In common with **2**, the Te–X bonds are rather long in these structures (Te–Cl 3.00(15), Te–Br 3.11(1) Å).

The $^{31}P\{^1H\}$ NMR spectrum of **2** reveals a single peak at $\delta = 26.9$, which is shifted to higher field than that exhibited by Ph_3PI_2 ($\delta = 44.2$), which is known to ionize in $CDCl_3$ to produce $[Ph_3PI]^+$. This result suggests that **2** may retain its molecular CT structure in solution, however, no ^{125}Te satellites were observed. Work is currently underway to investigate whether the reaction of compounds of the general type $R_3PTe(R)I$ with crude metal powders will provide a novel route to new tellurium-bound metal complexes, analogous to our recent results concerning the reaction of R_3EI_2 with metal powders: For example in the reaction of Me_3PI_2 with gold metal powder the novel trigonal-bipyramidal complex $AuI_3(PMe_3)_2$ is produced.^[20]

Experimental Section

1: Ph_2Te_2 (Aldrich; 1.00 g, 2.44 mmol) was dissolved in dry Et_2O (ca. 75 mL), and subsequently I_2 (0.62 g, 2.44 mmol) was added against a stream of dry N_2 . After stirring for about 2 d under N_2 , the dark purple **1** was isolated using standard Schlenk techniques. The yield was essentially quantitative. After drying in vacuo, **1** was transferred to predried nitrogen-filled ampoules, which were flame-sealed. Crystals suitable for X-ray diffraction were easily obtained by dissolving the bulk solid in refluxing Et_2O and leaving the mixture to cool to room temperature. A large crop of black crystals formed after about 3 d. Elemental analysis of $Ph_4Te_4I_4$: found (calcd): C 22.0 (21.8), H 1.9 (1.5), I 38.2 (38.4), Te 37.9 (38.3).

2: Compound **1** (0.5 g, 0.38 mmol) was dissolved in dry Et_2O (ca. 50 mL), and subsequently Ph_3P (0.40 g, 1.50 mmol) was added against a stream of dry N_2 . Almost instantaneously a color change from deep burgundy (due to **1**) to bright yellow was noted. After stirring for 1 d under N_2 , the bright yellow **2** was isolated using standard Schlenk techniques (yield ca. 90%). After drying in vacuo, **2** was transferred to predried nitrogen-filled ampoules, which were flame-sealed. Crystals suitable for X-ray diffraction were obtained by dissolution of the bulk solid in Et_2O/CH_2Cl_2 (1/1) at 50 °C. On cooling to room temperature large well-formed yellow-orange crystals were apparent in the reaction vessel after about 2 d.

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- [1] S. M. Godfrey, C. A. McAuliffe, R. G. Pritchard, S. Sarwar, *J. Chem. Soc. Dalton Trans.* **1997**, 1031.
- [2] S. M. Godfrey, C. A. McAuliffe, R. G. Pritchard, S. Sarwar, *J. Chem. Soc. Dalton Trans.* **1997**, 3504.
- [3] P. D. Boyle, W. I. Cross, S. M. Godfrey, C. A. McAuliffe, R. G. Pritchard, S. J. Teat, *J. Chem. Soc. Dalton Trans.* **1999**, 2845.
- [4] F. Bigoli, A. M. Pellinghelli, P. Deplano, F. A. Devillanova, V. Lippolis, M. L. Mercuri, E. F. Trogu, *Gazz. Chim. Ital.* **1994**, 124, 445.
- [5] F. Cristiani, F. Demartin, F. A. Devillanova, F. Isaia, V. Lippolis, G. Verani, *Inorg. Chem.* **1994**, 33, 6315.
- [6] P. D. Boyle, S. M. Godfrey, C. A. McAuliffe, R. G. Pritchard, J. M. Sheffield, *Chem. Commun.* **1999**, 2159.
- [7] B. Regelmann, K. W. Klinkhammer, A. Schmidt, *Z. Anorg. Allg. Chem.* **1997**, 623, 1633.
- [8] W. Fimml, F. Sladky, *Chem. Ber.* **1991**, 124, 1131.
- [9] S. Kubiniok, W. W. du Mont, S. Pohl, W. Saak, *Angew. Chem.* **1988**, 100, 438; *Angew. Chem. Int. Ed. Engl.* **1988**, 27, 431.
- [10] a) Crystal data for **1** ($C_{24}H_{20}I_4Te_4$): $M_r = 1326.40$, monoclinic, space group Cc , $a = 13.759(4)$, $b = 13.812(4)$, $c = 16.451(5)$ Å, $\beta = 101.62(3)^\circ$, $V = 3062.3(16)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 2.877$ Mg m⁻³, MoK_{α} radiation ($\lambda = 0.71073$ Å), crystal dimensions $0.25 \times 0.25 \times 0.10$ mm³. Of 6769 reflections collected on a Rigaku AFC 6S diffractometer, 3551 were observed ($R_{\text{int}} = 0.0994$) and used for all calculations (programme SHELXL 93). After absorption correction (psi scans) the structure was solved by direct methods and refined anisotropically on F^2 . Final residuals $R_1 = 0.0603$, $wR_2 = 0.1642$ ($I > 2\sigma(I)$); $R_1 = 0.0650$, $wR_2 = 0.1700$ (all data), 287 parameters. b) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-141 820 and -141 821. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [11] G. Llabres, O. Dideberg, L. Dupont, *Acta Crystallogr. Sect. B* **1972**, 28, 2438.
- [12] P. Schulz, G. Klar, *Z. Naturforsch. B* **1975**, 30, 40.
- [13] N. W. Alcock, W. D. Harrison, *J. Chem. Soc. Dalton Trans.* **1984**, 869.
- [14] J. Jeske, W. W. du Mont, P. G. Jones, *Angew. Chem.* **1996**, 108, 2822; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 2653.
- [15] O. Blacque, H. Brunner, M. M. Kubicki, B. Nuber, B. Stubenhofe, J. Wachter, B. Wrackmeyer, *Angew. Chem.* **1997**, 109, 362; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 352.
- [16] Crystal data for **2** ($C_{24}H_{20}IPTe$):^[10b] $M_r = 593.87$, triclinic, space group $P\bar{1}$, $a = 9.780(2)$, $b = 9.780(2)$, $c = 12.412(3)$ Å, $\alpha = 76.21(2)^\circ$, $\beta = 73.03(2)^\circ$, $\gamma = 87.79(2)^\circ$, $V = 1102.3(4)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.789$ Mg m⁻³, MoK_{α} radiation ($\lambda = 0.71073$ Å), crystal dimensions $0.30 \times 0.20 \times 0.20$ mm³. Of 4097 reflections collected on a Nonius MAC 3 CAD4 diffractometer, 3847 were observed ($R_{\text{int}} = 0.0739$) and used for all calculations (programme SHELXL 93). After absorption correction (psi scans) the structure was solved by direct methods and refined anisotropically on F^2 . Final residuals $R_1 = 0.0549$, $wR_2 = 0.1376$ ($I > 2\sigma(I)$); $R_1 = 0.0692$, $wR_2 = 0.1491$ (all data), 244 parameters.
- [17] S. M. Godfrey, D. G. Kelly, A. G. Mackie, C. A. McAuliffe, R. G. Pritchard, S. M. Watson, *J. Chem. Soc. Chem. Commun.* **1991**, 1163.
- [18] M. Petragnani, M. Moura Campos, *Tetrahedron* **1965**, 21, 13.
- [19] O. Foss, S. Husebye, *Acta Chem. Scand.* **1966**, 20, 132.
- [20] S. M. Godfrey, N. Ho, C. A. McAuliffe, R. G. Pritchard, *Angew. Chem.* **1996**, 108, 2495; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 2343.

Highly Enantioselective Aldol Reaction with 2-Trimethylsilyloxyfuran: The First Catalytic Asymmetric Autoinductive Aldol Reaction**

Magali Szlosek and Bruno Figadère*

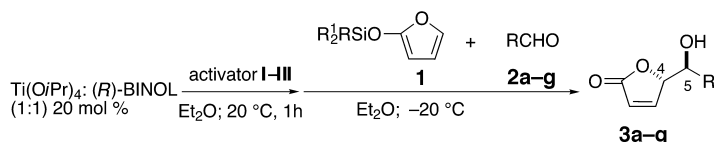
We report herein the first quantitative and highly enantioselective autoinductive aldol reaction (up to 96 % *ee*) between 2-trimethylsilyloxyfuran (TMSOF) **1a** and an achiral aldehyde **2**. The origin of homochirality of biomolecules (such as α -amino acids and sugars) remains a puzzling question.^[1] It is now well accepted that physical factors like circularly polarized light can induce chirality in molecules through photo-synthetic procedures.^[2, 3] However the *ee* values so obtained are very low and cannot be directly correlated with the enantiomeric purity of the known biomolecules. Thus, Soai et al. have shown that amplification of a slight enantiomeric imbalance in molecules could be due to an asymmetric autocatalytic system, and this was proved to be the case for the 1,2-addition of organozinc reagents to 2-methylpyrimidine-5-carbaldehyde.^[4] Since then, only catalytic asymmetric 1,2-addition of nucleophiles on aldehydes has been shown to be either autocatalyzed^[4–6] or autoinduced^[7–9] by the chiral product of the reaction, apart from several Diels–Alder reactions which were reported to be autoinduced by the cyclic adduct.^[10]

We recently disclosed that **1a** adds to aldehyde **2a** to afford the corresponding butenolides **3a** with good selectivity (76 to 90 % *ee*) when the (*R*)-(1,1'-bi-2-naphthol)₂Ti complex ((*R*)-BINOL₂Ti) was used (Scheme 1).^[11] In order to improve both

for 1 hour. Then 1 equivalent of **2a** (0.5 mmol) followed by 1.5 equivalents of **1a** were added. Butenolides **3a** so obtained were analyzed by ¹H NMR spectroscopy in the presence of chiral europium salt (Eu(hfc)₃). It should be noted that, in the presence of only Ti(OiPr)₄, **1a** does not add to **2a**.^[11] The first experiment shows that **1a** added to **2a**, with (*R*)-BINOL₂Ti as the catalyst gave, after 2 hours, **3a** with low d.r. (53:47), a good *ee* value (87 %) for the major *syn* product, and moderate 50 % yield (this is quite often encountered in such reactions^[13–15]). Under the same reaction conditions, but at lower temperature (–78 °C), the *ee* value of the *syn* butenolide **3a** was significantly increased (96 %) but to the detriment of the chemical yield (10 %). When (–)-TADDOL was added to the Ti(OiPr)₄ and (*R*)-BINOL (1:1:1 ratio), a similar yield and only a slight decrease in the *ee* value (70 relative to 87 %) were observed. We thought that these conditions may correspond to a mismatch case and so performed the same reaction with (+)-TADDOL and observed a quantitative reaction with formation of the same major diastereomer (*syn*) **3a** (d.r. = 70:30) with 82 % *ee*. In all cases the absolute configuration of the major isomer **3a** was *S,S* as determined by comparison with related products.^[11] The last two experiments gave good evidence that the (*R*)-BINOLTi(–)-TADDOL is less active than the (*R*)-BINOLTi(+)-TADDOL complex which is probably formed,^[13] and that the chiral induction is controlled by (*R*)-BINOL. Again a temperature effect was observed and, in the presence of a 1:1:1 mixture of Ti(OiPr)₄, (*R*)-BINOL, and (+)-TADDOL at –78 °C, **1a** added to **2a** to afford the major *syn* butenolide **3a** with a better *ee* value (90 relative to 82 %) but lower chemical yield (40 relative to 99 %).

It was now possible to perform the aldol reaction with a “reluctant” aldehyde, heptadecanal **2b**, in 55 % yield with a 75:25 d.r. and with an *ee* value of 75 % for the major *syn* isomer **3b** (see supporting information). So far, it is clear that BINOL is necessary for the aldol reaction to occur, and that addition of a second aliphatic alcohol (TADDOL for instance) could form an even more reactive complex with both titanium(IV) and BINOL. It was therefore important to check if the product of the reaction could interact with the catalytic species. This investigation led us to describe the first auto-inductive aldol reaction (Scheme 1 and Table 1).

The first experiment shows that, in the presence of 20 mol % of the catalyst (*R*)-BINOL₂Ti and 5 mol % of



Scheme 1. Activators: **I** = (*R*)-BINOL, **II** = (–)-TADDOL, **III** = (+)-TADDOL; **1a**: R¹ = R = Me; **1b**: R¹ = Me and R = *t*Bu; **1c**: R¹ = Ph and R = *t*Bu; **2a–g**: see Table 2.

the chemical yield and the *ee* value of this reaction, we studied the use of a second chiral ligand, as an activator, based on the chiral ligand acceleration concept.^[12–15] The catalysts were prepared in situ at 20 °C in Et₂O by mixing 20 mol % of Ti(OiPr)₄, (*R*)-BINOL, and the activator **I–III** in a 1:1:1 ratio

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

Table 1. 2-TMSOF (**1a**) addition to octanal (**2a**) in the presence of the BINOL₂Ti complex at –20 °C in Et₂O.

Entry	activator ^[a]	yield of 3a [%]	<i>syn:anti</i> ratio	<i>ee</i> of <i>syn</i> (abs. conf.)
1	(<i>S,S</i>)- 3a ^[b]	99	70:30	> 96 (<i>S,S</i>) ^[b]
2	(<i>R,R</i>)- 3a ^[c]	99	70:30	40 (<i>S,S</i>)
3	(<i>R,S,SR</i>)- 3a ^[d]	57	60:40	92 (<i>S,S</i>)
4	none ^[e]	99	70:30	70 (<i>S,S</i>)
5	none ^[f]	90	60:40 ^[g]	> 96 (<i>S,S</i>)

[a] 5 mol % solution; [b] (*S,S*)-**3a** with 82 % *ee*, and thus, the *ee* value for the butenolide **3a** is 96.88 %, as calculated in ref. [8]; [c] (*R,R*)-**3a** with > 96 % *ee*; [d] racemic *anti* butenolide **3a**; [e] after 24 hours; [f] with addition of a mixture of 2-TMSOF (**1a**) and octanal (**2a**) in four portions (ratios of **2a** to **1a** = 0.032:0.049, 0.093:0.139, 0.125:0.188, and 0.250:0.375 mmol with 15, 15, 30, and 60 min. of delay, respectively); [g] the *anti* product (4*R,5S*)-**3a** with an *ee* value of 90 %.

(*S,S*)-**3a** (82% *ee*), the reaction was quantitative with an *ee* value of 96% determined for all *syn* butenolide **3a** (which corresponds to 96.9% *ee* for the newly formed butenolide, see reference [8] for calculations). In the presence of the minor enantiomer (*R,R*)-**3a**, a much lower *ee* value was observed for **3a**, as expected (40% *ee*, entry 2, Table 1). These results suggested that the major chiral products formed in this reaction are incorporated into the active chiral catalyst to form new catalysts which are more active due to a ligand accelerating effect (leading to higher chemical yields) and which have a better asymmetric inductive effect (higher *ee* values observed). However, when 5 mol% of racemic *anti* isomer **3a** was introduced into a 20 mol% (*R*)-BINOL₂Ti catalyst solution followed by **2a** and then **1a**, the expected butenolides **3a** were obtained in 57% yield as a 60:40 *syn:anti* mixture, with an *ee* value of 92% for the major *syn* product. This important result shows that there is not a significant increase in the chemical yield (57 relative to 50%) or the *ee* value (92 in comparison with 87%). This is, furthermore, in accordance with the result obtained in the absence of added aldol, therefore, we assume that the *anti* isomer has a negligible influence on the course of the reaction.

We then examined the amplification of the *ee* values when the reaction is performed in a stepwise manner. Indeed, by adding the mixture of reagents **1a** and **2a** in four portions (ratios of **2a:1a** were 0.032:0.049, 0.093:0.139, 0.125:0.188, and 0.250:0.375 mmol with 15, 15, 30, and 60 minutes of delay, respectively) to the reaction medium containing 20 mol% of the (*R*)-BINOL₂Ti catalyst, we observed the formation of **3a** with excellent yield (90 relative to 50%) with an *ee* value of 96% after only 2 hours at -20°C ! This result may be rationalized as follows: After the first addition of 6.5 mol% of the mixed **1a** and **2a**, the major *syn* butenolide **3a** so formed can be incorporated into the new catalyst and gives identical results to those observed when *syn* **3a** is first added to the reaction mixture. The amplification can be shown by comparing the *ee* value obtained in the one addition protocol (87%) with the value of 96% obtained after the “stepwise” experiment. Indeed, these results are consistent with an autoinduced process with amplification^[16] of the *ee* value of the product. In this last experiment, the *ee* value of the *anti* aldol **3a** was measured (90% *ee*), and its absolute configuration was assumed to be (4*R*,5*S*) by comparison with the sign of the specific rotation of the dihydrogenated compound with epimuricatacin.^[17] Thus, epimerization at the allylic position of the *anti* aldol **3a** (for example, through Et₃N treatment^[18]) would eventually lead to the formation of the major *syn* aldol compound **3a**.

Several other aldehydes (**2c–g**) were added with **1a** in four portions as above (Scheme 1 and Table 2). It is noteworthy that aldehydes possessing either isolated double bonds, such as **2c**, or a long saturated aliphatic chain, like **2f**, gave the corresponding aldols with excellent yields and high *ee* values (**3c** and **3f**, 94 and 96% *ee*, respectively). In the case of α,β -unsaturated aldehyde **2e** (Entry 3), we were delighted to observe that the aldols **3e**, which resulted from 1,2-addition, were the major compounds formed (ratio of 1,2- to 1,4-addition^[19] = 70:30). However, in this latter case the major product is now the *anti* isomer, and the *syn* isomer only had an *ee* value of 52%; the absolute configuration of the major enantiomer was determined as (4*S*,5*S*). In the case of the aromatic aldehyde **2d**, the reaction was again quantitative (Entry 2) and the major *anti* aldol **3d** had an *ee* value of 90%, whereas the *syn* product only had a 60% *ee* value (absolute configurations have not yet been determined). The reverse diastereoselectivity, as well as a lower enantioselectivity in these two cases may be rationalized by including electronic factors. Finally, when a bulky aldehyde, such as cyclohexylcarbaldehyde **2g**, is involved (Entry 5) a 70:30 mixture of the *syn:anti* aldols **3g** was obtained in 70% yield. The major *syn* product had an *ee* value of 71%, whereas the *anti* isomer showed a 79% *ee* value. Thus, as far as chemical yields and *ee* values are concerned, the reaction seems sensitive to steric hindrance, which is often the case with aldol reactions.^[20]

In an attempt to improve the d.r., we turned our attention to the use of different silyloxyfurans, 2-*tert*-butyldimethylsilyloxyfuran (TBDMSOF) **1b**^[21] and 2-*tert*-butyldiphenylsilyloxyfuran (TBDPSOF) **1c**. When these silyloxyfurans were separately mixed with **2a** in the presence of a (*R*)-BINOL₂Ti complex solution (20 mol%) in Et₂O cooled to -20°C , no reaction occurred (after 12 hours). However, when the reaction was performed with either **1b** or **1c** in a stepwise fashion at -20°C (ratios of **2a** to **1b** or **1c** = 0.032:0.049, 0.093:0.139, 0.125:0.188, and 0.250:0.375 mmol with 30, 60, 90, and 180 min delay, respectively), the aldols **3a** were obtained in 60% yield as a 70:30 *syn:anti* mixture with *ee* values of 97% for the (4*S*,5*S*) isomer and 86% for the (4*R*,5*S*) isomer for the reaction involving **1b**, and 45% yield as a 86:14 *syn:anti* mixture with a value of 82% *ee* for the (4*S*,5*S*) isomer, for the reaction with **1c** (results not optimized). It is worth noting that the d.r. was improved from 60:40 to 70:30 and finally 86:14 by using the more bulky reagents.

In conclusion, the reaction of **1a** with achiral aldehydes in the presence of BINOL/titanium(IV) complexes is the first catalytic asymmetric autoinductive aldol reaction reported.^[22] Both yields and enantiomeric excess values are good to

Table 2. Additions of various couples of 2-TMSOF (**1a**) and aldehyde (**2c–g**) in the presence of the BINOL₂Ti complex at -20°C in Et₂O.

Entry	aldehyde	time [h]	yield [%] 3c–g	<i>syn:anti</i> ratio	<i>ee</i> of <i>syn</i> (abs. conf.)	<i>ee</i> of <i>anti</i> (abs. conf.)
1	<i>E</i> -decen-4-al (2c)	2	99	53:47	94 (<i>S,S</i>)	90 (<i>R,S</i>)
2	benzaldehyde (2d)	6	99	24:76	60 (ND) ^[a]	90 (ND) ^[a]
3	<i>E</i> -nonen-2-al (2e)	2	70 ^[b]	30:70	52 (<i>S,S</i>)	24 (<i>R,S</i>) ^[c]
4	tridecanal (2f)	6	80 ^[d]	60:40	> 96 (<i>S,S</i>)	90 (<i>R,S</i>)
5	cyclohexylcarbaldehyde (2g)	2	70	65:35	71 (<i>S,S</i>)	79 (<i>R,S</i>)

[a] ND = not determined; [b] 1,4-addition = 30%; [c] measured on the hydrogenated product by comparison with the specific rotation of an authentic compound; [d] when addition is performed in one portion: yield = 20%, d.r. = 60:40, *ee* = 90% (*syn S,S*).

excellent, which is unusual for such reactions. The stepwise procedure allows the highest *ee* values to be obtained together with high chemical yields.^[23] These results show the wide scope of such aldol reactions, with certain limitations for bulky and/or conjugated aldehydes (results not optimized). The use of bulkier trialkylsilyloxyfurans slightly improved the d.r.; further optimization studies are in progress. Furthermore these findings may provide some evidence for amplification of *ee* values of biomolecules (by chemical reactions) in the chemical origins of life. Indeed, conditions for aldol reactions may have been found in prebiotic systems. More experiments are nevertheless needed to set a mathematical model for such a mechanism.^[24] Such a process could also be involved in other asymmetric reactions.^[23, 25, 26]

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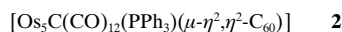
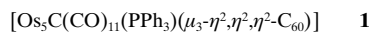
- [1] K. Soai, T. Shibata, H. Morioka, K. Choji, *Nature* **1995**, 378, 767.
- [2] a) N. P. M. Huck, W. F. Jager, B. de Lange, B. L. Feringa, *Science* **1996**, 273, 1686; b) Y. Zhang, G. B. Schuster, *J. Org. Chem.* **1995**, 60, 7192.
- [3] O. Buchardt, *Angew. Chem.* **1974**, 86, 222; *Angew. Chem. Int. Ed. Engl.* **1974**, 136, 179.
- [4] T. Shibata, J. Yamamoto, N. Matsumoto, S. Yonekubo, S. Osanai, K. Soai, *J. Am. Chem. Soc.* **1998**, 120, 12157.
- [5] T. Shibata, H. Morioka, T. Hayase, K. Choji, K. Soai, *J. Am. Chem. Soc.* **1996**, 118, 471.
- [6] T. Shibata, T. Hayase, J. Yamamoto, K. Soai, *Tetrahedron: Asymmetry* **1997**, 8, 1717.
- [7] A. H. Alberts, H. Wynberg, *J. Am. Chem. Soc.* **1989**, 111, 7265.
- [8] L. Shengjian, J. Yaozhong, M. Aiqiao, Y. Guishu, *J. Chem. Soc. Perkin Trans 1* **1993**, 885.
- [9] H. Danda, H. Nishikawa, K. Otaka, *J. Org. Chem.* **1991**, 56, 6740.
- [10] a) F. Rebiere, O. Riant, H. B. Kagan, *Tetrahedron: Asymmetry* **1990**, 1, 199; b) D. P. Heller, D. R. Goldberg, W. D. Wulff, *J. Am. Chem. Soc.* **1997**, 119, 10551.
- [11] M. Szlosek, X. Franck, B. Figadère, A. Cavé, *J. Org. Chem.* **1998**, 63, 5169.
- [12] D. J. Berrisford, C. Bolm, K. B. Sharpless, *Angew. Chem.* **1995**, 107, 1159; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 1059.
- [13] K. Mikami, S. Matsukawa, T. Volk, M. Terada, *Angew. Chem.* **1997**, 109, 2936; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 2768.
- [14] K. Mikami, S. Matsukawa, *Nature* **1997**, 385, 613.
- [15] M. Chavarot, J. J. Byrne, P. Y. Chavant, J. Pardillos-Guindet, Y. Vallée, *Tetrahedron: Asymmetry* **1998**, 9, 3889.
- [16] a) C. Puchot, O. Samuel, E. Dunach, S. Zhao, C. Agami, H. B. Kagan, *J. Am. Chem. Soc.* **1986**, 108, 2353; b) N. Oguni, Y. Matsuda, T. Kaneko, *J. Am. Chem. Soc.* **1988**, 110, 7877; c) M. Kitamura, S. Okada, S. Suga, R. Noyori, *J. Am. Chem. Soc.* **1989**, 111, 4028; d) C. Girard, H. B. Kagan, *Angew. Chem.* **1998**, 110, 3088; *Angew. Chem. Int. Ed.* **1998**, 37, 2922; e) B. L. Feringa, R. A. van Delden, *Angew. Chem.* **1999**, 111, 3624; *Angew. Chem. Int. Ed.* **1999**, 38, 3418.
- [17] B. Figadère, J.-C. Harmange, A. Laurens, A. Cavé, *Tetrahedron Lett.* **1991**, 32, 7539.
- [18] B. Figadère, C. Chaboche, J. F. Peyrat, A. Cavé, *Tetrahedron Lett.* **1993**, 34, 8093.
- [19] H. Nishikori, K. Ito, T. Katsuki, *Tetrahedron: Asymmetry* **1998**, 9, 1165.
- [20] S.-I. Kiyooka, H. Maeda, M. Abu Hena, M. Uchida, C.-S. Kim, M. Horiike, *Tetrahedron Lett.* **1998**, 39, 8287.
- [21] G. Rassu, F. Zanardi, L. Battistini, E. Gaetani, G. Casiraghi, *J. Med. Chem.* **1997**, 40, 168.
- [22] A. H. Alberts, H. Wynberg, *J. Chem. Soc. Chem. Commun.* **1990**, 453.
- [23] S. Kobayashi, Y. Fujishita, T. Mukaiyama, *Chem. Lett.* **1990**, 1455.
- [24] E. F. Kogut, J. C. Thoen, M. A. Lipton, *J. Org. Chem.* **1998**, 63, 4604.
- [25] M. Pichon, J. C. Jullian, B. Figadère, A. Cavé, *Tetrahedron Lett.* **1998**, 39, 1755.
- [26] D. A. Evans, M. C. Kozlowski, J. A. Murry, C. S. Burgey, K. R. Campos, B. T. Connell, R. J. Staples, *J. Am. Chem. Soc.* **1999**, 121, 669.

Interconversion between μ - η^2 , η^2 -C₆₀ and μ_3 - η^2 , η^2 , η^2 -C₆₀ on a Carbido Pentaosmium Cluster Framework**

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Exohedral metallofullerenes have recently attracted much attention concerning the effects of metal coordination on the chemical and physical properties of C₆₀.^[1] Most approaches to forming metal complexes have been based on metal–C₆₀ π -complex chemistry, which has resulted in η^2 -C₆₀, μ - η^2 , η^2 -C₆₀, and μ_3 - η^2 , η^2 , η^2 -C₆₀ ligands in monometallic (for most metals),^[2] bimetallic (Re₂, Ru₂, Ir₂),^[3] and metal cluster complexes (Ru₃, Os₃, Ru₅C, Ru₆C, PtRu₅C),^[4, 5] respectively. Metal clusters can potentially accommodate all these C₆₀ bonding modes, but the interaction of C₆₀ with cluster frameworks has been, thus far, dominated by the face-capping cyclohexatriene-like bonding mode, μ_3 - η^2 , η^2 , η^2 -C₆₀. The μ - η^2 , η^2 -C₆₀ bonding mode has never been observed on a cluster framework, although it has been postulated as an intermediate for the transformation of [Os₃(CO)₁₁(η^2 -C₆₀)] to [Os₃(CO)₉(μ_3 - η^2 , η^2 , η^2 -C₆₀)] by loss of carbonyl ligands.^[5c] The interconversion among the three kinds of the C₆₀ ligands remains to be established in the area of C₆₀–metal cluster chemistry. We have recently observed the elusive μ - η^2 , η^2 -C₆₀ bonding mode on an Os₅C cluster framework, and furthermore demonstrated that the two C₆₀ bonding modes μ - η^2 , η^2 and μ_3 - η^2 , η^2 , η^2 are interconvertible.

Reaction of [Os₅C(CO)₁₂(PPh₃)(NCMe)₂] with C₆₀ in refluxing C₆H₆ produced a mixture of **1** and **2** (see the



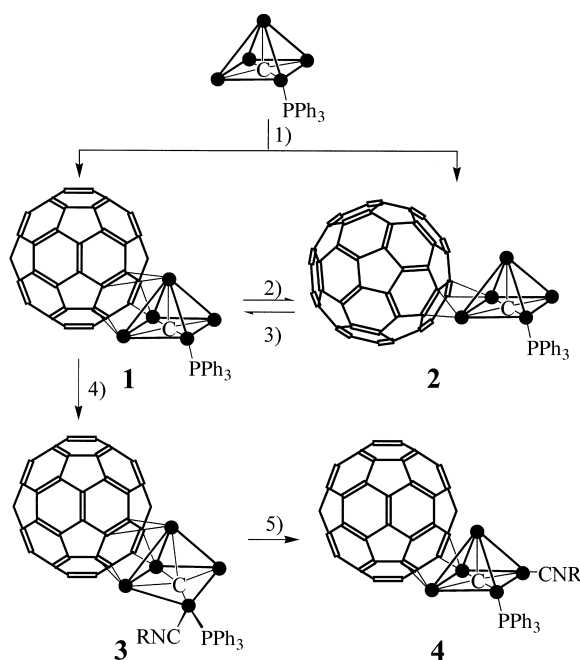
Experimental Section). The conversion of **1** into **2** could be effected by heating a solution of **1** in C₆H₆ at 80 °C under 1 atm of carbon monoxide. Upon thermolysis at 132 °C, **2** was cleanly reconverted into **1** by loss of a carbonyl ligand (Scheme 1).

The solid-state structure of **1** is isomorphous to that of the ruthenium analogue [Ru₅C(CO)₁₁(PPh₃)(μ_3 - η^2 , η^2 , η^2 -C₆₀)].^[4b] The structure of **2** (Figure 1) reveals a very intriguing feature

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Scheme 1. 1) 1.2 Me₃NO, CH₃CN/CH₂Cl₂ (1/2), room temperature, 2 h; 2) C₆₀, ClC₆H₅, 132 °C, 1 h, 19% for **1** and 13% for **2**; 2) CO (1 atm), ClC₆H₅, 80 °C, 30 h, 72%; 3) ClC₆H₅, 132 °C, 6 h, 73%; 4) RNC, ClC₆H₅, room temperature, 15 h, 84%; 5) ClC₆H₅, 100 °C, 12 h, 64%. R = CH₂C₆H₅.

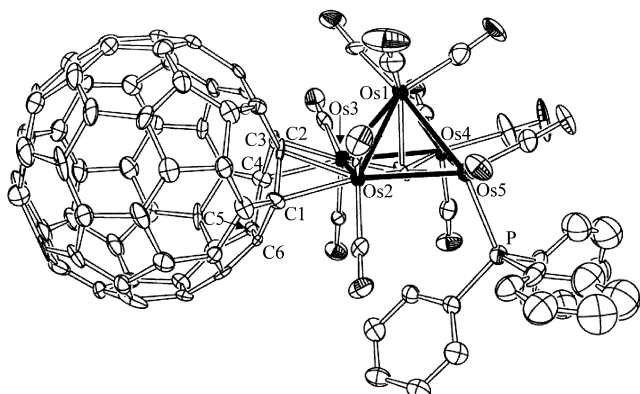
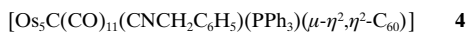
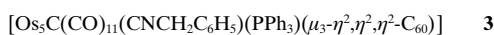


Figure 1. Molecular structure of **2**. Selected bond lengths [Å]: C1–C2 1.39(2), C2–C3 1.53(2), C3–C4 1.41(2), C4–C5 1.49(2), C5–C6 1.41(2), C6–C1 1.45(2), Os2–C1 2.23(2), Os2–C2 2.18(2), Os3–C3 2.19(1), Os3–C4 2.27(2).

of the C₆₀ ligand disposition. Two adjacent double bonds in a C₆ ring of the C₆₀ ligand bridge the basal Os2 and Os3 atoms in a μ-η²,η² mode. Interestingly, the third uncoordinated double bond in the C₆ ring is disposed away from the Os1–Os2–Os3 triangle; the C₆₀ ligand has apparently rotated by 180° upon the center of the Os2–Os3 bond during **1** → **2** conversion as compared to the C₆₀ position in **1**.

This is, to our knowledge, the first example of interconversion between μ-η²,η²- and μ₃-η²,η²,η²-C₆₀ bonding modes. To understand in detail the pathways of the interconversion and the apparent accompanying C₆₀ rotation on the cluster framework, the interaction of **1** with a benzyl isocyanide ligand was investigated. This would provide insight into the fate of the incoming carbonyl ligand in the transformation of **1** to **2**. Reaction of **1** with benzyl isocyanide at room temper-

ature gave the addition product **3**. Thermolysis of this kinetic product at 100 °C resulted in the isomer **4** in high yield (Scheme 1, see the Experimental Section).



The molecular structure of the kinetic isomer **3** is shown in Figure 2. The μ₃-η²,η²,η²-C₆₀ bonding mode is unaffected, but one Os_{apical}–Os_{basal} bond in the square-pyramidal Os₅C framework of **1** has been cleaved by the addition of the two-electron

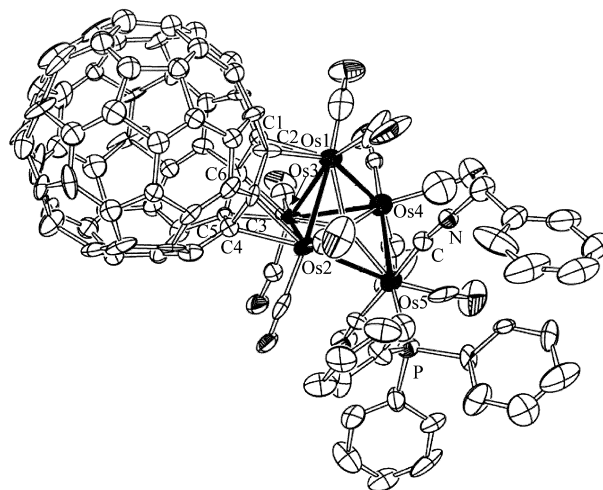


Figure 2. Molecular structure of **3**. Selected bond lengths [Å]: C1–C2 1.51(4), C2–C3 1.41(4), C3–C4 1.46(4), C4–C5 1.52(4), C5–C6 1.40(4), C6–C1 1.45(4), Os1–C1 2.27(2), Os1–C2 2.19(3), Os2–C3 2.26(3), Os2–C4 2.30(3), Os3–C5 2.17(3), Os3–C6 2.24(3).

donor benzyl isocyanide. The four Os atoms Os1, Os2, Os3, and Os4 form a “butterfly” framework, and the remaining osmium atom, coordinated with phosphine and isocyanide ligands, bridges the two “wing-tip” atoms Os2 and Os4. The coordination sphere of the carbido atom can be regarded as a distorted trigonal bipyramid. A similar structural feature has been previously observed in several M₅C clusters, such as [Os₅C(CO)₁₃H{OP(OMe)₂}[P(OMe)₃]],^[6] [Os₅C(CO)₁₅I][–],^[7] and [Ru₅C(CO)₁₅(NCMe)].^[8] It has been proposed that the site of attack by the incoming ligand is the basal metal atom in the reaction of [Ru₅C(CO)₁₅] and MeCN.^[8] Similarly, it can be envisaged that the Os1–Os5 bond in **1** is cleaved by attack of the benzyl isocyanide ligand on the most basal Os5 center with a phosphine ligand to form **3** (Scheme 1).

The molecular structure of the thermodynamic isomer **4** (Figure 3) shows that the μ₃-η²,η²,η²-C₆₀ ligand in **3** has been converted into a μ-η²,η²-C₆₀ ligand on a square-pyramidal Os₅C cluster framework; the two adjacent double bonds in a C₆ ring of the C₆₀ ligand bridge the two basal Os2 and Os3 atoms. The two electrons needed to remove one Os–C₂ (C₆₀) bonding interaction in **3** have been provided by the formation of an Os–Os bond between two nonbonding osmium atoms. The benzyl isocyanide ligand occupies an equatorial position on the basal Os4 atom not bound by either C₆₀ or PPh₃ ligands. In contrast to **2**, however, the C₆ ring positions centrally over

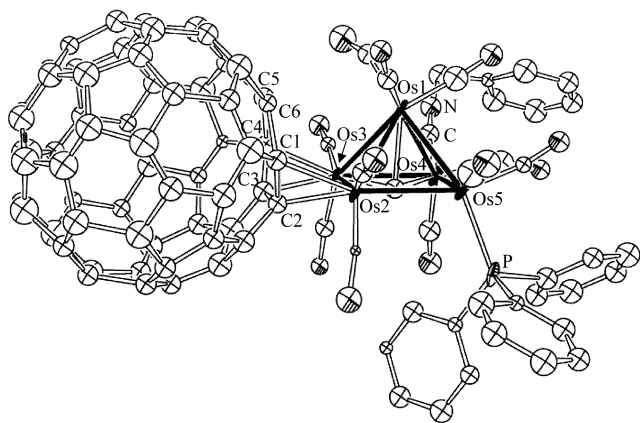


Figure 3. Molecular structure of **4**. Selected bond lengths [Å]: C1–C2 1.40(9), C2–C3 1.45(4), C3–C4 1.45(9), C4–C5 1.55(3), C5–C6 1.29(9), C6–C1 1.54(10), Os2–C1 2.27(8), Os2–C2 2.16(6), Os3–C3 2.33(8), Os3–C4 2.17(4).

the Os₃ triangle, as observed in **1**. Detailed speculation concerning the mechanism of formation of **4** from **3** is unwarranted, but the likely pathway would involve reformation of the Os1–Os5 bond, consecutive migration of the benzyl isocyanide ligand from Os5 to Os4 and of a carbonyl ligand from Os4 to Os1, and subsequent rupture of the Os1–C₂ (C₆₀) interaction.

Importantly, two different orientations of the $\mu\text{-}\eta^2\text{-}\eta^2\text{-C}_{60}$ ligand have been observed in **2** and **4**, which are related by 180° rotation with respect to the center of the two C₆₀-coordinated metal atoms. The reason for the two different orientations is not clear at the moment, but it seems to have an electronic origin since the steric environments around the C₆₀ ligand appear to be essentially identical in **2** and **4**.

In conclusion, we have shown the first examples of the $\mu\text{-}\eta^2\text{-}\eta^2\text{-C}_{60}$ bonding mode on an Os₃C cluster framework (**2** and **4**), and demonstrated interconversion of the $\mu\text{-}\eta^2\text{-}\eta^2\text{-C}_{60}$ and $\mu_3\text{-}\eta^2\text{-}\eta^2\text{-}\eta^2\text{-C}_{60}$ ligands (**1** $\rightleftharpoons$ **2**) by carbonyl addition and elimination reactions. The two different dispositions of the $\mu\text{-}\eta^2\text{-}\eta^2\text{-C}_{60}$ ligand in **2** and **4** may occur by C₆₀ rotation due to electronic reasons. Efforts are currently underway to understand the origin of the different structural features in **2** and **4** and to explore further reactivities of C₆₀–Os₃C clusters.

Experimental Section

Details on the syntheses of **1–4** as well as the interconversion of **1** and **2** are given in the Supporting Information. For the X-ray structure analyses, data were collected at $T=293(2)$ K on a CCD diffractometer with MoK α radiation ($\lambda=0.71073$ Å) using ω scans. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-136396 (**1**), -136394 (**2**), -136397 (**3**), and -136395 (**4**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

1: Elemental analysis calcd for C₉₀H₁₅O₁₁POs₅: C 47.96, H 0.67; found: C 48.13, H 0.78; IR (CS₂): $\tilde{\nu}=2073(\text{s})$, 2032(s), 2021(s), 2011(s) cm⁻¹ (CO); ¹H NMR (300 MHz, CS₂/external CD₂Cl₂): $\delta=7.83\text{--}7.73$ (m, 15H); ³¹P NMR (122 MHz, Cl₂C₆H₄/CDCl₃ 1/1): $\delta=6.09$; FAB-MS (negative-ion): m/z : 2261.8 [M^-].

X-ray crystal data for **1**: Dark red, thin, needlelike crystals were obtained by slow evaporation of a solution of **1** in CS₂ at room temperature. The

crystal (0.14 $\times$ 0.18 $\times$ 0.20 mm) used for data collection contained one molecule of CS₂ and a half a molecule of H₂O (C₉₀H₁₅O₁₁POs₅·CS₂·0.5 H₂O, $M_r=2440.12$): triclinic, space group $P\bar{1}$, $a=10.0093(2)$, $b=13.5022(2)$, $c=26.9630(3)$ Å, $\alpha=77.415(1)^\circ$, $\beta=80.705(1)^\circ$, $\gamma=70.586(1)^\circ$, $V=3338.5(1)$ Å³, $Z=2$, $\rho_{\text{calcd}}=2.326$ g cm⁻³. The structure was solved by direct methods and refined by full-matrix least-squares analysis to give $R=0.1280$ and $R_w=0.3107$ (based on F^2) for 502 variables and 5553 observed reflections with $I>2\sigma(I)$ and $0.78<\theta<22.00^\circ$.

2: Elemental analysis calcd for C₉₁H₁₅O₁₂POs₅: C 47.87, H 0.66; found: C 47.41, H 0.79; IR (CS₂): $\tilde{\nu}=2072(\text{s})$, 2040(m), 2028(m, sh), 2022(s), 2007(m), 1996(w) cm⁻¹ (CO); ¹H NMR (300 MHz, CS₂/external CD₂Cl₂): $\delta=7.79\text{--}7.59$ (m, 15H); ³¹P NMR (122 MHz, CS₂/external CD₂Cl₂): $\delta=-3.87$; FAB-MS (negative-ion): m/z : 2289.8 [M^-].

X-ray crystal data for **2**: Green-brown, platelike crystals (0.13 $\times$ 0.22 $\times$ 0.80 mm) were obtained by slow diffusion of C₆H₁₂ into solution of **2** in CS₂ at room temperature (C₉₁H₁₅O₁₂POs₅, $M_r=2282.00$): monoclinic, space group $C2/c$, $a=41.821(8)$, $b=19.375(4)$, $c=18.550(4)$ Å, $\beta=116.04(3)^\circ$, $V=13504(5)$ Å³, $Z=8$, $\rho_{\text{calcd}}=2.245$ g cm⁻³; $R=0.0606$ and $R_w=0.1535$ (based on F^2) for 942 variables and 6750 observed reflections with $I>2\sigma(I)$ and $1.08<\theta<23.00^\circ$.

3: Elemental analysis calcd for C₉₈H₂₂NO₁₁POs₅: C 49.64, H 0.94, N 0.59; found: C 50.49, H 0.83, N 0.55; IR (CH₂Cl₂): $\tilde{\nu}=2064(\text{s})$, 2046(m), 1997(s), 1990(s), 1955(w), 1933(w) cm⁻¹ (CO); ¹H NMR (300 MHz, CD₂Cl₂): $\delta=7.63\text{--}6.98$ (m, 20H), 4.78 (d, $J=16.4$ Hz, 1H), 4.49 (d, $J=16.4$ Hz, 1H); ³¹P NMR (122 MHz, Cl₂C₆H₄/CDCl₃ 1/1): $\delta=-8.42$; FAB-MS (positive-ion): m/z : 2378.9 [M^+].

X-ray crystal data for **3**: Dark red-brown, needlelike crystals (0.28 $\times$ 0.29 $\times$ 0.78 mm) were obtained by slow diffusion of MeOH into a solution of **3** in CS₂/CH₂Cl₂ (1/1) at room temperature (C₉₈H₂₂NO₁₁POs₅, $M_r=2371.14$): orthorhombic, space group $Fdd2$, $a=48.766(10)$, $b=55.356(11)$, $c=10.054(2)$ Å, $V=27140(9)$ Å³, $Z=16$, $\rho_{\text{calcd}}=2.321$ g cm⁻³; $R=0.0738$ and $R_w=0.1760$ (based on F^2) for 985 variables and 7846 observed reflections with $I>2\sigma(I)$ and $1.11<\theta<23.36^\circ$.

4: Elemental analysis calcd for C₉₈H₂₂NO₁₁POs₅: C 49.64, H 0.94, N 0.59; found: C 50.35, H 0.95, N 0.58; IR (CH₂Cl₂): $\tilde{\nu}=2046(\text{m})$, 2024(vs), 2010(s), 1991(w, br) cm⁻¹ (CO); ¹H NMR (300 MHz, CS₂/external CD₂Cl₂): $\delta=7.68\text{--}7.60$ (m, 20H), 5.54 (s, 1H), 5.47 (s, 1H); ³¹P NMR (122 MHz, CS₂/external CD₂Cl₂): $\delta=-2.87$; FAB-MS (positive-ion): m/z : 2378.9 [M^+].

X-ray crystal data for **4**: Brown-green, thin, platelike crystals were obtained by slow diffusion of $n\text{-C}_7\text{H}_{16}$ into a solution of **4** in CS₂ at room temperature. The crystal (0.12 $\times$ 0.15 $\times$ 0.21 mm) used for data collection contained a half a molecule of CS₂ (C₉₈H₂₂NO₁₁POs₅·0.5 CS₂, $M_r=2409.20$): triclinic, space group $P\bar{1}$, $a=9.970(3)$, $b=17.383(6)$, $c=21.365(10)$ Å, $\alpha=101.62(6)^\circ$, $\beta=99.09(3)^\circ$, $\gamma=98.49(3)^\circ$, $V=3519(2)$ Å³, $Z=2$, $\rho_{\text{calcd}}=2.274$ g cm⁻³; $R=0.1696$ and $R_w=0.4094$ (based on F^2) for 441 variables and 6164 observed reflections with $I>2\sigma(I)$ and $0.99<\theta<22.00^\circ$.

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- [1] a) P. J. Fagan, J. C. Calabrese, B. Malone, *Acc. Chem. Res.* **1992**, 25, 134–142; b) J. R. Bowser, *Adv. Organomet. Chem.* **1994**, 36, 57–94; c) W. Sliwa, *Transition Met. Chem.* **1996**, 21, 583–592; d) A. Stephens, M. L. H. Green, *Adv. Inorg. Chem.* **1997**, 44, 1–43; e) A. L. Balch, M. M. Olmstead, *Chem. Rev.* **1998**, 98, 2123–2165.
- [2] a) P. J. Fagan, J. C. Calabrese, B. Malone, *Science* **1991**, 252, 1160–1161; b) A. L. Balch, J. W. Lee, B. C. Noll, M. M. Olmstead, *Inorg. Chem.* **1993**, 32, 3577–3578; c) R. E. Douthwaite, M. L. H. Green, A. H. H. Stephens, J. F. C. Turner, *J. Chem. Soc. Chem. Commun.* **1993**, 1522–1523; d) H.-F. Hsu, Y. Du, T. E. Albrecht-Schmitt, S. R. Wilson, J. R. Shapley, *Organometallics* **1998**, 17, 1756–1761; e) H. Song, K. Lee, J. T. Park, I.-H. Suh, *J. Organomet. Chem.* **1999**, 584, 361–365.
- [3] a) M. Rasinkangas, T. T. Pakkanen, T. A. Pakkanen, M. Ahlgrén, J. Rouvinen, *J. Am. Chem. Soc.* **1993**, 115, 4901; b) I. J. Mavunkal, Y. Chi, S.-M. Peng, G.-H. Lee, *Organometallics* **1995**, 14, 4454–4456; c) A. N. Chernega, M. L. H. Green, J. Haggitt, A. H. H. Stephens, *J. Chem. Soc. Dalton Trans.* **1998**, 755–767.

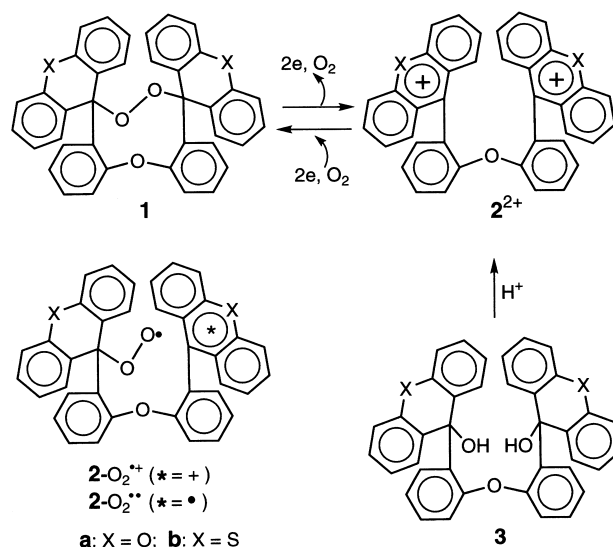
- [4] a) H.-F. Hsu, J. R. Shapley, *J. Am. Chem. Soc.* **1996**, *118*, 9192–9193; b) K. Lee, H.-F. Hsu, J. R. Shapley, *Organometallics* **1997**, *16*, 3876–3877; c) K. Lee, J. R. Shapley, *Organometallics* **1998**, *17*, 3020–3026.
- [5] a) J. T. Park, J.-J. Cho, H. Song, *J. Chem. Soc. Chem. Commun.* **1995**, 15–16; b) J. T. Park, J.-J. Cho, H. Song, C.-S. Jun, Y. Son, J. Kwak, *Inorg. Chem.* **1997**, *36*, 2698–2699; c) J. T. Park, H. Song, J.-J. Cho, M.-K. Chung, J.-H. Lee, I.-H. Suh, *Organometallics* **1998**, *17*, 227–236; d) H. Song, K. Lee, J. T. Park, M.-G. Choi, *Organometallics* **1998**, *17*, 4477–4488.
- [6] J. M. Fernandez, B. F. G. Johnson, J. Lewis, P. R. Raithby, *J. Chem. Soc. Dalton Trans.* **1981**, 2250–2257.
- [7] P. F. Jackson, B. F. G. Johnson, J. Lewis, J. N. Nicholls, M. McPartlin, W. J. H. Nelson, *J. Chem. Soc. Chem. Commun.* **1980**, 564–566.
- [8] B. F. G. Johnson, J. Lewis, J. N. Nicholls, J. Puga, P. R. Raithby, M. J. Rosales, M. McPartlin, W. Clegg, *J. Chem. Soc. Dalton Trans.* **1983**, 277–290.

Preparation, Structure, and Redox Reactions of Nine-Membered Cyclic Peroxides: A Novel Electrochromic System Undergoing Reversible Extrusion and Trapping of O₂**

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Cyclic peroxides having a medium-size ring skeleton have recently attracted much attention from a pharmacological standpoint^[1] and the mechanistic studies of their electron-transfer reactions^[2,3] have provided the basis for a better understanding of their biological activities. The nine-membered peroxides, however, are so rare^[4] that their chemistry has not been well developed. During the course of our study on molecular systems in which the structures and properties can be controlled by electron transfer (ET)^[5] we have found that the dihydrotrioxonin derivatives **1**, a new class of nine-membered cyclic peroxides having a hitherto unknown 1,2,6-trioxacyclononane skeleton, could be generated very easily. Here we report the preparation and X-ray structure of **1** along with their unique electron-transfer behavior. It is worth noting that ET induces the oxidative deoxygenation of peroxides **1** to form the dicationic dyes **2**²⁺, from which compounds **1** are

recovered upon 2e reduction in the presence of O₂ (Scheme 1). This system could be used to electrochemically control O₂ concentration.



Scheme 1. Interconversion between **1** and **2**²⁺ by the oxidative-deoxygenation and reductive-oxygenation reactions.

Diols **3a** and **3b**^[6] were prepared in 54 and 41 % yield, respectively, by the reaction of 2,2'-dilithiodiphenyl ether^[7] with xanthone and thioxanthone. Deeply colored salts **2a**-(BF₄)₂^[6] [λ_{max} (lg ϵ) 480 (sh, 3.73), 450 (3.84), 378 (4.71), 260 nm (4.86) in MeCN] and **2b**-(BF₄)₂^[6] [531 (sh, 3.78), 498 (3.88), 387 (4.47), 282 (5.09)] were obtained in 94 and 91 % yield, respectively, by treating these diols with HBF₄ in (EtCO)₂O.

According to the voltammetric analyses the dicationic dye undergoes facile and reversible 2e reduction (**2a**²⁺: $E_{\text{red}}^{\text{red}} = +0.29$ V (2e); **2b**²⁺: $E_{\text{red}}^{\text{red}} = +0.26$, $E_{\text{red}}^{\text{red}} = +0.05$ V versus the saturated calomel electrode (SCE)),^[8] which indicates that the diradical **2**^{••} is a long-lived species in an argon atmosphere. In contrast, only irreversible reduction waves were observed in both cases when the same solutions were saturated with O₂ (Figure 1). On the basis of a detailed examination of the cyclic voltammograms, it seems likely that the cation radical intermediate **2**^{•+} is readily trapped by O₂ to form (**2-O**)^{•+}, which is further reduced to (**2-O**)^{••} at a slightly more negative potential than **2**²⁺ (ECE process). The resultant diradical (**2-O**)^{••} undergoes ring closure to the entropically disfavored nine-membered ring with unexpectedly high efficiency. Thus, peroxides **1a**^[6] [λ_{max} (lg ϵ) 291 (3.92), 284 nm (sh, 3.90) in MeCN] and **1b**^[6] [285 (sh, 4.02), 267 (4.28)] were isolated as colorless crystals in 80 and 71 % yield, respectively, when the **2**-(BF₄)₂ salts were reduced with Zn powder in aerated THF.^[9]

X-ray analyses^[10] on the novel heterocycles **1** (Figure 2) have revealed the extended conformation of the peroxide moiety (torsion angle for the C-O-O-C unit: 160.8(3)° for **1a**; 154.6(2)° for **1b**), and their O–O bond lengths (1.507(4) Å for **1a**; 1.502(3) Å for **1b**) are two of the longest values ever reported^[11]. Yet, the electrochemical oxidation does not break this weak O–O bond, but instead breaks the adjacent C–O bonds.^[3] It is also interesting to note that deoxygenation and

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

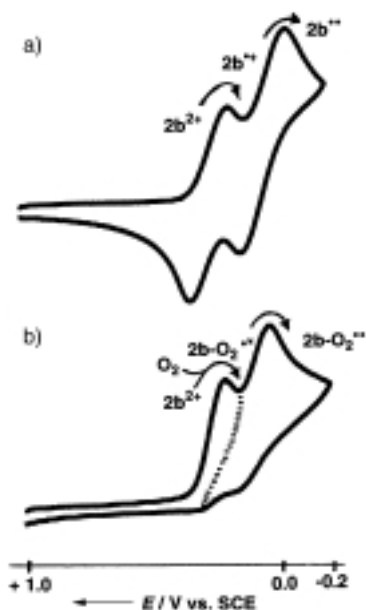


Figure 1. Cyclic voltammogram of dication **2b**²⁺ a) under Ar and b) under O₂ (in CH₂Cl₂ containing 0.1 mol dm⁻³ nBu₄NBF₄, Pt electrode, scan rate 500 mV s⁻¹). The first reduction wave at +0.21 V in (b) is irreversible even when the scanning was reversed at +0.15 V just after the 1e reduction of **2b**²⁺ (dotted line). The voltammogram of **2a**²⁺ measured under O₂ also shows two well separated, irreversible peaks at +0.15 and -0.06 V. Full details of the voltammograms of **1** and **2**²⁺ are given in the Supporting Information.

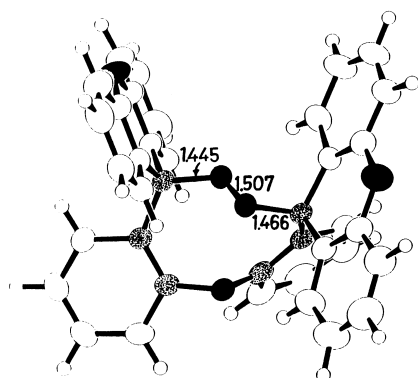


Figure 2. Molecular geometry of peroxide **1a** determined by X-ray analysis with the selected bond lengths. Filled ellipsoids correspond to O atoms and dotted ones indicate C atoms comprising the nine-membered ring. X-ray analysis showed that **1b** adopts a similar molecular structure to **1a** although the deformation of the thioxanthene rings into a butterfly shape is much more pronounced.

not oxygenation is induced upon oxidation. Thus, the voltammograms of **1** show the irreversible oxidation waves (**1a**: +2.08 V; **1b** +1.89 V versus SCE in CH₂Cl₂), and their corresponding reduction peaks appear in the far cathodic region, which were assigned to those of the deoxygenated dications **2**²⁺. Furthermore, dication salts **2a**-(BF₄)₂ and **2b**-(BF₄)₂ were isolated in 85 and 65 % yield, respectively, when the peroxides **1** were treated with two equivalents of NOBF₄ in CH₂Cl₂. When 1.00 mmol of **1a** was treated with 2.06 equivalents of (2,4-Br₂C₆H₃)₃N⁺SbCl₆⁻ in 1,2-dichloroethane **2a**-(SbCl₆)₂ was obtained in 99 % yield. At the same time, 21.5 mL of gas evolved. This amount corresponds to 96 % of

the calculated volume of O₂ that would be released from **1a**.^[12]

These results indicate that **1** and **2**²⁺ constitute a novel redox system that undergoes reversible extrusion and trapping of O₂. The large changes in the UV/Vis spectrum upon electrolysis show the electrochromic nature^[13] of their interconversion (Figure 3). The several isosbestic points observed

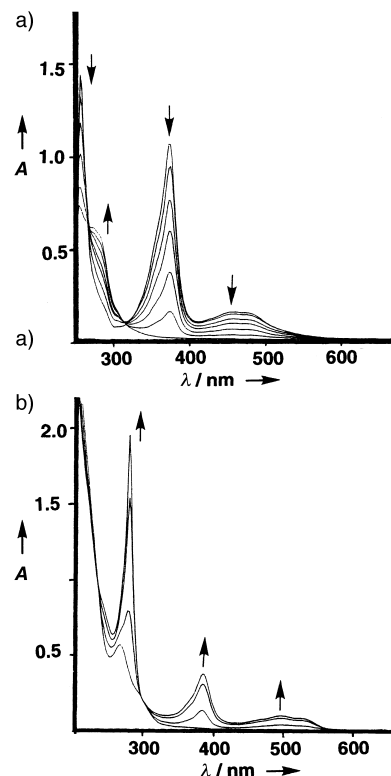


Figure 3. a) Changes in the UV/Vis spectrum of **2a**²⁺ (3.5 mL; 2.07×10^{-5} mol dm⁻³ in MeCN containing 0.05 mol dm⁻³ nBu₄NBF₄) upon constant-current electrochemical reduction (40 μA) at 2 min intervals. b) Changes in the UV/Vis spectrum of **1b** (3.5 mL; 2.24×10^{-5} mol dm⁻³ in MeCN containing 0.05 mol dm⁻³ nBu₄NBF₄) upon constant-current electrochemical oxidation (70 μA) at 6 min intervals.

are indicative of the clean and quantitative transformation between **1** and **2**²⁺ although elucidation of the detailed reaction mechanism awaits further experiments.^[14]

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- [1] A. Robert, B. Meunier, *Chem. Soc. Rev.* **1998**, 27, 273; C. W. Jefford, S. Kohmoto, D. Jaggi, G. Timári, J.-C. Rossier, M. Rudaz, O. Barbuzzi, D. Gérard, U. Burger, P. Kamalaprija, J. Mareda, G. Bernardinelli, I. Manzanares, C. J. Canfield, S. L. Fleck, B. L. Robinson, W. Peters, *Helv. Chim. Acta* **1995**, 78, 647; G. H. Posner, J. N. Cumming, P. Ploypradith, C. H. Oh, *J. Am. Chem. Soc.* **1995**, 117, 5885.
- [2] S. Antonello, M. Musumeci, D. D. M. Wayner, F. Maran, *J. Am. Chem. Soc.* **1997**, 119, 9541; R. L. Donkers, J. Tse, M. S. Workentin, *Chem. Commun.* **1999**, 135.
- [3] Y. Takahashi, K. Wakamatsu, S. Morishima, T. Miyashi, *J. Chem. Soc. Perkin Trans. 2* **1993**, 243; Y. Takahashi, S. Morishima, K. Wakamatsu, T. Suzuki, T. Miyashi, *J. Chem. Soc. Chem. Commun.* **1994**, 13.
- [4] Y. Ushigoe, Y. Torao, A. Masuyama, M. Nojima, *J. Org. Chem.* **1997**, 62, 4949; Y. Takahashi, M. Ando, T. Miyashi, *Tetrahedron Lett.* **1995**, 36, 1889; W. Fenical, *J. Am. Chem. Soc.* **1974**, 96, 5580.

1 $\xrightarrow[2. \text{Ph}_3\text{P}=\text{CH}_2]{1. \text{R}^*\text{ONa}}$ **(+)-2**

(+)-2 + $\xrightarrow[3. \text{MeI}]{1. \text{Yb}(\text{OTf})_3, \text{CH}_2\text{Cl}_2, \text{SO}_2, -90^\circ\text{C}, 24 \text{ h}; 2. \text{Bu}_4\text{NF}}$

3 R = Ph
9 R = Me

$\text{CF}_3\text{COOH} \begin{cases} \text{(-)-4 R = Ph, R}' = \text{R}^* (79\%, \text{d.r.} = 25:1) \\ \text{(-)-5 R = Ph, R}' = \text{H} (88\%) \\ \text{(-)-10 R = Me, R}' = \text{R}^* (86\%, \text{d.r.} = 5.2:1) \\ \text{(-)-11 R = Me, R}' = \text{H} (83\%) \end{cases}$

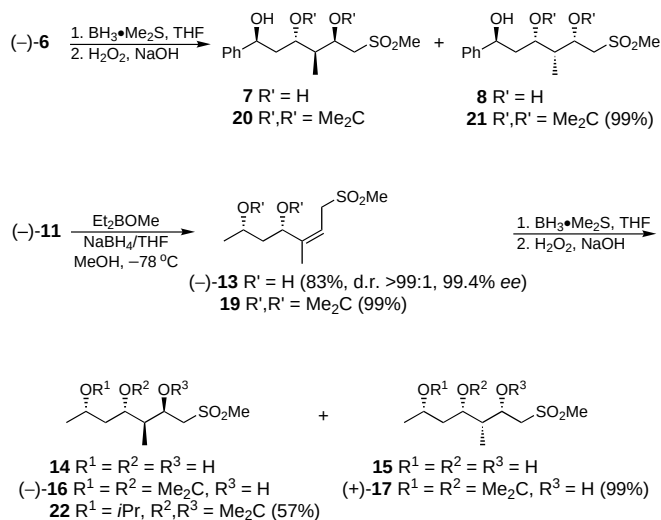
(-)-5 or (-)-11 $\xrightarrow[-40^\circ\text{C}]{(\text{Me}_4\text{N})(\text{AcO})_3\text{BH}, \text{MeCN}, \text{AcOH}}$

(-)-6 R = Ph, R' = H (79%, 99.4% ee)
12 R = Me, R' = H (22-49%, d.r. = 5:1)
18 R = Ph, R', R' = Me₂C (99%)

an excess of SO₂, (+)-**2** reacted with enoxysilane **3** to give a trimethylsilyl sulfinate, which was desilylated with Bu₄NF and treated with MeI to afford a 25:1 mixture of sulfone (–)-**4** and its diastereomer (79% yield, recovery of 20% of (+)-**2**).^[5]

[**] This work was supported by the Swiss National Science Foundation.

Pure (–)-**4** was obtained by flash chromatography on silica gel. Trifluoroacetylation^[6] of (–)-**4** gave (–)-**5** (88% yield), which was reduced with (Me₄N)(AcO)₃BH in MeCN/AcOH (–40 °C, 15 h)^[7] to produce *anti*-diol (–)-**6** (79% yield). Hydroboration of (–)-**6** with BH₃·Me₂S (THF, –8 °C, 24 h) provided a 3.9:1 mixture of triols **7** and **8** (91% yield, Scheme 2).



Scheme 2. Synthesis of polyketides; see text for details.

Under similar conditions, (+)-**2** and SO₂ reacted with enoxysilane **9** to give a 5.2:1 mixture of (–)-**10** and its diastereomer (86% yield). Pure (–)-**10** was obtained by flash chromatography on silica gel. Trifluoroacetylation of (–)-**10** gave aldol (–)-**11**, which was reduced with (Me₄N)(AcO)₃BH in MeCN/AcOH^[7] to form **12** as the major *anti*-diol (Scheme 1). Alternatively, reduction of (–)-**11** with Et₂BOMe/NaBH₄^[8] afforded *syn*-diol (–)-**13** (83% yield, Scheme 2), the hydroboration of which with BH₃·Me₂S in THF (0 °C, 24 h) led to a 2.2:1 mixture of triols **14** and **15** (83%). Their acetonides **16** and **17**, obtained under standard conditions ((Me₂O)₂CMe₂/acetone/pyridinium *p*-toluenesulfonate (PPTS)), could be separated by flash chromatography. The modest diastereoselectivities for the hydroborations of (–)-**6** and (–)-**13** might be due to competing effects arising from A^{1,2} and A^{1,3} allylic strain.^[9]

The structures of (–)-**4** and (–)-**10** were established by single-crystal X-ray radiocrystallography.^[10] The enantiomeric purity (99.4% ee) of diols (–)-**6** and (–)-**13** was determined from the ¹⁷F NMR spectra (¹³C satellite for C–F coupling) of their Mosher diesters^[11] derived from (*S*)- α -methoxy- α -phenylacetyl chloride. The *anti* and *syn* relative configurations of (–)-**6** and (–)-**13**, respectively, were confirmed by the ¹³C NMR spectra of their acetonides **18** (δ = 25.0, 24.7; Me₂C) and **19** (δ = 30.2, 19.6; Me₂C),^[12] obtained upon treatment with (MeO)₂CMe₂ and PPTS. Under similar conditions, triols **7** and **8** were converted selectively into acetonides **20** and **21**; their ¹³C NMR and 2D NOESY ¹H NMR spectra of the latter proved the structures of the former. Treatment of **16** and **17** with acid (AcOH/H₂O) provided pure triols **14** and **15**. Hydroboration of **19** provided

16 as the major alcohol. Further treatment of **16** with BH₃·THF led to a diol that gave acetonide **22** on treatment with (MeO)₂CMe₂/acetone/PPTS (δ = 23.7, 24.9; Me), establishing the relative configurations of **14** and **15**.

An asymmetric version of our four component reaction (1-alkoxy-1,3-diene + SO₂ + enoxysilane + alkyl iodide) has been realized. Exploration of its versatility and its application to the synthesis of polyketides and analogues, as well as studies of its mechanism,^[13] are underway in our laboratory.^[14]

Experimental Section

(–)-**4**: A mixture of (+)-**2** (192 mg, 0.6 mmol), **3** (0.6 mL, 3 mmol), and Yb(OTf)₃ (298 mg, 0.48 mmol) in anhydrous CH₂Cl₂ (6 mL) was degassed on the vacuum line. SO₂, purified by flowing through a column of basic alumina (Merck, activity Is, 1.28 g, 20 mmol), was added at –196 °C. The mixture was stirred at –90 °C for 24 h, and SO₂ was pumped away at –90 °C and then at –65 °C. 1M Bu₄NF in THF (6 mL, 6 mmol) and MeI (1.2 mL, 20 mmol) were added under an argon atmosphere, and the mixture stirred at 0 °C for 1 h and then at 20 °C for 15 h. After addition of H₂O (30 mL), the mixture was extracted with CH₂Cl₂ (5 × 20 mL). The combined organic extracts were dried over MgSO₄. Solvent evaporation and flash chromatography on silica gel (CH₂Cl₂/Et₂O 24/1; R_f ((–)-**4**) = 0.35) afforded (–)-**4** (244 mg, 79%) and (+)-**2** (36 mg, 20%); colorless crystals were obtained by recrystallization from MeOH, m.p. 116–118 °C (MeOH). [α]_D²⁵ = –0.7 (*c* = 1.0, CHCl₃); UV (MeCN): λ_{max} (ϵ) = 241 (5700), 205 nm (12500); IR (KBr): $\tilde{\nu}$ = 3055, 2965, 1685, 1420, 1305, 1265, 895, 745, 705 cm^{–1}; ¹H NMR (400 MHz, CDCl₃): δ = 7.86–7.41 (m, 5H), 7.06–6.98 (m, 2H), 5.55 (ddq, ³J(H-5,H-6) = 9.4, ³J(H-5,H'-6) = 4.7, ⁴J(H-5,Me-C(4)) = 1.3 Hz, H-5), 5.01 (q, ³J(H-1',H-2') = 6.7 Hz, H-1'), 4.70 (dd, ³J(H-3,H-2) = 8.8, ³J(H-3,H'-2) = 4.0 Hz, H-3), 4.03 (dd, ²J(H,H) = 15.2, ³J(H-6,H-5) = 9.4 Hz, H-6), 3.93 (sept, CH*i*Pr), 3.47 (dd, ²J(H,H) = 17.3, ³J(H-2,H-3) = 8.8 Hz, H-2), 3.25 (dd, ²J(H,H) = 15.2, ³J(H-6,H-5) = 4.7 Hz, H'-6), 3.19 (dd, ²J(H,H) = 17.3, ³J(H-2,H-3) = 4.0 Hz, H'-2), 3.12, 2.85 (2 sept, 2 CH*i*Pr), 2.78 (s, MeSO₂), 1.88 (d, ⁴J(Me-C(4),H-5) = 1.3 Hz, Me-C(4)), 1.54 (d, ³J(H-1',H-2') = 6.7 Hz, H₃C(2')), 1.40–1.13 (m, 2 Me₂C), 1.26 (d, ³J(Me,CH) = 6.9 Hz, *i*Pr); elemental analysis calcd for C₃₂H₄₈O₅S ((–)-**4**·MeOH): C 70.55, H 8.88, S 5.88; found: C 70.86, H 8.60, S 5.99.

(–)-**5**: A mixture of (–)-**4** (149 mg, 0.29 mmol), CH₂Cl₂ (4.5 mL), and CF₃COOH (0.3 mL) was stirred at 20 °C for 40 min. A saturated aqueous solution of NaHCO₃ (30 mL) was added, and the mixture extracted with CH₂Cl₂ (3 × 20 mL). The combined organic phases were dried over MgSO₄. Solvent evaporation and flash chromatography on silica gel (CH₂Cl₂/EtOAc) afforded (–)-**5** (72 mg, 88%) as a yellow oil after removal of solvent. [α]_D²⁵ = –52 (*c* = 1.0, CHCl₃).

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- [1] B. Deguin, J.-M. Roulet, P. Vogel, *Tetrahedron Lett.* **1997**, 32, 6197–6200.
- [2] J.-M. Roulet, G. Puh, P. Vogel, *Tetrahedron Lett.* **1997**, 38, 6201–6204.
- [3] P. Delair, A. M. Kanazawa, M. B. M. de Azevedo, A. E. Greene, *Tetrahedron: Asymmetry* **1996**, 7, 2707–2710; A. Kanazawa, P. Delair, M. Pourashraf, A. E. Greene, *J. Chem. Soc. Perkin Trans. 1* **1997**, 1911–1912.
- [4] R. Rieger, E. Breitmaier, *Synthesis* **1990**, 697–701.
- [5] The use of other chiral alcohols such as (*R*)-1-phenyl-1-propanol, (*R*)-1-(2-naphthyl)ethanol, (*R*)-1-phenylethanol, and (*S*)-1-(pentafluorophenyl)ethanol led to lower diastereoselectivities.
- [6] G. H. Posner, D. G. Wettlaufer, *J. Am. Chem. Soc.* **1986**, 108, 7373–7377.
- [7] D. A. Evans, K. T. Chapman, E. M. Carreira, *J. Am. Chem. Soc.* **1988**, 110, 3560–3578.
- [8] K. Nasaraka, F. G. Pai, *Tetrahedron* **1984**, 40, 2233–2238; K. M. Chen, G. E. Hardtmann, K. Prasad, O. Repic, M. J. Shapiro, *Tetrahedron Lett.* **1987**, 28, 155–158.
- [9] R. W. Hoffmann, *Chem. Rev.* **1989**, 89, 1841–1860.

- [10] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-134999 and -135000. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [11] T. A. Dale, H. S. Mosher, *J. Am. Chem. Soc.* **1973**, *95*, 512–519; T. A. Dale, D. L. Dull, H. S. Mosher, *J. Org. Chem.* **1969**, *34*, 2543–2549.
- [12] S. D. Rychnovsky, B. N. Rogers, T. I. Richardson, *Acc. Chem. Res.* **1998**, *31*, 9–17, and references therein.
- [13] For preliminary studies, see T. Fernández, J. A. Sordo, F. Monnat, B. Deguin, P. Vogel, *J. Am. Chem. Soc.* **1998**, *120*, 13276–13277; T. Fernández, D. Suárez, J. A. Sordo, F. Monnat, E. Roversi, A. Estrella de Castro, K. Schenk, P. Vogel, *J. Org. Chem.* **1998**, *63*, 9490–9499; E. Roversi, F. Monnat, K. Schenk, P. Vogel, P. Braña, J. A. Sordo, *Chem. Eur. J.*, in press.
- [14] Preliminary experiments have shown that (*E*)-1-alkoxybutadienes and (*E,E*)-1-alkoxy-2-methylpenta-1,3-dienes can be combined with enoxysilanes **3**, **9**, and others, and with carbon electrophiles different from MeI.

Metamorphic Channels in Periodic Mesoporous Methylenesilica**

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Since the discovery of mesoporous silica, MCM-41, in 1992,^[1, 2] the synthesis of mesoporous inorganic materials using supramolecular organic templates continues to draw a great deal of attention due to the potential application of these materials in catalysis,^[3] nanoelectronics,^[4] separation science,^[5] and environmental remediation.^[6] The composition of mesoporous materials span a variety of inorganic compounds, including a range of oxides,^[7, 8] Pt metal,^[9] phosphates,^[10] CdS,^[11] and M/Ge₄S₁₀,^[12] but, until very recently,^[13–16] none have included organic moieties as a structural component of the framework.

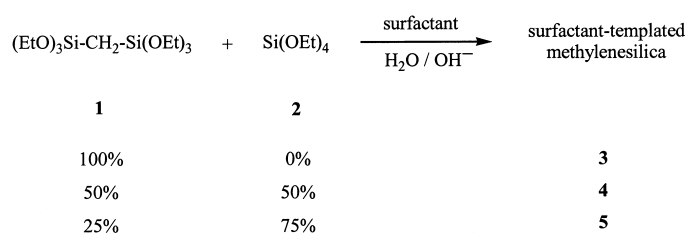
A vast number of organic, inorganic, organometallic, and polymeric species have been included inside the periodic hexagonal channels of MCM-41.^[17] In particular, many species have been anchored or grafted to the channel wall of the silica by synthesis or postsynthesis treatment with RSi(OR')₃ compounds.^[17, 18] These organic groups, which protrude into the channels, have potential for many useful transformations, catalysis, separations, and other interesting chemistry.

We and others have recently reported an interesting class of materials possessing organic groups fused “within” the mesoporous frameworks.^[13–16] These novel organic–inorganic hybrid materials, denoted periodic mesoporous organosilicas (PMOs), are prepared through the surfactant-templated condensation of bifunctional organosiloxane precursors, (R'O)₃SiRSi(OR')₃.^[19] This new approach extends the realm of mesoporous materials to “chemistry of the channel walls” rather than limiting it to “chemistry of the void space”. In contrast to mesoporous materials that have terminally bonded organic groups dangling into the channels, PMOs offer a unique opportunity to affect the chemistry of the framework through organic transformations. The physical and chemical properties of these materials may be modified with suitable choice of organic and organometallic groups (R) inside the channel wall of the PMO. Examples of PMOs containing ethane, ethene, acetylene, ferrocene, thiophene, and benzene inside the framework have been reported.^[13–16] Here we report the discovery of thermally robust periodic mesoporous methylenesilica and demonstrate a unique thermal transformation in which bridging methylene groups “inside the channel walls” convert into terminally bound methyl groups residing “within the channel spaces”.

Methylene-bridged PMOs are also an interesting class of materials from the point of view of fundamental theory and applications. As the simplest organic group, methylene is a useful moiety to study for sophisticated ab initio calculations.^[20, 21] Methylene is also useful for the preparation of silicon carbides and oxycarbides.^[22, 23] Since methylene is isoelectronic with oxygen, periodic mesoporous methylenesilica is a useful analogue for structural, mechanical, and electronic comparison with the widely studied periodic mesoporous silica. Moreover, periodic mesoporous methylenesilica may be useful as a precursor for additional chemical modifications.

To prepare hexagonal mesoporous methylenesilica, we used a procedure similar to that of other PMOs.^[13, 14] With a surfactant as the template, samples were synthesized with different proportions of methylene groups (Scheme 1). For this, a mixture of two precursors was used: bis(triethoxysilyl)methane (**1**), containing the bridging methylene groups, and tetraethoxysilane (**2**). The cationic cetyltrimethylammonium surfactant template was then removed from the samples by solvent extraction with methanol/hydrochloric acid.

The mesoporous methylenesilica materials were investigated by powder X-ray diffraction (PXRD) and transmission electron microscopy (TEM) before and after solvent extrac-



Scheme 1. Synthesis of the hexagonal mesoporous methylenesilica. The ratio of **1** and **2** corresponds to the molar contribution of Si from each reagent (i.e., **5** was prepared from BTM **1** and TEOS **2** in a molar ratio of 1:6; see the Experimental Section).

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tion. The surfactant-free material was also investigated by gas adsorption. Figure 1 shows PXRD patterns of as-synthesized and solvent-extracted materials and a representative TEM image of as-synthesized **3**. The presence of (100), (110), and (200) peaks in the diffraction patterns is characteristic of well-ordered, hexagonal mesoporous materials. Moreover, the PXRD peak intensities decreased as the relative amount of

pore diameter of 31 Å, which in conjunction with the unit cell dimension of 46 Å estimated from PXRD and TEM provide a wall thickness of 15 Å.

Fourier transform micro-Raman spectroscopy (FT-MRS) and solid-state cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP-MAS and ^{29}Si MAS NMR) confirmed that the methylene groups survive hydrolysis, condensation and solvent-extraction conditions, and afterwards retain their integrity inside the channel walls of the PMO. The Raman spectrum of **3** showed the $\nu(\text{C-Si})$ bond stretching mode at 860 cm^{-1} , which remains unchanged after solvent extraction. The intensity of this peak, when compared with other resonances, increased with the relative amount of bis(triethoxysilyl)methane **1** (BTM) used in the synthesis. The ^{13}C CP-MAS NMR spectrum also indicates the presence of methylene groups in the materials. A broad resonance at $\delta \approx 0$ corresponds to the $\text{Si-CH}_2\text{-Si}$ carbon atoms, whose relative intensity with respect to the surfactant increases with the relative amount of **1** used in the synthesis. The peak is broad, consistent with methylene groups present in a variety of microenvironments inside a rigid methylenesilica framework. These data show that the methylene groups are incorporated into the materials and that the Si-C bond remained intact under the synthesis conditions. The latter was also confirmed by ^{29}Si MAS NMR spectroscopy, in which only T sites (T_x , $\text{RSi}(\text{OSi})_x(\text{OH})_{3-x}$) and no Q sites (Q_x , $\text{Si}(\text{OSi})_x(\text{OH})_{4-x}$) were observed in sample **3**. Furthermore, ^{29}Si MAS NMR spectroscopy confirmed that the Si-C bond is stable to solvent extraction.

The thermal properties of the materials were studied using a combination of thermogravimetric analysis (TGA) under N_2 and solid-state NMR spectroscopy. The TGA of **3** shows a weight loss at $35\text{--}80^\circ\text{C}$ corresponding to the evolution of adsorbed water and a weight loss at $150\text{--}300^\circ\text{C}$ corresponding to the removal of surfactant. Unlike periodic mesoporous ethylenesilica we previously prepared,^[13] the periodic mesoporous methylenesilicas **3–5** showed little weight loss above 300°C , indicating the stability of the methylene spacers at higher temperatures. ^{13}C CP-MAS NMR spectroscopy of samples **3** and **4** treated at 400°C for 4 h in air showed a peak at $\delta \approx -3$ corresponding to hydrocarbons attached to silicon and a small peak at $\delta \approx 53$ attributed to a trace of surfactant. Figure 2A shows the ^{13}C CP-MAS NMR spectra of **4** after treatment at different temperatures. After thermal treatment at 500°C for 4 h, all surfactant peaks had disappeared and only the peak at $\delta \approx -3$ remained. This peak appeared even after the same sample was treated in air at 550 and 600°C for 4 h. The ^{29}Si MAS/CP-MAS NMR spectra of the same samples showed the presence of T sites after treatment at 400 , 550 , and 600°C (Figure 2B). Pyrolysis mass spectrometry of a sample of **3** (with surfactant completely removed by thermal treatment in air for 7 h at 400°C) showed fragments consistent with the elimination of small organic and organosiloxane molecules at 550°C , which could be satisfactorily associated with the thermal decomposition of methylenesilica.

These results indicated that the materials still contained stable organic groups attached to silicon atoms when treated thermally in air at temperatures as high as 600°C . There was, however, a continuous conversion of framework T sites into Q

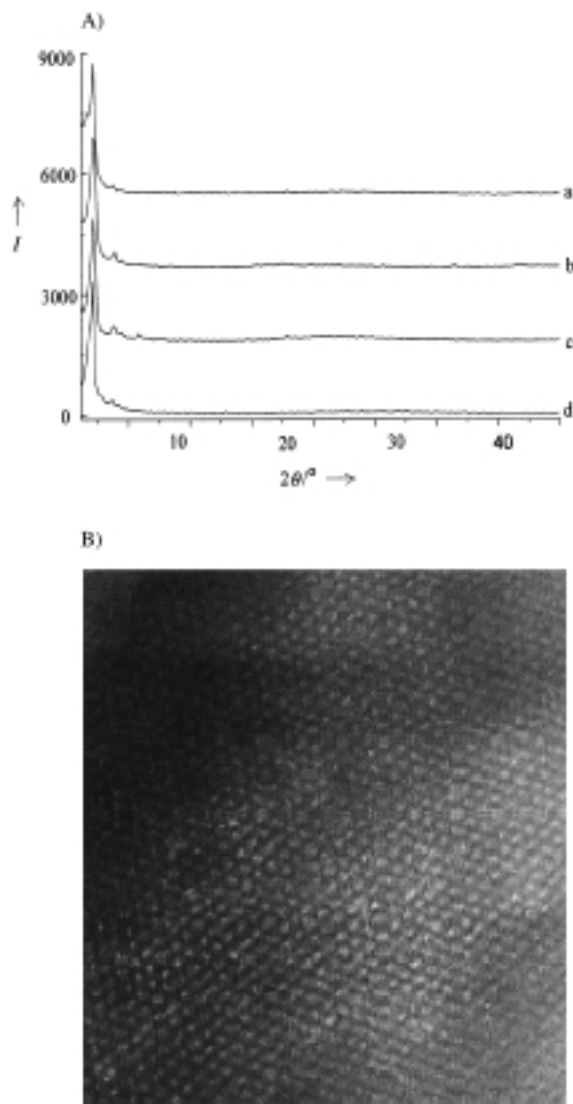


Figure 1. A) PXRD patterns for as-synthesized samples of **3** (a), **4** (b), and **5** (c), and solvent-extracted **3** (d). B) TEM image of as-synthesized **3**.

the methylene precursor used increased. This is consistent with the decreased electron contrast expected between the wall and the channel with the incorporation of methylene species inside the silica framework. After solvent extraction, the materials showed an enhanced intensity diffraction pattern (Figure 1A). The TEM investigation of the PMOs confirmed that they were well organized and have the hexagonal structure (Figure 1B). The N_2 adsorption isotherm of the materials showed a type IV isotherm with little hysteresis revealing the mesoporosity of the materials with a Brunauer–Emmett–Teller (BET) surface area over $900\text{ m}^2\text{ g}^{-1}$, a total pore volume of $0.72\text{ cm}^3\text{ g}^{-1}$, and an average

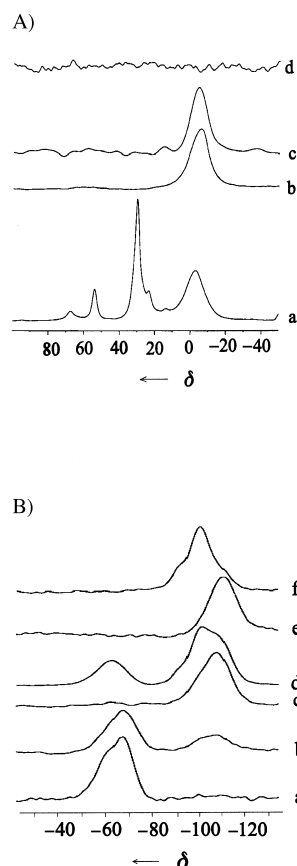


Figure 2. A) ^{13}C CP-MAS NMR spectra of as-synthesized **4** (a) and **4** thermally treated in air to 400 (b), 550 (c), and 700 °C (d). B) ^{29}Si MAS NMR spectra of as-synthesized **4** (a) and **4** thermally treated in air to 400 (b), 550 (c) and 700 °C (e); CP-MAS NMR spectra of **4** thermally treated in air to 550 (d) and 700 °C (f). Thermal treatment was performed sequentially for the same sample in air for 4 h at each temperature.

control, an NQS experiment performed on the as-synthesized sample **4** did not show a methylene peak near $\delta = 0$, but showed only resonances for the surfactant carbon atoms. With the same parameters, the ^{13}C CP-MAS NQS NMR spectrum of the pyrolyzed sample **4** showed a peak at $\delta = -6$, which was unequivocally assigned to methyl carbon atoms attached to silicon (Figure 3) since the methylene carbon atoms are not observable under these conditions. The normal ^{13}C CP-MAS NMR spectra of the same pyrolyzed sample **4** displayed a relatively broader peak from $\delta = -3$ to -6 , showing that both methyl and methylene groups are present in the sample. These results demonstrate that an interesting transformation began at around 400 °C. Cleavage of one $\text{Si}-\text{CH}_2$ bond of bridging methylene groups upon addition of residual silanol groups or water in air yielded methyl groups terminally bonded to the silicon, (Scheme 2). This is the first observation of a chemical transformation from a bridging organic group bonded inside the framework of a mesoporous material to a terminally bound organic suspended within the channel space.^[24] To

sites, as shown by the increase in intensity of signals for Q sites at the expense of those for T sites as the treatment temperature increased from 400 to 700 °C. Complete conversion occurred between 600 and 700 °C as there were no observable T sites for the sample treated at 700 °C, as shown by the ^{29}Si CP-MAS NMR spectrum. The meso-scale periodicity of the materials, however, was maintained after each of the thermal treatment steps with a concomitant decrease in d spacing from about 40 to 35 Å after pyrolysis at 700 °C for 4 h.

As the quantity of Q sites increased at the expense of T sites, we were curious about the nature of the organic species present in the thermally treated materials. Due to the breadth of the peak and similarity in chemical shift for $\text{Si}-\text{CH}_2-\text{Si}$ and $\text{Si}-\text{CH}_3$, a challenge remained to assign the broad peak observed at $\delta \approx -3$ in the ^{13}C CP-MAS NMR spectra to methylene and/or methyl groups. For this, we applied a nonquaternary suppression (NQS) pulse sequence to the ^{13}C CP-MAS NMR spectrum for the selective observation of methyl groups. As a control,

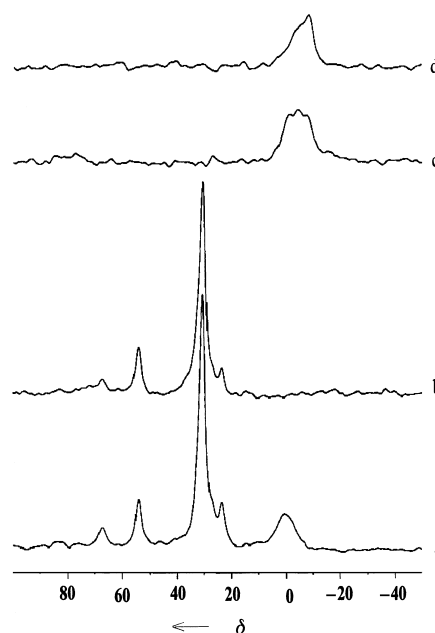
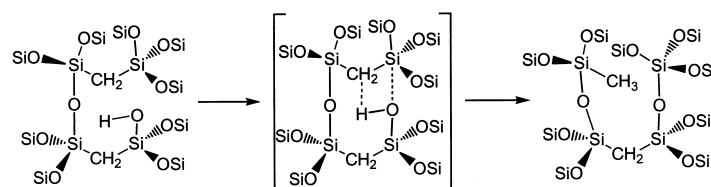


Figure 3. ^{13}C CP-MAS NMR (a) and ^{13}C NQS CP-MAS NMR (b) spectra of **4** before being thermally treated in air and ^{13}C CP-MAS NMR (c) and ^{13}C NQS CP-MAS NMR (d) after thermal treatment in air for 4 h at 550 °C.



Scheme 2. Schematic representation of the cleavage of one $\text{Si}-\text{CH}_2$ bond to a bridging methylene group, leading to methyl groups terminally bonded to silicon.

prove that the methyl groups originated from the bridging methylene spacers inside the channel walls and not from the surfactant imbibed within the channel void space, we pyrolysed an as-synthesized mesoporous silica (MCM-41) sample under the same conditions—the product did not show any trace of methyl or methylene groups.

Although the formation of methyl from methylene groups upon thermal treatment in air was probed with the NQS experiment, quantification of the methyl and methylene moieties was not possible with this CP experiment. It is noteworthy that the pyrolysis of periodic mesoporous methylenesilica under Ar resulted in a similar transformation of the methylene spacers, the organic moieties again being stable up to 700 °C. However, other pyrolysis products, such as aromatic species, were also observed in the sample under these conditions. The arenes most likely originated from the methylene/methyl groups, as they appeared in the ^{13}C CP-MAS NMR spectra ($\delta = 128$) only above temperatures at which the surfactant was thermally decomposed.

In conclusion, we have prepared a new PMO material, periodic mesoporous methylenesilica, with bridging methylene spacers incorporated “inside” the walls of the hexagonally close packed channels. This new material shows enhanced chemical and thermal stability relative to other PMOs

prepared to date. We have also described the first example of “channel metamorphosis” of a PMO; in this case, the chemical transformation occurs from organosilica channels containing bridging organic moieties to ones having terminally bonded organic groups located in the mesopores.

Experimental Section

3–5: A base-catalyzed surfactant-templating sol–gel technique was employed using NH_4OH (14.18 g, 0.122 mol) as base, cetyltrimethylammonium bromide (CTAB; 0.672 g, 0.00184 mol), H_2O (26.73 g, 1.485 mol), and **1** and **2** as silica sources (molar ratio $\text{Si}:\text{CTAB}:\text{NH}_4\text{OH}:\text{H}_2\text{O} = 1.00:0.12:15.0:114$). These reagents were combined and stirred for 30 min to form a homogeneous solution. To that solution, Si (0.015 mol) from a mixture of BTM **1** and tetraethoxysilane (TEOS) **2** (100 % BTM for **3**, 50 % BTM for **4**, and 25 % BTM for **5**) was slowly added. BTM (Gelest, used without further purification) was considered to provide twice as many moles of Si as moles of TEOS in the above composition (i.e., the sample of 50 % BTM (**4**) was prepared from 1.32 g of BTM (3.86 mmol) and 1.61 g of TEOS (7.72 mmol)). The resulting solution was stirred for 30 min and then aged at 80 °C for 4 d. After filtration and washing, the product was dried under ambient conditions. Yields: **3**: 1.40 g, **4**: 1.19 g, **5**: 1.36 g. To prepare the solvent-extracted materials, a sample of **3–5** (0.5 g) was stirred in a solution of 10 M HCl (12 g) and methanol (70 g) for 24 h at 40 °C. The product was filtered, washed with methanol, and dried under ambient conditions. Yield: **3**: 0.32 g.

The PXRD, TEM, TGA, and pyrolysis mass spectrometry investigations were carried out as reported previously.^[12c] The N_2 adsorption and desorption measurements were conducted at 77 K with a Quantachrome Autosorb 1 instrument by immersing the sample in liquid nitrogen. The FT micro-Raman spectra were obtained on a S.A. LabRam confocal Raman microscope. The signal collected was transmitted through a fiber optic cable into a single grating spectrometer equipped with a 1024×256 element CCD. Raman spectra were collected by manually placing the probe tip, with micron spatial resolution, near the desired point of the sample on a glass slide. Identical Raman spectra were obtained over the entire area of the sample, confirming the homogeneous distribution of methylene groups in the material. The ^{13}C (100.6 MHz) and ^{29}Si (79.5 MHz) solid-state NMR spectra were obtained with a Bruker DSX400 spectrometer. Experimental conditions: ^{13}C CP-MAS NMR: 6.5 kHz spin rate, 2.5 ms contact time, 3 s recycle delay, 20000 scans; ^{29}Si MAS NMR: 6.5 kHz spin rate, 100 s recycle delay, 800 scans; ^{13}C NQS CP-MAS NMR: 6.5 kHz spin rate, 30 s recycle delay, 30000 scans.

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- [1] C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature* **1992**, 359, 710–712.
- [2] J. S. Beck, J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T.-W. Chu, D. H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, J. L. Schlenker, *J. Am. Chem. Soc.* **1992**, 114, 10834–10843.
- [3] a) J. P. G. Pater, P. A. Jacobs, J. A. Martens, *J. Catal.* **1999**, 184, 262–267; b) M. S. Morey, A. Davidson, G. D. Stucky, *J. Porous Mater.* **1998**, 5, 262–267.
- [4] a) J. C. Vartulli, S. S. Shih, C. T. Kresge, J. S. Beck, *Mesoporous Mol. Sieves* **1998**, 117, 13–21; b) L. Z. Wang, J. L. Shi, J. Yu, D. S. Yan, *J. Inorg. Mater.* **1999**, 14, 333–342.
- [5] a) H. Hata, S. Saeki, T. Kimura, Y. Sugahara, K. Kuroda, *Chem. Mater.* **1999**, 11, 1110–1119; b) K. M. Kemner, X. Feng, J. Liu, G. E. Fryxell, L. Q. Wang, A. Y. Kim, M. Gong, S. Mattigod, *J. Synchrotron Radiat.* **1999**, 6, 633–635; c) D. Y. Zhao, P. D. Yang, Q. S. Huo, B. F. Chmelka, G. D. Stucky, *Curr. Opin. Solid State Mater. Sci.* **1998**, 3, 111–121.
- [6] a) X. Feng, G. E. Fryxell, L.-Q. Wang, A. Y. Kim, J. Liu, K. M. Kemner, *Science* **1997**, 276, 923–926; b) L. Mercier, T. J. Pinnavaia, *Adv. Mater.* **1997**, 9, 500–503.

- [7] D. M. Antonelli, J. Y. Ying, *Angew. Chem.* **1996**, 108, 461–464; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 426–430.
- [8] Z.-R. Tian, W. Tong, J. Y. Wang, N. G. Duan, V. V. Krishnan, S. L. Suib, *Science* **1997**, 276, 926–930.
- [9] G. S. Attard, C. G. Göltner, J. M. Corker, S. Henke, R. H. Templer, *Angew. Chem.* **1997**, 109, 1372–1374; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1315–1317.
- [10] U. Ciesla, S. Schacht, G. D. Stucky, K. K. Unger, F. Schüth, *Angew. Chem.* **1996**, 108, 597–600; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 541–543.
- [11] P. V. Braun, P. Osenar, S. I. Stupp, *Nature* **1996**, 380, 325–328.
- [12] a) M. J. MacLachlan, N. Coombs, G. A. Ozin, *Nature* **1999**, 397, 681–684; b) K. K. Rangan, S. J. L. Billinge, V. Petkov, J. Heising, M. G. Kanatzidis, *Chem. Mater.* **1999**, 11, 2629–2632; c) M. J. MacLachlan, N. Coombs, R. L. Bedard, S. White, L. K. Thompson, G. A. Ozin, *J. Am. Chem. Soc.* **1999**, 121, 12005–12017.
- [13] T. Asefa, M. J. MacLachlan, N. Coombs, G. A. Ozin, *Nature* **1999**, 402, 867–871.
- [14] C. Yoshina-Ishii, T. Asefa, N. Coombs, M. J. MacLachlan, G. A. Ozin, *Chem. Commun.* **1999**, 2539–2540.
- [15] S. Inagaki, S. Guan, Y. Fukushima, T. Oshuna, and O. Terasaki, *J. Am. Chem. Soc.* **1999**, 121, 9611–9614.
- [16] B. J. Melde, B. T. Holland, C. F. Blanford, A. Stein, *Chem. Mater.* **1999**, 11, 3302–3308.
- [17] a) G. A. Ozin, E. Chomski, D. Khushalani, M. J. MacLachlan, *Curr. Opin. Interf. Sci.* **1998**, 3, 181–193; b) K. Moller, T. Bein, *Chem. Mater.* **1998**, 10, 2950–2963.
- [18] For example, see a) S. L. Burkett, S. D. Sims, S. Mann, *Chem. Commun.* **1996**, 11, 1367–1368; b) M. Koya, H. Nakajima, *Mesoporous Mol. Sieves* **1998**, 117, 243–248; c) M. H. Lim, C. F. Blanford, A. Stein, *J. Am. Chem. Soc.* **1997**, 119, 4090–4091.
- [19] Bifunctional precursors, such as $(\text{R}'\text{O})_3\text{SiSi}(\text{OR}')_3$, have previously been incorporated into amorphous xerogels. See, for example, a) G. Cerveau, R. J. P. Corriu, *Coord. Chem. Rev.* **1998**, 80, 1051–1071; b) D. A. Loy, G. M. Jamison, B. M. Baugher, S. A. Myers, R. A. Assink, K. J. Shea, *Chem. Mater.* **1996**, 8, 656–663; c) G. Cerveau, R. J. P. Corriu, N. Costa, *J. Non-Cryst. Solids* **1993**, 163, 226–235; d) D. A. Loy, G. M. Jamison, B. M. Baugher, E. M. Russick, R. A. Assink, S. Prabakar, K. J. Shea, *J. Non-Cryst. Solids* **1995**, 186, 44–53.
- [20] J. F. Harrison, *Acc. Chem. Res.* **1968**, 1, 17–22.
- [21] W. S. Staker, K. D. King, G. J. Gutsche, W. D. Lawrance, *Ber Bunsen-Ges. Phys. Chem.* **1998**, 102, 238–243.
- [22] R. J. P. Corriu, D. Leclercq, P. H. Mutin, A. Vioux, *Chem. Mater.* **1992**, 4, 711–716.
- [23] Q. Liu, W. Shi, F. Babonneau, L. V. Interrante, *Chem. Mater.* **1997**, 9, 2434–2441.
- [24] Similar chemistry has been observed in the thermolysis of hydrolyzed poly(dialkoxysilylenemethylene), $[(\text{RO})_2\text{SiCH}_2]_n$, a polymeric analogue of the methylenesilica; see a) Q. Liu, W. Shi, F. Babonneau, L. V. Interrante, *Chem. Mater.* **1997**, 9, 2434–2441; b) G. D. Sorarú, Q. Liu, L. V. Interrante, T. Apple, *Chem. Mater.* **1998**, 10, 4047–4054.

Electropolymerization of π -Conjugated Oligomers Containing Phosphole Cores and Terminal Thienyl Moieties: Optical and Electronic Properties**

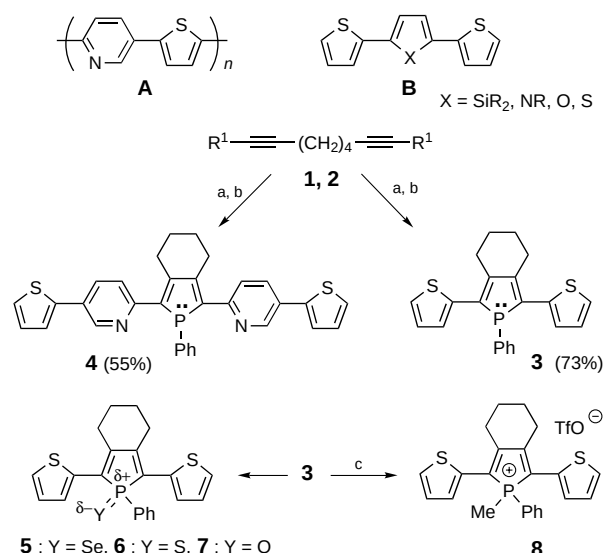
Caroline Hay, Cédric Fischmeister, Muriel Hissler, Loïc Toupet, and Régis Réau*

Dedicated to Professor Pierre H. Dixneuf on the occasion of his 60th birthday

Thiophene-based π -conjugated oligomers and polymers have recently emerged as very promising organic devices for electronic and optical technologies.^[1] The optoelectronic properties of these materials vary with the degree of extended conjugation and the inherent electron density of the π -conjugated backbone. A recent strategy to modulate these two parameters involves the synthesis of macromolecules featuring more than one type of conjugated-ring unit along the main chain.^[2] Increased conjugation can be achieved in alternating copolymers such as **A** (Scheme 1) because of an intramolecular charge transfer (ICT) from the electron-donor thienyl to the electron-acceptor pyridinyl moieties.^[3] The derivatives of **B**, the 2,5-bis(2-thienyl)heterocyclopentadienes,

are not only versatile synthons for the preparation of copolymers,^[2c, 4] but also act as well defined models suitable for studying the dramatic influence of the electronic properties of the central heteroatom and its effect on the degree of conjugation between the consecutive subunits. Silole derivatives of **B** ($X = \text{SiR}_2$) possess a higher degree of conjugation than thiophene and pyrrole analogues ($\Delta\lambda_{\text{max}} = 60 \text{ nm}$) due to ICT involving the electron-accepting silicon-containing heterocyclopentadiene.^[4e, 5] Phosphole-containing π -conjugated systems have been poorly investigated,^[6] in spite of the phospholes possessing limited aromatic character and a reactive phosphorus atom,^[7] which affords a family of chromophores with different electronic properties. Herein, we describe new chromophores featuring phosphole-based cores and terminal thienyl moieties, and the preparation of the first phosphole-containing polymers through electro-oxidation.

Besides 2,5-bis(2-thienyl)phosphole **3** (Scheme 1), a phosphorus-containing derivative of **B**, the mixed oligomer **4** was chosen as a target, since **4** should possess alternating donor and acceptor units as phospholes are generally considered electron-rich heterocyclopentadienes.^[8] Derivatives **3** and **4** were prepared through an intramolecular coupling of diynes **1**^[9a] and **2**^[9b] via a zirconacyclopentadiene intermediate^[5, 10] and addition of PhPBr_2 (Scheme 1). Phospholes **3** and **4** are obtained as air-stable orange solids after purification by flash chromatography on alumina (yields: **3**: 73%; **4**: 55%). Their NMR spectra show typical resonance patterns and the simplicity of the ^{13}C NMR spectra indicates a symmetric structure.^[11] The endocyclic phosphole carbon atoms give two signals at low field, whereas only one set of signals is observed for the two substituents at the 2,5 positions. The proposed structures were confirmed by X-ray analysis performed on the free phosphole **3** (Figure 1) and $\text{W}(\text{CO})_5$ -coordinated **4**^[12]



Scheme 1. Synthesis of the chromophores featuring phosphole cores and terminal thienyl moieties. Synthesis: a) $[\text{Cp}_2\text{ZrCl}_2]$, 2 BuLi, THF, -78°C ; b) PhPBr_2 , THF; c) MeOTf. Cp = C_5H_5^- ; OTf = OSO_2CF_3 ; R = alkyl, aryl; R¹ = 2-thienyl (**1**), 5-(2-thienyl)-2-pyridyl (**2**).

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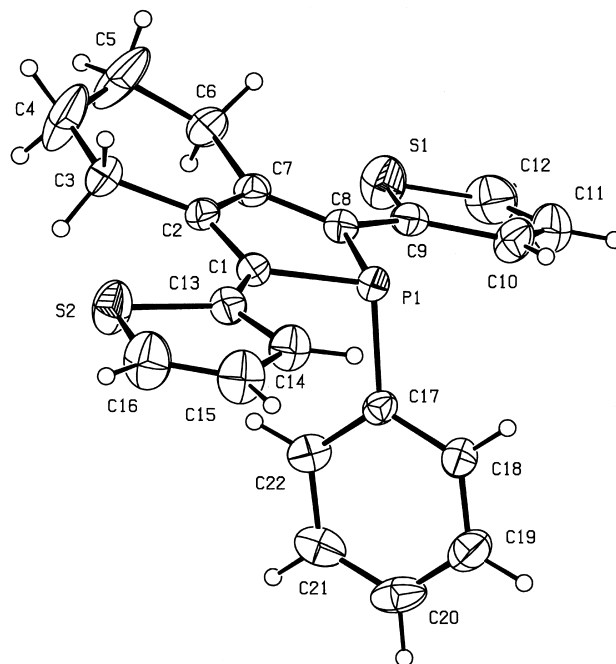


Figure 1. X-ray structure analysis of **3**. Hydrogen atoms have been omitted for clarity. Selected bond lengths [$\text{\AA}$] and angles [$^\circ$]: P1-C1 1.817(4), C1-C2 1.336(6), C2-C7 1.465(7), C7-C8 1.356(6), C1-C13 1.436(6), C8-C9 1.457(6); C1-P1-C8 90.9(2), C1-P1-C17 104.3(2), C8-P1-C17 104.1(2).

(Figure 2).^[13] In both cases, the thienyl rings exhibit a statistical disorder (90–60% for the prevailing conformations), a feature which is typical for linear oligomers that bear terminal thiophene rings.^[3a, 4d, 14] For both compounds, the

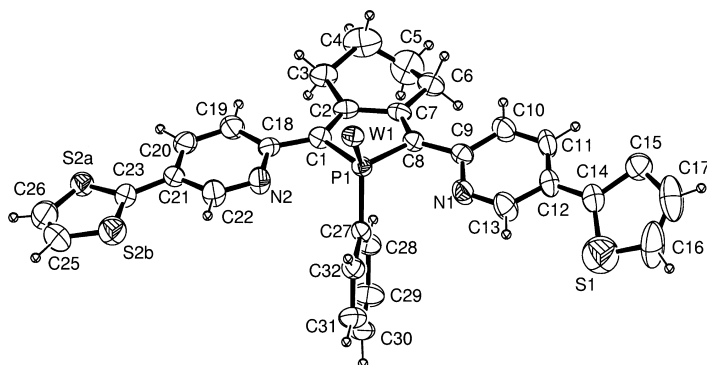


Figure 2. X-ray structure analysis of $[\text{W}(\text{CO})_5(\mathbf{4})]$. Hydrogen atoms and carbonyl ligands have been omitted for clarity. Selected bond lengths [Å] and angles [°]: P1–C1 1.822(9), C1–C2 1.362(12), C2–C7 1.466(13), C7–C8 1.341(12), C1–C18 1.444(12), C8–C9 1.476(12), C12–C14 1.445(14), C21–C23 1.469(13), P1–W1 2.534(2); C1–P1–C8 90.7(4), C1–P1–C27 104.3(4), C8–P1–C27 104.4(4).

twist angles between two adjacent rings are small (**3**: 16.7(5)° and 12.5(5)°; $[\text{W}(\text{CO})_5(\mathbf{4})]$: 1.8(16)–18.3(17)°) and allow an extended π conjugation. The C–C links between the rings (**3**: 1.436(6)–1.457(6) Å; $[\text{W}(\text{CO})_5(\mathbf{4})]$: 1.444(12)–1.476(12) Å) and the endocyclic C2–C7 bond (**3**: 1.465(7) Å; $[\text{W}(\text{CO})_5(\mathbf{4})]$: 1.466(13) Å) are relatively short, whereas the endocyclic P–C bond lengths (**3** and $[\text{W}(\text{CO})_5(\mathbf{4})]$: 1.817(4)–1.822(9) Å) approach that of a P–C single bond (1.84 Å). These solid-state data are in favour of an extended delocalisation path involving the dienic moiety of the phosphole rings and the two thienyl or 5-(2-thienyl)pyridyl units.

The absorption maximum in the UV/Vis spectrum of compound **4** ($\lambda_{\text{max}} = 427$ nm) is at a notably longer wavelength than that recorded for 2,5-bis(2-dipyridyl)phosphole ($\lambda_{\text{max}} = 390$ nm^[15]) and clearly indicates an extended π conjugation. The fluorescence spectrum of this oligomer shows an emission maximum at 570 nm, which reveals a large Stokes shift ($\Delta\lambda = 143$ nm). Remarkably, **3** shows an absorption at 412 nm, a wavelength only slightly shorter than that of **4**^[16] but considerably longer than those observed for the related derivatives of **B** that feature central electron-rich rings, such as pyrrole derivatives ($\text{X} = \text{N}-\text{CH}_3$, $\lambda_{\text{max}} = 322$ nm)^[4d] or group 16 heterocyclopentadienes ($\lambda_{\text{max}} = 366$ (X = O),^[2e] 356 nm (X = S)^[5b]). This unexpected, relatively high value for λ_{max} is within the range recorded for 2,5-bis(2-thienyl)siloles (X = SiPh₂, $\lambda_{\text{max}} = 418$ nm), which possess an unusually low HOMO–LUMO gap due to ICT.^[5] This surprising analogy between silicon- and phosphorus-containing heterocyclopentadienes^[17] should be even more pronounced for σ^4, λ^4 -phospholes, which have no aromatic character and feature an electropositive tetrahedral heteroatom. We prepared electrically neutral σ^4, λ^4 -phospholes with polarised $\text{P}^{\delta+}=\text{Y}^{\delta-}$ bonds (Y = O, S, Se) and cationic σ^4, λ^4 -phospholes in order to evaluate the influence of the electron density of the phosphorus atom on the optical and electronic properties of the chromophore **B**. Derivative **3**

was treated with selenium, elemental sulfur, bis(trimethylsilyl)peroxide, and methyl trifluorosulfonate affording σ^4, λ^4 -phospholes **5** (91% yield), **6** (93% yield), **7** (95% yield), and **8** (95% yield), respectively (Scheme 1). The λ_{max} of derivatives **5–8** are red shifted with respect to that of **3** and, suggesting ICT, this effect is weak for the $\text{P}^{\delta+}=\text{Y}^{\delta-}$ derivatives **5–7** and stronger for the phospholium salt **8** (Table 1). Compounds **5–8** have comparable high energy absorptions, comparable with

Table 1. UV/Vis, fluorescence, and electrochemical data for **3–8**.

Compound	3	4	5	6	7	8
λ_{max} [nm] ^[a]	412	427	423	432	434	442
lg ϵ	3.94	4.53	4.09	3.98	3.97	3.87
λ_{em} [nm] ^[b]	501	570	547	548	556	593
E_{pa} [V] ^[c]	+1.01	+1.10	+1.28	+1.27	+1.26	+1.52

[a] In THF. [b] Maximum wavelength in emission spectra; in THF. [c] Sample concentration 1 mM; solution 0.1M LiClO₄ in MeCN; scan rate 100 mV s^{−1}. Oxidation potential (versus standard calomel electrode (SCE)) measured against a ferrocene internal standard ($E_{\text{p1/2}} = 0.5$ V versus SCE, $\Delta E_{\text{p}} = 70$ mV).

those of reported chromophores **B** bearing a central thiophene-S-oxide unit ($\lambda_{\text{max}} = 424–428$ nm).^[10c] The tuning of the emission properties through derivatization is more pronounced. Oxidation of the phosphorus atom causes a bathochromic shift of λ_{em} in the fluorescence spectra ($\Delta\lambda_{\text{em}} = 46–92$ nm; Table 1) and a notable increase of the Stokes shifts (**3**: $\Delta\lambda = 89$ nm; **5–8**: $\Delta\lambda = 116–151$ nm). The wavelengths of the emission bands of chromophores **3–8** cover a range of approximately 92 nm.

Electropolymerization of **B** derivatives is usually accomplished by oxidation proceeded by coupling of cation radicals.^[4c] Indeed, derivatives **3** and **5–8** were oxidized irreversibly and with anodic potentials clearly related to the electron density of the central phosphole rings (Table 1). The anodic peak potentials (E_{pa}) range from +1.01 V for σ^3, λ^3 -phosphole **3** to +1.52 V for cation **8**, whereas compounds **5–7** show intermediate E_{pa} values (+1.27 V). Derivatives **5–8** are oxidized at higher potentials than **4** bearing the terminal thienylpyridyl groups (+1.10 V, irreversible). These data in total clearly show that the optical and electronic properties of phosphorus-containing chromophores **B** are considerably influenced by the properties of the central phosphorus atom.

No films formed on the surface of the platinum working electrode after oxidation of monomers **3** and **4** (containing a σ^3, λ^3 -phosphole unit) or of cation **8**. In contrast, films formed on the electrode surface with compounds **5–7** (a typical polymerization voltammogram is shown in Figure 3a). Repeated scan cycling results in increased activity of the electrode surface, which is indicative of electroactive polymer growth. Interestingly, the polymers formed have E_{pa} values (Figure 3b) dependent on the nature of the Y chalcogen substituent ($E_{\text{pa}} = +1.63$ (Y = Se), +1.54 (Y = S), +1.38 V (Y = O)). It should be noted that to deposit films, applied potentials must lie between those of the monomer and of the corresponding polymer. This behaviour is probably related to the fact that the radical cations involved in the polymerization^[4c] are not stabilized by delocalisation on the positively charged phosphole rings.

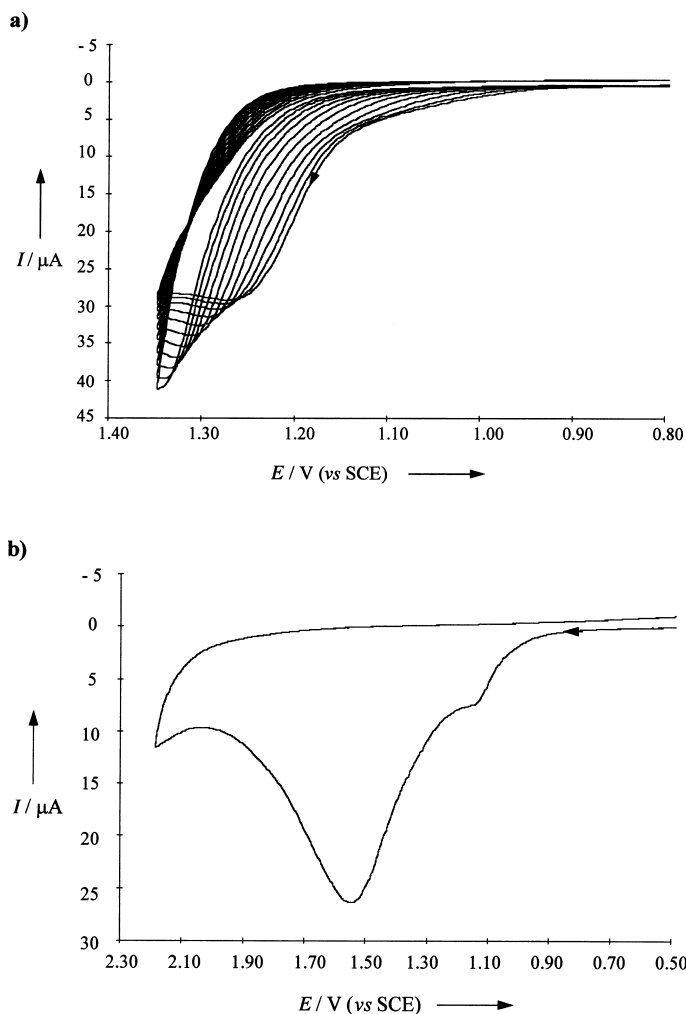


Figure 3. a) Anodic polymerization of **6** and b) anodic cyclic voltammogram of the corresponding polymer using a 0.03 cm² Pt button electrode in 0.1 mM LiClO₄ in MeCN solution at scan rate of 100 mV s⁻¹.

In conclusion, we have prepared a new family of mixed donor–acceptor molecules with chromophores possessing tunable optical and electronic properties that may be modified via chemical procedures. The unprecedented electropolymerization of phosphole-containing derivatives opens the route to a range of new materials upon functionalization of the initial σ^3, λ^3 -phosphole cores. Metal-containing polymers are now important targets.^[18]

Experimental Section

A solution of BuLi in hexanes (5.32 mL, 1.6 M, –78 °C) was added to a solution of [Cp₂ZrCl₂] in THF (1.18 mg, 4.06 mmol, 40 mL). The mixture was allowed to warm to –60 °C and a solution of **1** in THF (1.0 g, 3.70 mmol, 40 mL) was added dropwise. The mixture warmed to room temperature and neat PhPBr₂ (0.84 mL, 4.07 mmol) was added. The solution was stirred for 4 h and the solvent removed under vacuum. Phosphole **3** was isolated by flash column chromatography on basic alumina (THF, R_f = 0.7) as an orange solid (1.02 g, 73 %, m.p. 205 °C (dec.)).

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- [1] For recent reviews, see: a) A. Kraft, A. C. Grimsdale, A. B. Holmes, *Angew. Chem.* **1998**, *110*, 416; *Angew. Chem. Int. Ed.* **1998**, *37*, 402; b) *Electronic Materials: The Oligomer Approach* (Eds.: K. Müllen, G. Wegner), WILEY-VCH, Weinheim, **1998**; c) J. Roncali, *Chem. Rev.* **1997**, *97*, 173; d) F. Garnier, *Acc. Chem. Res.* **1998**, *32*, 209.
- [2] For recent contributions, see: a) N. C. Greenham, S. C. Moratti, D. D. C. Bradley, R. H. Friend, A. B. Holmes, *Nature* **1993**, *365*, 628; b) Q. T. Zhang, J. T. Tour, *J. Am. Chem. Soc.* **1998**, *120*, 5355; c) T. Yamamoto, Z. Zhou, T. Kanbara, M. Shimura, K. Kizu, T. Muruyama, Y. Nakamura, T. Fukada, B. Lee, N. Ooba, S. Tomaru, T. Kurihara, T. Kaino, K. Kubota, S. Sasaki, *J. Am. Chem. Soc.* **1996**, *118*, 10389; d) F. Demanze, A. Yassar, F. Garnier, *Macromolecules* **1996**, *29*, 4267; e) A. Hücke, M. P. Cava, *J. Org. Chem.* **1998**, *63*, 7413.
- [3] a) D. J. Irvin, C. J. Dubois, J. R. Reynolds, *Chem. Commun.* **1999**, 1211; b) Z. Zhou, T. Maruyama, T. Kanbara, T. Ikeda, K. Ichimura, T. Yamamoto, K. Tokuda, *J. Chem. Soc. Chem. Commun.* **1991**, 1210.
- [4] For electrochemical routes, see: a) H. Röckel, J. Huber, R. Gleiter, W. Schuhmann, *Adv. Mater.* **1994**, *6*, 568; b) P. Audebert, J. M. Catel, G. Le Coustumer, V. Duchenet, P. Hapiot, *J. Phys. Chem. B* **1998**, *102*, 8661; c) M. Tsionsky, A. J. Bard, D. Dini, F. Decker, *Chem. Mater.* **1998**, *10*, 2120; d) R. E. Niziurski, C. C. Scordilis-Kelley, T. L. Liu, M. P. Cava, R. T. Carlin, *J. Am. Chem. Soc.* **1993**, *115*, 887. For chemical routes, see: e) K. Tamao, S. Yamagushi, M. Shiozaki, Y. Nakagawa, Y. Ito, *J. Am. Chem. Soc.* **1992**, *114*, 5867; f) R. E. Niziurki-Mann, M. P. Cava, *Adv. Mater.* **1993**, *5*, 547; g) A. H. Mustafa, M. K. Shepherd, *Chem. Commun.* **1998**, 2743.
- [5] a) S. Yamagushi, K. Tamao, *J. Chem. Soc. Dalton Trans.* **1998**, 3693; b) S. Yamagushi, Y. Itami, K. Tamao, *Organometallics* **1998**, *17*, 4910.
- [6] a) E. Deschamps, L. Ricard, F. Mathey, *Angew. Chem.* **1994**, *106*, 1214; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1158; b) S. H. Mao, T. D. Tilley, *Macromolecules* **1997**, *30*, 5566; Mathey et al. have prepared derivatives containing α -thienyl- α -phospholyl links: c) M.-O. Bevierre, F. Mercier, L. Ricard, F. Mathey, *Angew. Chem.* **1990**, *102*, 672; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 655; d) E. Deschamps, F. Mathey, *J. Org. Chem.* **1990**, *55*, 2494; e) E. Deschamps, L. Ricard, F. Mathey, *Heteroatom Chem.* **1991**, *3*, 377.
- [7] a) F. Mathey, *Chem. Rev.* **1988**, *88*, 437; b) L. D. Quin, *Comprehensive Heterocyclic Chemistry* (Ed.: A. R. Katritzky), Pergamon, Oxford, **1996**, p. 757.
- [8] I. Albert, T. Marks, M. Ratner, *J. Am. Chem. Soc.* **1997**, *119*, 6575.
- [9] a) S. Yamagushi, R. Z. Jin, K. Tamao, F. Sato, *J. Org. Chem.* **1998**, *63*, 10060. b) Diyne **2** was obtained in 50 % yield through a Stille coupling involving 2-trimethylstannylthiophene and 1,8-bis[2-(4-bromopyridyl)]-hexa-1,7-diyne.
- [10] a) P. J. Fagan, W. A. Fagan, J. C. Calabrese, *J. Am. Chem. Soc.* **1994**, *116*, 1880; b) X. Sava, N. Mézaille, N. Maigrot, F. Nief, L. Ricard, F. Mathey, P. Le Floch, *Organometallics* **1999**, *18*, 4205; c) B. J. Jiang, T. D. Tilley, *J. Am. Chem. Soc.* **1999**, *121*, 9744.
- [11] Selected physical data: **3**: ¹³C NMR (75.469 MHz, CD₂Cl₂, 25 °C, TMS): δ = 23.1 (s, CH₂), 29.3 (s, CH₂), 124.8 (d, J(P,C) = 2.0 Hz, SCH), 125.6 (d, J(P,C) = 10.2 Hz, SCHCHCH), 126.8 (s, SCHCH), 128.7 (d, J(P,C) = 9.2 Hz, phenyl C_{meta}), 129.6 (s, phenyl C_{para}), 133.5 (d, J(P,C) = 13.3 Hz, phenyl C_{ipso}), 133.9 (d, J(P,C) = 19.5 Hz, phenyl C_{ortho}), 135.7 (s, PCCCH₂), 139.7 (d, J(P,C) = 22.1 Hz, SCCP), 144.4 (d, J(P,C) = 9.1 Hz, PCCCH₂); ³¹P NMR (81.014 MHz, CD₂Cl₂, 25 °C, 80 % H₃PO₄): δ = +12.7 (s); HR-MS (EI): m/z: calcd: 378.0651; found: 378.0666 [M]⁺. **4**: ¹³C NMR (75.469 MHz, C₆D₆, 25 °C, TMS): δ = 23.2 (s, CH₂), 29.8 (s, CH₂), 123.3 (d, J(P,C) = 10.2 Hz, py CH), 123.6, 125.2, 128.0 (3 × s, thienyl CH), 128.6 (d, J(P,C) = 7.8 Hz, phenyl C_{meta}), 129.2 (s, phenyl C_{para}), 132.5 (s, py CH), 133.6 (d, J(P,C) = 14.0 Hz, phenyl C_{ipso}), 134.3 (d, J(P,C) = 18.8 Hz, phenyl C_{ortho}), 141.0 (s, thienyl C_{ipso}), 144.4 (d, J(P,C) = 3.9 Hz, PCCCH₂), 146.5 (d, J(P,C) = 1.5 Hz, NCH), 149.08 (d, J(P,C) = 9.4 Hz, PCCCH₂), 154.9 (d, J(P,C) = 20.3 Hz, NC_{ipso}), NCC_{ipso} is not observed; ³¹P NMR (81.014 MHz, C₆D₆, 25 °C, 80 % H₃PO₄): δ = +11.3 (s); HR-MS (FAB): m/z: calcd: 533.1274; found: 533.1275 [M+H]⁺.
- [12] The UV/Vis spectra of oligomer **4** in the free state and in the coordination sphere of W(CO)₅ have similar λ_{max} . Single crystal X-ray diffraction studies of complex [W(CO)₅(**3**)] (to be published) revealed minor modifications of the geometric data compared to the free phosphole **3**.

- [13] X-ray structural analysis. **3**: $C_{22}H_{19}S_2P$, $0.40 \times 0.35 \times 0.33$ mm, hexagonal, space group $R\bar{3}$, $a = 33.059(5)$, $c = 9.001(2)$ Å, $V = 8519(3)$ Å³, $Z = 18$, $\rho_{\text{calcd}} = 1.328$ mgm⁻³, $2\theta_{\text{max}} = 54^\circ$, $\lambda(\text{MoK}\alpha) = 0.71073$ Å, ω scan, 293 K, 2319 measured reflections, 2307 independent, observed $I > 2\sigma(I)$, Lorentz and polarization correction ($\mu = 3.67$ cm⁻¹), 227 refined parameters with $R1 = 0.0582$ and $wR2 = 0.1649$, max./min. residual electron density $0.335/-0.304$ e Å⁻³. **4**: $C_{37}H_{25}N_3O_3PS_2W$, $0.45 \times 0.40 \times 0.40$ mm, monoclinic, space group $C2/c$, $a = 26.378(7)$, $b = 22.272(2)$, $c = 15.479(7)$ Å, $\beta = 123.99(2)^\circ$, $V = 7540.4(4)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.571$ mgm⁻³, $2\theta_{\text{max}} = 54^\circ$, $\lambda(\text{MoK}\alpha) = 0.71073$ Å, ω scan, 293 K, 8218 measured reflections, 8044 independent, observed $I > 2\sigma(I)$, Lorentz and polarization correction ($\mu = 33.31$ cm⁻¹), 437 refined parameters with $R1 = 0.0573$ and $wR2 = 0.1615$. After Lorentz and polarization corrections with HELENA, the structures were solved with SIR-97, and refined with SHELX93 by full-matrix least squares on F^2 . Programs: HELENA: A. L. Spek, 1997, Utrecht University, The Netherlands; SIR-97: A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **1998**, *31*, 74; SHELX93: G. M. Sheldrick, 1993, Universität Göttingen, Germany. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137515 and -137516. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [14] G. Barbarella, M. Zambianchi, L. Antolini, P. Ostojia, P. Maccagnari, A. Bongini, E. A. Marseglia, E. Tedesco, G. Gigli, R. Cingolani, *J. Am. Chem. Soc.* **1999**, *121*, 8920, and references therein.
- [15] D. Le Vilain, C. Hay, V. Deborde, L. Toupet, R. Réau, *Chem. Commun.* **1999**, 345.
- [16] Theoretical calculations in order to understand the effect of the central phosphorus atom on the relative properties of 2,5-bis(2-pyridyl)- and 2,5-bis(2-thienyl)phospholes are in progress.
- [17] Recent theoretical works have predicted comparable electronic properties for polyphosphole and polysilole: U. Salzner, J. B. Lagowski, P. G. Pickup, R. A. Poirier, *Synth. Met.* **1998**, *96*, 177.
- [18] R. P. Kingsborough, T. M. Swager, *J. Am. Chem. Soc.* **1999**, *121*, 8825.

The First Structurally Characterized Metal–SeH Compounds: [LAl(SeH)₂] and [L(HSe)AlSeAl(SeH)L]**

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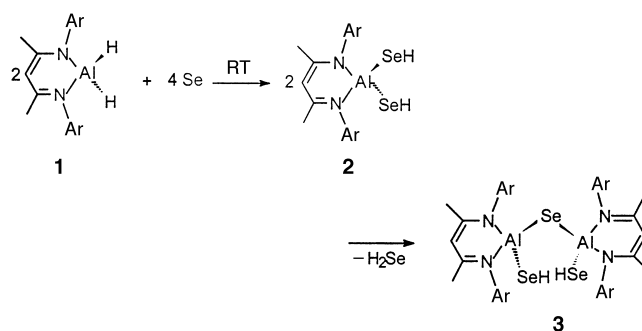
Dedicated to Professor Jürgen Troe
on the occasion of his 60th birthday

Aluminum compounds containing Group 16 elements have been widely studied due to their important applications in chemical vapor deposition (CVD) and catalysis.^[1] We have recently shown that organoaluminum dihydrides are very

useful precursors for preparing aluminum chalcogenides of the formula [(RAIE)_n] (R = organic ligand; E = S, Se, Te; $n = 2, 4$).^[2] A few novel intramolecularly stabilized aluminum chalcogenides, [[N(SiMe₃)C(Ph)C(SiMe₃)₂AlE]₂]^[2a] and [(ArAlE)₂] (Ar = 2,6-(Et₂NCH₂)₂C₆H₃, 2-Et₂NCH₂-6-MeC₆H₃; E = Se, Te),^[2b] have been synthesized. We became interested in employing the same synthetic route using other ligand systems with the aim of studying the mechanism of the reaction and synthesizing monomeric aluminum chalcogenides [RAIE]. Previously we predicted that this reaction proceeds through hydroselenide (SeH) or hydrotelluride (TeH) intermediates.^[2] Barron et al. and Oliver et al. discussed the same intermediates for the reaction of Group 13 trialkyl compounds with H₂Se.^[3] However, no stable compounds of Group 13 elements with the SeH ligand have been isolated so far. In fact, only a few transition metal complexes (Co, Cr, Fe, Ir, Mn, Pt, Re, Ta, Ti) containing the SeH ligand (both terminal and bridging) are known, but they have not been structurally characterized.^[4] Herein we report on the first stable aluminum SeH compounds with the β-diketiminato ligand.

Reaction of the β-diketimine LH (L = N(Ar)C(Me)-CHC(Me)N(Ar), Ar = 2,6-*i*Pr₂C₆H₃)^[5] with AlH₃·NMe₃ at room temperature in *n*-hexane leads to the dihydride [LAlH₂] (**1**; see Scheme 1) in high yield. The IR spectrum shows typical asymmetric and symmetric absorptions^[6] for Al–H at 1832 and 1795 cm⁻¹, and the mass spectrum gives the highest peak at $[M^+ - H]$, indicating the formation of a monomeric dihydride. Compound **1** is related to [(*i*Pr)₂(ati)AlH₂] (ati = 2-aminotroponimine), which has a monomeric structure in the solid state.^[6b]

Compound **1** reacts smoothly with two equivalents of elemental selenium at room temperature to afford [LAl(SeH)₂] (**2**) in modest yield (Scheme 1). It is noteworthy that **2** is formed even when only one equivalent of Se is used for the



Scheme 1. Synthesis of **2** and **3**. Ar = 2,6-*i*Pr₂C₆H₃.

reaction. Compound **2** is not stable in solution (*n*-hexane, THF) over a longer period of time at room temperature, and it slowly eliminates H₂Se to give pale yellow [L(HSe)AlSeAl(SeH)L] (**3**). Compound **3** can also be obtained by the reaction of **1** with two equivalents of Se at 60 °C.

Compounds **2** and **3** have been characterized by multinuclear NMR spectroscopy, mass spectrometry as well as elemental analysis. The ¹H NMR spectra of **2** and **3** show a high-field singlet at $\delta = -2.82$ and -2.83 , respectively, which

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[**] L = N(Ar)C(Me)CHC(Me)N(Ar), Ar = 2,6-*i*Pr₂C₆H₃. This work was supported by the Deutsche Forschungsgemeinschaft

can be assigned to SeH.^[7] The IR spectra also give weak SeH stretching frequencies at 2318 cm⁻¹ for **2** and at 2276 and 2292 cm⁻¹ for **3**.^[4c] The ¹H NMR spectrum of **3** shows two septets for the CHMe₂ protons (*i*Pr groups), indicating C_s symmetry of the molecule. The ⁷⁷Se NMR spectrum of **3** gives two broad signals ($\delta = -341$ and -535) in an approximate intensity ratio of 2:1; the former can be assigned to SeH.^[8]

The molecular structures of **2** and **3** were determined by X-ray crystallography.^[9] Yellow crystals of **2** suitable for X-ray structure analysis were grown from *n*-hexane at -30°C . Unfortunately, the quality of the crystal structure did not allow location of the hydrogen atoms on the selenium atoms. Nevertheless, the presence of terminal SeH groups was established unequivocally by IR and ¹H NMR spectroscopy. The molecular structure of **2** is shown in Figure 1. The Al–Se bond lengths (234.0 and 233.1 pm) are in line with those of the dimeric $[[[N(\text{SiMe}_3)\text{C}(\text{Ph})\text{C}(\text{SiMe}_3)_2]\text{AlSe}]_2]$ (av 235 pm).^[2] The Al–N bond distances of **2** are equal, and the Al atom deviates slightly from the NCCCN plane of the ligand.

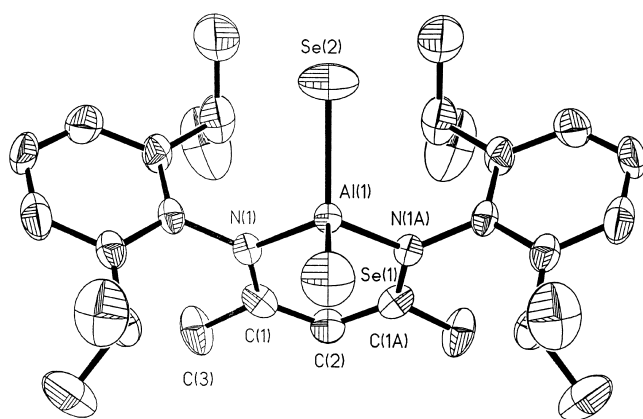


Figure 1. Molecular structure of **2** in the crystal. Hydrogen atoms have been omitted for clarity. Selected bond distances [pm] and angles $^\circ$: Al(1)–N(1) 189.9(6), Al(1)–N(1A) 189.9(6), Al(1)–Se(2) 233.1(3), Al(1)–Se(1) 234.0(3); N(1)–Al(1)–N(1A) 97.1(4), N(1)–Al(1)–Se(2) 113.5(2), N(1A)–Al(1)–Se(2) 113.5(2), N(1)–Al(1)–Se(1) 114.8(2), N(1A)–Al(1)–Se(1) 114.8(2), Se(2)–Al(1)–Se(1) 103.73(12).

The structure of compound **3** is shown in Figure 2.^[9] The Al–Se bond lengths (232.6 and 233.3 pm) of the Al–Se–Al unit are among the shortest reported so far. They are slightly shorter than those of the Al–SeH units (237.5 and 237.1 pm) due to the more highly polarized Al–Se–Al bonds. The internal angle at Se(2) (116.88°) is much more open than those of compounds with the dimeric Al₂Se₂ core (av 78°),^[2] and is only slightly larger than that of $[[[N(\text{SiMe}_3)_2\text{CH}]_2\text{Al}]_2\text{Se}]$ (114.71°),^[10] probably due to the bulky nature of the ligand L. The hydrogen atoms could be located, by a ΔF synthesis, at the selenium atoms (Se–H ca. 150 pm); free refinement, however, shortens this distance (as expected for hydrogen atoms on heavy atoms). There is very little information in the literature on the Se–H bond. The Se–H bond lengths computed (with the MINI-1, 3-21-G/LWD and STD-3G basis sets) for organic RSeH compounds range from 142 to 157 pm.^[11] Compounds **2** and **3** are well separated monomers

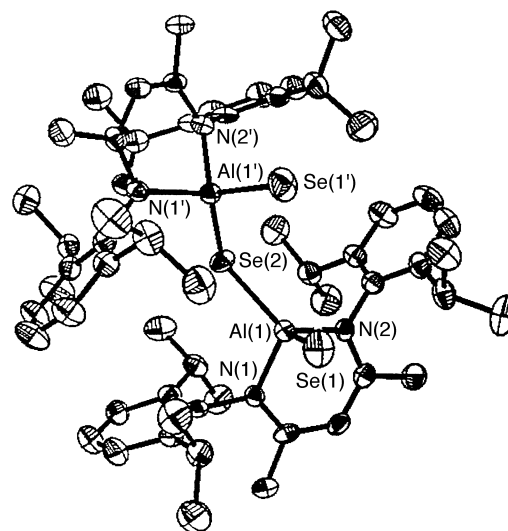


Figure 2. Molecular structure of **3** in the crystal. Hydrogen atoms have been omitted for clarity. Selected bond distances [pm] and angles $^\circ$: Al(1)–N(2') 191.2, Al(1)–N(1') 191.8(5), Al(1)–Se(2) 232.6(2), Al(1)–Se(1') 237.5(2), Al(1)–N(2) 190.6(5), Al(1)–N(1) 190.6(5), Al(1)–Se(2) 233.3, Al(1)–Se(1) 237.1(2); N(2)–Al(1)–N(1') 96.7(2), N(2)–Al(1)–Se(2) 108.2(2), N(1)–Al(1)–Se(1') 109.5(2), N(1)–Al(1)–Se(1) 106.0(2), Se(2)–Al(1)–Se(1') 117.29(9), N(2)–Al(1)–N(1) 97.3(2), N(2)–Al(1)–Se(2) 115.4(2), N(1)–Al(1)–Se(2) 108.5(2), N(2)–Al(1)–Se(1) 107.0(2), N(1)–Al(1)–Se(1) 107.5(2), Se(2)–Al(1)–Se(1) 118.69(8), Al(1)–Se(2)–Al(1) 116.77(7).

in the solid state, which excludes the presence of any intermolecular hydrogen bonds. Furthermore, **2** and **3** are the first structurally characterized organometallic compounds which contain metal–SeH units.

The X-ray structural analysis of **2** and **3** disclose the lability of the Se–H bonds,^[12] and provide direct evidence that the tendency to form hydrogen bonds is much lower than for the lighter congeners (S, O).^[13] The stabilization of **2** and **3** might be attributed to the bulky chelating ligand. Owing to the acidic nature of the SeH moiety, compounds **2** and **3** might be used as synthons for preparing Se-bridged heterometallic compounds. Moreover, the synthesis of **2** and **3** suggests that the selenium atom can insert very easily into the Al–H bond.

An explanation for why the reaction of $[\text{LiAlH}_2]$ with one equivalent of selenium yields **2** instead of $[\text{LiAl}(\text{H})\text{SeH}]$ is presently not available. It is difficult to monitor the reaction by NMR spectroscopy due to its heterogeneous nature. However, it is supposed that the Se₂ unit is involved in this reaction. It has been argued^[14c] that reactions of diorgano-diselenides with $\text{AlH}_3 \cdot \text{NMe}_3$ proceed by initial complexation of a selenium species, creating a hypervalent aluminum center.^[14a, 14b] According to this argument the reaction of $[\text{LiAlH}_2]$ with selenium proceeds by coordination of a Se₂ unit to the aluminum center, followed by a concerted reduction of the activated Se₂ by the two hydride ligands to cleave the Se₂ unit. The conversion of **1** to **2** demonstrates the ‘umpolung’ of the hydridic to the protonic form of the hydrogen atoms. This assumption can well explain our experimental results that **2** is always the product formed at room temperature, regardless of the amount of selenium used. The conversion of **2** into **3** clearly indicates that intermolecular elimination of H₂Se is favored over intramolecular elimination.

Experimental Section

1: A solution of LH (2.50 g, 5.98 mmol) in *n*-hexane (40 mL) was added at room temperature to a solution of $\text{AlH}_3 \cdot \text{NMe}_3$ (0.56 g, 6.29 mmol) in *n*-hexane (10 mL), and the resulting mixture was stirred for 48 h. All volatile components were removed in vacuo, and the crude product was crystallized from *n*-hexane to afford colorless crystals of **1** (2.3 g, yield 86.2% based on LH); m.p. 194 °C. ^1H NMR (200 MHz, C_6D_6): δ = 7.18 (d, 4H, aromatic H of Ar group), 7.10 (m, 2H, aromatic H of Ar group), 4.86 (s, 1H, γ -CH), 3.42 (sept, 4H, CHMe_2), 1.54 (s, 6H, CMe), 1.37, 1.12 (d, 24H, CHMe_2); ^{13}C NMR (125.75 MHz, C_6D_6): δ = 170.2 (CN), 144.6, 139.4, 124.8 (Ar), 96.2 (γ -CH), 28.48 (CHMe_2), 25.5, 24.5 (CHMe_2), 22.9 (CMe); IR (KBr, Nujol): $\tilde{\nu}$ = 1832, 1795 cm^{-1} (m, Al–H); EI-MS: m/z (%): 445 (100, $[\text{M}^+ - \text{H}]$); elemental analysis calcd for $\text{C}_{29}\text{H}_{43}\text{AlN}_3$: C 78.0, H 9.7, N 6.3; found: C 78.2, H 9.7, N 6.7.

2: To a mixture of **1** (0.45 g, 1 mmol) and selenium (0.16 g, 2 mmol) was added toluene (20 mL). The mixture was stirred at room temperature for 15 h, and a light orange solution formed with trace amounts of unchanged selenium. After filtration, the solvent was removed and the residue extracted with *n*-hexane (20 mL). The extract was stored at –30 °C for 2 d to give yellow crystals of **2** (0.35 g, yield 58%); m.p. 220 °C (decomp.). ^1H NMR (500 MHz, C_6D_6): δ = 7.15–7.09 (m, 6H, aromatic H of Ar group), 4.93 (s, 1H, γ -CH), 3.52 (sept, $^3J_{\text{HH}} = 6.8$ Hz, 4H, CHMe_2), 1.51 (s, 6H, CMe), 1.35 (d, $^3J_{\text{HH}} = 6.8$ Hz, 12H, CHMe_2), 1.10 (d, $^3J_{\text{HH}} = 6.8$ Hz, 6H, CHMe_2), –2.82 (s, 2H, SeH); ^{27}Al NMR (65.17 MHz, C_6D_6): δ = 122 ($\nu_{1/2} = 260$ Hz); EI-MS: m/z (%): 604 (8, $[\text{M}^+ - \text{H}]$), 525 (20, $[\text{M}^+ - \text{SeH}]$), 403 (100, $[\text{L}^+ - \text{Me}]$); IR (KBr, Nujol): $\tilde{\nu}$ = 2318 cm^{-1} (w, SeH); elemental analysis calcd for $\text{C}_{29}\text{H}_{43}\text{AlN}_2\text{Se}_2$: C 57.6, H 7.2, N 4.6; found: C 58.0, H 7.5, N 4.6.

3: This compound was prepared in a similar manner as **2** except that the mixture was stirred at 60 °C for 15 h. After removal of the solvent, the residual was recrystallized from THF at –30 °C to give pale yellow crystals of **3** (0.26 g, yield 46%); m.p. 250 °C (decomp.). ^1H NMR (500 MHz, CDCl_3): δ = 7.13, 7.03, 6.92 (m, 12H, aromatic H of Ar group), 5.17 (s, 2H, γ -CH), 3.21 (sept, $^3J_{\text{HH}} = 6.7$ Hz, 4H, CHMe_2), 2.98 (sept, $^3J_{\text{HH}} = 6.8$ Hz, 4H, CHMe_2), 1.61 (s, 12H, CMe), 1.10 (d, $^3J_{\text{HH}} = 6.75$ Hz, 12H, CHMe_2), 0.95 (d, $^3J_{\text{HH}} = 6.65$ Hz, 12H, CHMe_2), 0.90 (d, $^3J_{\text{HH}} = 6.82$ Hz, 12H, CHMe_2), 0.84 (d, $^3J_{\text{HH}} = 6.82$ Hz, 12H, CHMe_2), –2.83 (s, 2H, SeH); ^{27}Al NMR (65.17 MHz, CDCl_3): δ = 116 ($\nu_{1/2} = 680$ Hz); ^{77}Se NMR (C_6D_6 , 500 MHz): δ = –341 (s, SeH, $\nu_{1/2} = 120$ Hz), –535 (s, Al_2Se , $\nu_{1/2} = 90$ Hz); EI-MS: m/z (%): 1126 (7, $[\text{M}^+ - 2\text{H}]$), 1048 (90, $[\text{M}^+ - \text{H} - \text{SeH}]$), 443 (100, $[\text{LAl}^+ + \text{H}]$); IR (KBr, Nujol): $\tilde{\nu}$ = 2276, 2292 cm^{-1} (w, SeH); elemental analysis calcd for $\text{C}_{38}\text{H}_{84}\text{Al}_2\text{N}_4\text{Se}_3$: C 61.8, H 7.5, N 5.0; found: C 62.2, H 7.1, N 5.0.

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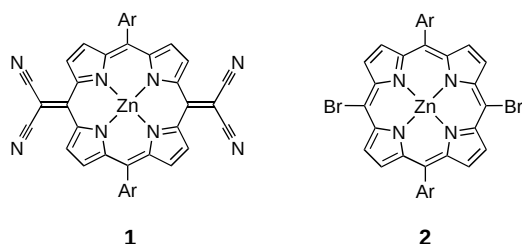
- [1] Reviews: a) A. R. Barron, *Adv. Mater. Opt. Electron.* **1995**, 5, 245; b) J. L. Atwood in *Coordination Chemistry of Aluminum* (Ed.: G. H. Robinson), VCH, New York, **1993**, pp. 197–232.
- [2] a) C. Cui, H. W. Roesky, M. Noltemeyer, H.-G. Schmidt, *Organometallics* **1999**, 18, 5120; b) C. Cui, H. W. Roesky, M. Noltemeyer, H.-G. Schmidt, unpublished results.
- [3] a) M. B. Power, J. W. Ziller, A. N. Tyler, A. R. Barron, *Organometallics* **1992**, 11, 1055; b) R. Kumar, D. G. Dick, S. U. Ghazi, M. Taghiof, M. J. Heeg, J. P. Oliver, *Organometallics* **1995**, 14, 1601.
- [4] a) J. H. Shin, G. Parkin, *Organometallics* **1995**, 14, 1104; b) F. Bottomley, R. W. Day, *Organometallics* **1991**, 10, 2560; c) W. Hoffmann, H. Werner, *Angew. Chem.* **1981**, 93, 1088; *Angew. Chem. Int. Ed. Engl.* **1981**, 20, 1014; d) H. J. Gysling in *The Chemistry of Organic Selenium and Tellurium Compounds, Vol. 1* (Eds.: S. Patai, Z. Rappoport), Wiley, New York, **1986**, pp. 741–754, and references therein.
- [5] B. Qian, D. L. Ward, M. R. Smith III, *Organometallics* **1998**, 17, 3070.
- [6] a) H. S. Isom, A. H. Cowley, A. Decken, F. Sissingh, S. Corbelin, R. J. Lagow, *Organometallics* **1995**, 14, 2400; b) H. V. R. Dias, W. Jin, R. E. Ratcliff, *Inorg. Chem.* **1995**, 34, 6100.

- [7] U. Svanholm in *Organic Selenium Compounds: Their Chemistry and Biology* (Eds.: D. L. Klayman, W. H. H. Günther), Wiley-Interscience, New York, **1973**, pp. 903–932.
- [8] Se satellites cannot be observed due to the broadness of the signals. A ^{77}Se NMR spectrum of **2** could not be obtained because of decomposition of the compound in solution.
- [9] The data were collected on a Stoe-Siemens four-circle diffractometer using MoK_α radiation. The intensities were recorded with φ and ω scans. The structures were solved by direct methods (SHELXS-96)^[15] and refined against F^2 using SHELXL-97.^[16] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in the refinement in geometrically ideal positions. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-139091 (**2**) and -139092 (**3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). Crystallographic data for **2** ($\text{C}_{29}\text{H}_{43}\text{AlN}_2\text{Se}_2$): $M_r = 604.55$, orthorhombic, space group $Pnma$, $a = 1306.5(3)$, $b = 2204.9(4)$, $c = 1055.7(2)$ pm, $\alpha = \beta = \gamma = 90^\circ$, $V = 3.0467(11)$ nm³, $Z = 4$, $\rho_{\text{calcd}} = 1.318$ Mg m^{–3}, $F(000) = 1248$, $\lambda = 71.073$ pm, $T = 203(2)$ K, $\mu = 2.475$ mm^{–1}; crystal dimensions $1.00 \times 0.80 \times 0.40$ mm, $7.26 \leq 2\theta \leq 50.02^\circ$; of 2769 reflections collected, 2760 were independent and were used in the structure refinement of 165 parameters; $R1 = 0.1020$ ($I > 2\sigma(I)$), $wR2 = 0.2787$ (all data); min./max. residual electron density 1706/–1408 e nm^{–3}. Crystallographic data for **3** ($\text{C}_{38}\text{H}_{84}\text{Al}_2\text{N}_4\text{Se}_3$): $M_r = 1128.13$, monoclinic, space group $P2_1/n$, $a = 1225.5(3)$, $b = 2734.7(6)$, $c = 1775.3(4)$ pm, $\alpha = 90^\circ$, $\beta = 98.29(3)$, $\gamma = 90^\circ$, $V = 5.888(2)$ nm³, $Z = 4$, $\rho_{\text{calcd}} = 1.273$ Mg m^{–3}, $F(000) = 2352$, $\lambda = 71.073$ pm, $T = 203(3)$ K, $\mu = 1.942$ mm^{–1}; crystal dimensions $1.00 \times 0.80 \times 0.40$ mm, $7.06 \leq 2\theta \leq 45.06^\circ$; of 8425 reflections collected, 7712 were independent and were used in the structure refinement of 630 parameters; $R1 = 0.0595$ ($I > 2\sigma(I)$), $wR2 = 0.1253$ (all data); min./max. residual electron density 1847/900 e nm^{–3}.
- [10] W. Uhl, R. Gerding, I. Hahn, S. Pohl, W. Saak, *Polyhedron* **1996**, 15, 3987.
- [11] R. A. Poirier, I. G. Csizmadia in *The Chemistry of Organic Selenium and Tellurium Compounds, Vol. 1* (Eds.: S. Patai, Z. Rappoport), Wiley, New York, **1986**, pp. 30–45, and references therein.
- [12] The bond energy of Se–H in H_2Se is 73 kcal mol^{–1}; see S. R. Gunn, *J. Phys. Chem.* **1964**, 68, 949.
- [13] For example, $[\{2,6-(\text{Me}_2\text{NCH}_2)_2\text{C}_6\text{H}_3\}\text{Ga}(\text{OH})_2] \cdot 10\text{H}_2\text{O}$ has an infinite structure with hydrogen bonds; see A. H. Cowley, F. P. Gabbai, D. A. Atwood, C. J. Carrano, L. M. Mokry, M. R. Bond, *J. Am. Chem. Soc.* **1994**, 116, 1559.
- [14] a) C. Jones, G. A. Koutsantonis, C. L. Raston, *Polyhedron* **1993**, 12, 1829; b) C. L. Raston, *J. Organomet. Chem.* **1994**, 475, 15; c) M. G. Gardiner, C. L. Raston, V.-A. Tolhurst, *J. Chem. Soc. Chem. Commun.* **1995**, 1457.
- [15] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, 46, 467.
- [16] G. M. Sheldrick, SHELXL, Program for Crystal Structure Refinement, Universität Göttingen, Germany, **1997**.

Synthesis and Crystal Structure of a Cumulenetic Quinoidal Porphyrin Dimer with Strong Electronic Absorption in the Infrared**

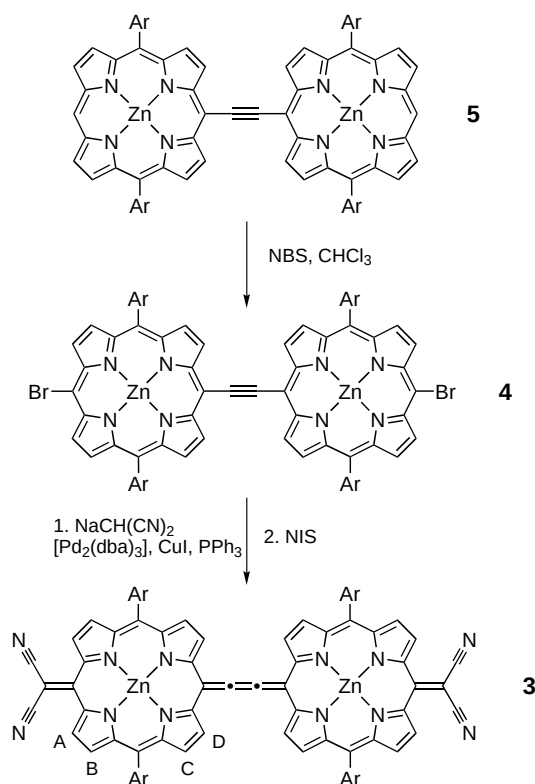
Iain M. Blake, Leigh H. Rees, Tim D. W. Claridge, and Harry L. Anderson*

The remarkable optoelectronic and nonlinear-optical behavior of *meso*-alkyne-linked porphyrin oligomers can be attributed to the contribution of cumulenetic quinoidal mesomeric forms, particularly in the excited states.^[1] Recently we reported the synthesis of the quinoidal 5,15-dialkylideneporphyrin **1**, from dibromoporphyrin **2**, as a first step towards the



preparation of fully quinoidal porphyrin oligomers.^[2] The C(CN)₂ end groups stabilize this quinoidal structure, just as they stabilize 7,7,8,8-tetracyano-*p*-quinodimethane (TCNQ).^[3] We have now extended this approach to gain access to a cumulenetic quinoidal porphyrin dimer **3** (see Scheme 1). The phenomenal conjugation in this compound results in amazingly intense and red-shifted electronic absorption ($\lambda_{\text{max}} = 1080 \text{ nm}$, $\epsilon = 1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$).

The cumulenetic porphyrin dimer **3** was prepared from dibromoporphyrin dimer **4**, as summarized in Scheme 1. After exploring several routes to **4**, we were surprised to discover that it is formed in near-quantitative yield by direct bromination of **5** (prepared using the route of Therien et al.)^[1b] with *N*-bromosuccinimide (NBS) in chloroform at 0 °C.^[4] Bromination occurs exclusively at the two free *meso* positions, with no competing substitution at the β -pyrrolic positions or addition to the alkyne. Dibromo dimer **4** was converted into **3** in 34% yield by palladium/copper catalyzed Takahashi coupling^[2, 5] with the malononitrile anion followed by oxidation with *N*-iodosuccinimide (NIS).^[6] This sequence is similar to the reaction used to prepare **1** from **2**, except in that case no



Scheme 1. Synthesis of **3**. dba = dibenzylideneacetone.

oxidant was required as the intermediate spontaneously oxidized in air, whereas the synthesis of **3** requires NIS.

The ¹H and ¹³C NMR spectra of **3** were assigned using a combination of NOESY, HMQC, and HMBC experiments. Crucially, a HSQC-NOESY experiment^[7] was used to observe an NOE between the equivalent H_D protons across the symmetry plane at the center of the molecule (by observing an NOE between H_D and the ¹³C satellites of this same resonance),^[8] which allowed the pairs H_A/H_D and H_B/H_C to be unambiguously distinguished (Scheme 1). Part of the ¹H NMR spectra of **4** and **3** are compared in Figure 1 a, b; all the β -pyrrolic resonances are shifted up-field in **3** because of the absence of an aromatic porphyrin ring current, as observed previously in **1**.^[2] The crystal structure^[9] of **3** · 2C₅H₅N, illustrated in Figure 2, shows that the 62-atom chromophore adopts a bow-shaped geometry in the solid state, with both pyridine molecules coordinated to the concave surface. The bond lengths in each macrocycle are similar to those in **1** · C₅H₅N and the bond lengths in the central cumulene unit (marked on Figure 2b) are typical for a cumulene.^[10]

Success in preparing the cumulenetic dimer **3** encouraged us to attempt the synthesis of a shorter quinoidal porphyrin dimer **6** (conceptually derived from **3** by deletion of the two central carbon atoms). At first sight, **6** might appear to be an unrealistic synthetic target since the clash between the inner β -pyrrolic protons could make it difficult to form the central *meso*–*meso* double bond, but strained alkenes of this type^[11] have been prepared, such as 29,29,30,30-tetracyanobianthraquinodimethane (TBAQ).^[12] We found that the *meso*–*meso* linked dibromo dimer **7** (prepared by oxidative coupling of

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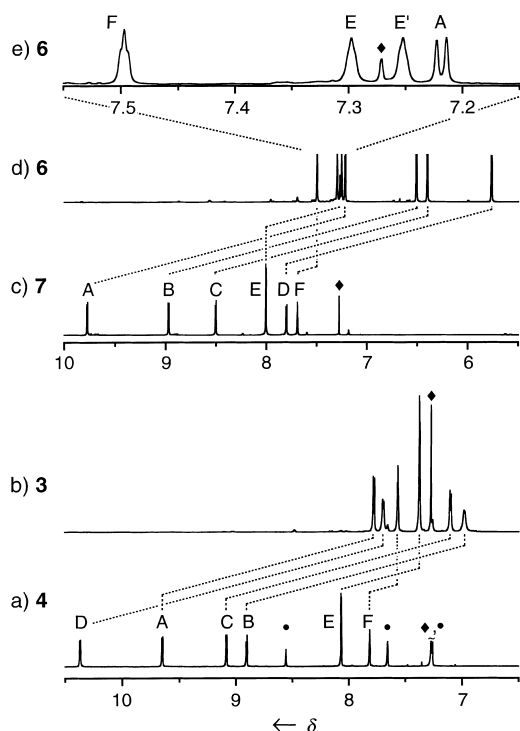


Figure 1. NMR spectra of a) **4**, b) **3**, c) **7**, and d, e) **6** in $C_5D_5N/CDCl_3$ (♦ and ● indicate $CHCl_3$ and C_5D_4HN , respectively).

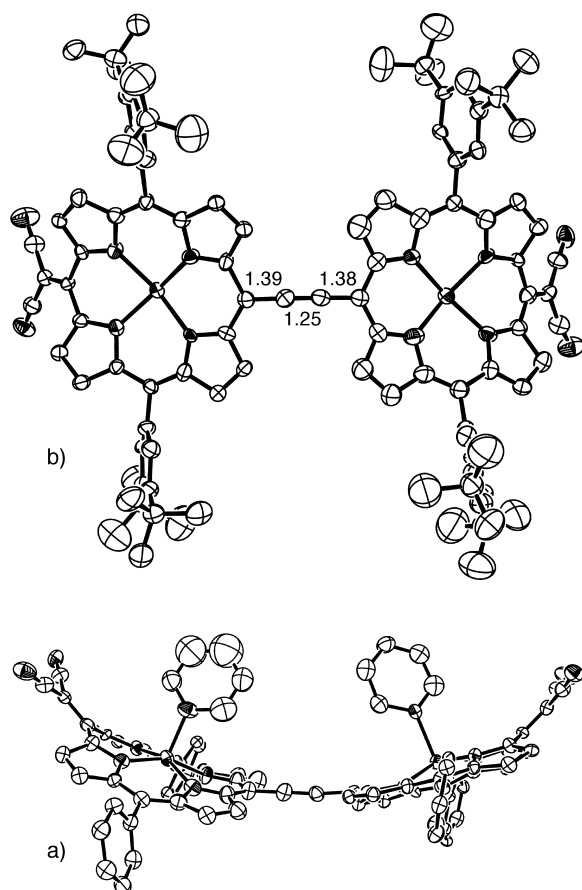
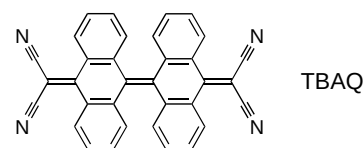
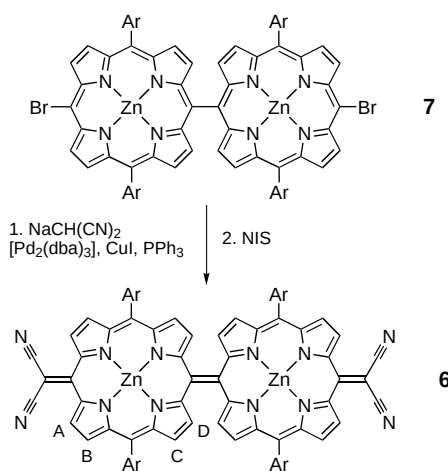


Figure 2. Structure of **3** · 2 C_5H_5N in the solid state, with 40% probability ellipsoids: a) side view (omitting *t*Bu groups) and b) plan view (omitting pyridine ligands) showing the bond lengths [Å] in the central cumulene unit; the estimated standard deviation in these bond lengths is 0.03 Å.

the monobromoporphyrin as reported by Osuka and co-workers)^[13] could be converted into **6** in 36% yield



(Scheme 2) using identical reaction conditions to those used to prepare **3** from **4**. The ^1H NMR spectrum of **6** (Figure 1 d, e) is shifted up-field relative to **7**, similar to that of **3** relative to **4**.^[14] Further evidence for the quinoidal nature of **6** comes from the observation of a ^{13}C signal at $\delta = 72.6$ in the



Scheme 2. Synthesis of **6**.

^{13}C NMR spectrum corresponding to the central carbon atom of the $C(CN)_2$ unit; this resonance occurs at $\delta = 76.5$ and 73.5 in **1** and **3**, respectively. Whereas the ^1H and ^{13}C NMR spectra of **3** are consistent with a time-averaged planar D_{2h} geometry, those of **6** show the molecule is nonplanar, as anticipated from the clash between the inner β -pyrrolic protons: the resonances of the aryl protons *ortho* to the porphyrin (E, E') and the *tert*-butyl protons are both split into two signals of equal intensity (Figure 1 e). Corresponding splittings are also observed by ^{13}C NMR spectroscopy. The C_{2h} and D_2 nonplanar geometries shown in Figure 3 are both consistent with these NMR spectra. Molecular mechanics calculations^[15] indicate that the D_2 geometry is more stable by 105 kJ mol^{-1} ; these calculations correctly predict that the C_{2h} geometry of TBAQ is preferred,^[12] although in this case the C_{2h}/D_2 energy difference is only 23 kJ mol^{-1} .

The electronic absorption spectra of **1**, **3**, and **6** are compared with those of **2**, **4**, and **7** in Figure 4, and illustrate the amazing increase in conjugation accompanying quinoidization. The less bathochromic absorption of **6** (compared to **3**) is probably a consequence of its nonplanarity, which must reduce the π overlap. The efficient conjugation in **3** shifts its optoelectronic activity into a region where few chromophores operate. The absorption spectrum of **3** resembles those of the electrogenerated porphyrin dimer dianions of Arnold, Heath,

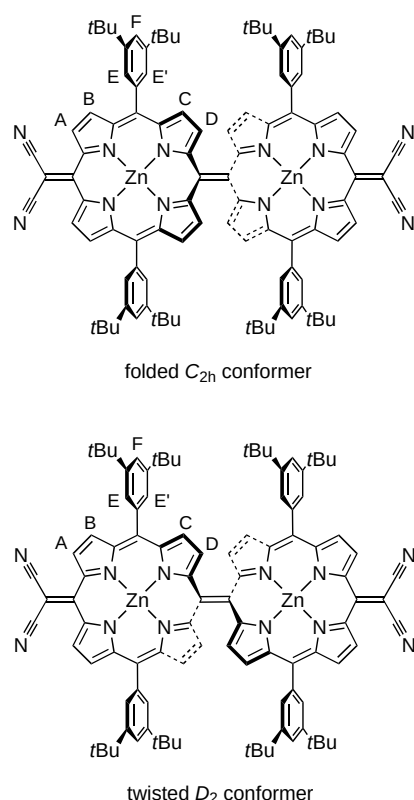


Figure 3. The C_{2h} and D_2 conformers of **6** are both consistent with its NMR spectra.

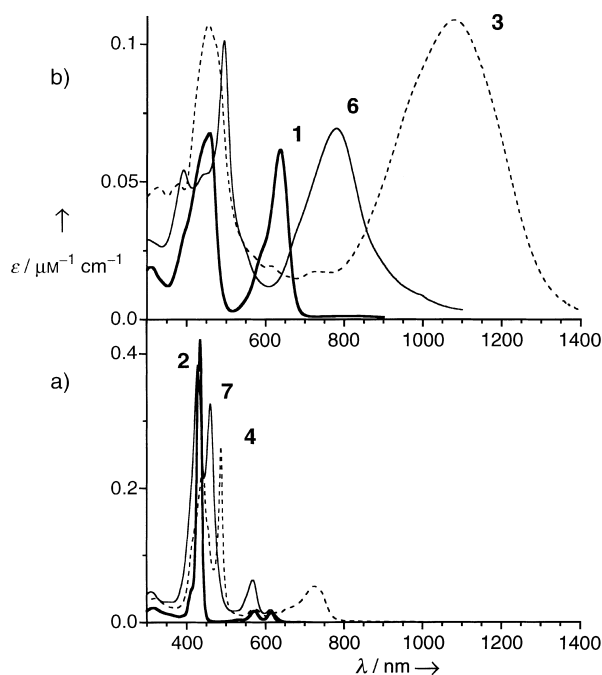


Figure 4. Absorption spectra of a) dibromoporphyrins **2** (bold), **4** (dashed), and **7** (plain) and b) quinoidal compounds **1** (bold), **3** (dashed) and **6** (plain) in 1% C_5H_5N/CH_2Cl_2 .

and James,^[1c] which also have strong bands around 1000 nm, yet **3** is a stable neutral compound. This near-infrared region is important because of the widespread use of neodymium yttrium aluminium garnet (Nd-YAG) lasers, at 1064 nm, and

because fibre-optic telecommunication systems operate in a nearby region (1300 nm). The optoelectronic and nonlinear optical properties of these new quinoidal porphyrin dimers are now under investigation.

Experimental Section

^1H and ^{13}C spectra were recorded in $C_5D_5N/CDCl_3$ at 500 MHz and 126 MHz respectively; UV/Vis spectra were recorded in 1% C_5H_5N/CH_2Cl_2 .

4: NBS (26 mg, 0.14 mmol) was added to a solution of dimer **5** (105 mg, 0.069 mmol) in $CHCl_3$ (75 mL) and pyridine (0.75 mL) at 0 °C. The mixture was stirred for 15 min then quenched with acetone (10 mL), evaporated and washed with methanol to yield the pure product as a black solid (100 mg, 86%).

3: Dibromo dimer **4** (40 mg, 0.024 mmol), $[Pd_2(dba)_3]$ (2.2 mg, 0.0024 mmol), PPh_3 (5.0 mg, 0.019 mmol), and CuI (1.8 mg, 0.0095 mmol) were dissolved in dry THF (25 mL) and pyridine (0.25 mL). Malononitrile (16 mg, 0.24 mmol) was added and the solution degassed before the addition of NaH (60% suspension in oil, 14 mg, 0.36 mmol). The mixture was heated to reflux under nitrogen for 15 h, then HOAc (20 μ L, 0.36 mmol) was added and the volume reduced to 5 mL before being passed through a size exclusion column (1% cross-linked polystyrene/divinylbenzene SX1 Bio-Beads from Bio-Rad, 1% pyridine/toluene). The fractions containing the intermediate were evaporated and redissolved in $CDCl_3$ (600 μ L) with C_5D_5N (10 μ L) in a 5-mm NMR tube. NIS (6 mg, 0.03 mmol) was added and the cumulene was seen to form cleanly and instantly by ^1H NMR spectroscopy. The oxidized product was precipitated, after transfer from the NMR tube, by layered addition of methanol. The precipitate was washed with methanol to yield pure **3** as a black solid (14 mg, 34%). ^1H NMR: δ = 7.77 (d, J = 4.4 Hz, 4H), 7.69 (d, J = 4.4 Hz, 4H), 7.56 (t, J = 1.5 Hz, 4H), 7.37 (d, J = 1.5 Hz, 8H), 7.10 (d, J = 4.4 Hz, 4H), 6.97 (brd, 4H), 1.39 (s, 72H); ^{13}C NMR: δ = 157.6, 150.1, 149.8, 149.4, 147.5, 146.0, 138.5, 134.1, 131.7, 129.9, 129.2, 126.1, 125.2, 122.9, 122.7, 118.0, 112.0, 73.5, 35.0, 31.6; UV/Vis: λ_{max} (log ϵ) = 455 (5.03), 1080 nm (5.04); MALDI-TOF-MS: m/z : 1650.9 [MH^+].

6: Dibromo dimer **7** (80 mg, 0.048 mmol) was converted into quinoidal dimer **6** (22 mg, 36% overall yield) using essentially the above procedure, except in this case the intermediate (51 mg, 65% yield) was crystallized from methanol prior to oxidation. ^1H NMR: δ = 7.50 (t, J = 1.7 Hz, 4H), 7.30 (brd, 4H), 7.25 (brd, 4H), 7.22 (d, J = 4.4 Hz, 4H), 6.51 (d, J = 4.4 Hz, 4H), 6.40 (d, J = 4.4 Hz, 4H), 5.76 (d, J = 4.4 Hz, 4H), 1.39 (s, 36H), 1.32 (s, 36H); ^{13}C NMR: δ = 162.6, 155.6, 150.2, 150.1, 150.0, 149.8, 147.0, 146.2, 137.8, 132.7, 132.1, 130.1, 125.7 ($\times 2$), 125.2, 123.1, 121.2, 117.3, 72.6, 35.3, 35.2, 31.9, 31.8; UV/Vis: λ_{max} (log ϵ) = 392 (4.73), 494 (5.00), 780 nm (4.84); MALDI-TOF-MS: m/z : 1625.4 [MH^+].

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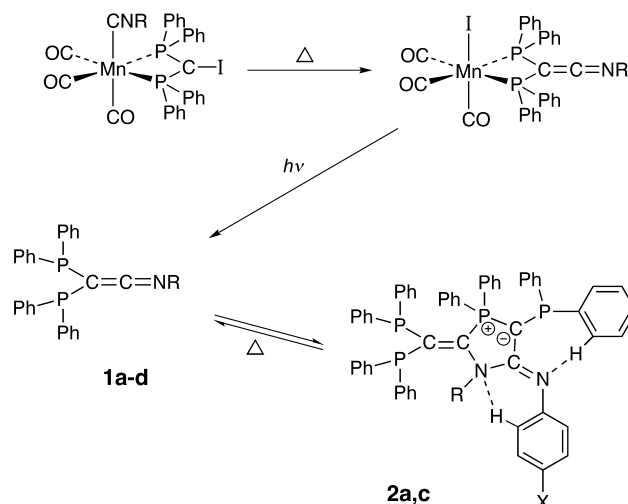
- [1] a) H. L. Anderson, *Chem. Commun.* **1999**, 2323–2330; b) V. S.-Y. Lin, S. G. DiMaggio, M. J. Therien, *Science* **1994**, 264, 1105–1111; c) D. P. Arnold, G. A. Heath, D. A. James, *New J. Chem.* **1998**, 1377–1387; d) H. L. Anderson, S. J. Martin, D. D. C. Bradley, *Angew. Chem.* **1994**, 106, 711–713; *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 655–657; e) P. N. Taylor, A. P. Wylie, J. Huuskonen, H. L. Anderson, *Angew. Chem.* **1998**, 110, 1033–1037; *Angew. Chem. Int. Ed.* **1998**, 37, 986–989; f) S. M. Kuebler, R. G. Denning, H. L. Anderson, *J. Am. Chem. Soc.* **2000**, 122, 339–347; g) J. J. Piet, P. N. Taylor, H. L. Anderson, A. Osuka, J. M. Warman, *J. Am. Chem. Soc.* **2000**, 122, 1749–1757.
- [2] I. M. Blake, H. L. Anderson, D. Beljonne, J.-L. Brédas, W. Clegg, *J. Am. Chem. Soc.* **1998**, 120, 10764–10765. For related work on 5,15-dialkylidene porphyrins see T. G. Traylor, K. B. Nolan, R. Hildreth, *J. Am. Chem. Soc.* **1983**, 105, 6149–6151; L. R. Milgrom, *Tetrahedron* **1983**, 39, 3895–3898; N. Y. Nelson, C. J. Medforth, R. G. Khoury, D. J. Nurco, K. M. Smith, *Chem. Commun.* **1998**, 1687–1688.
- [3] T. Itoh, H. Okuno, T. Hishida, A. Inokuchi, N. Kamei, T. Sato, M. Kubo, S. Iwatsuki, *Tetrahedron* **1997**, 53, 15247–15261.

- [4] G. S. Wilson, H. L. Anderson, *Chem. Commun.* **1999**, 1539–1540; R. Schlözer, J.-H. Fuhrhop, *Angew. Chem.* **1975**, *87*, 388; *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 363; L. R. Nudy, H. G. Hutchinson, C. Schieber, F. R. Longo, *Tetrahedron* **1984**, *40*, 2359–2363.
- [5] M. Uno, K. Seto, M. Masuda, W. Ueda, S. Takahashi, *Tetrahedron Lett.* **1985**, *26*, 1553–1556.
- [6] J. Diekmann, W. R. Hertler, R. E. Benson, *J. Org. Chem.* **1963**, *28*, 2719–2724.
- [7] R. Wagner, S. Berger, *Magn. Reson. Chem.* **1997**, *35*, 199–202; J. Kawabata, E. Fukushi, J. Mizutani, *J. Am. Chem. Soc.* **1992**, *114*, 1115–1117.
- [8] NOESY and HSQC-NOESY experiments were carried out with a 300 ms mixing time at 294 K on a Bruker DRX500 instrument. All NOEs were negative.
- [9] Crystals were grown by slow diffusion of *n*-pentane into a solution of $3 \cdot 2\text{C}_5\text{H}_5\text{N}$ in CHCl_3 , over two weeks. Crystal data for $3 \cdot 2\text{C}_5\text{H}_5\text{N} \cdot 0.5\text{C}_5\text{H}_{12} \cdot 3\text{H}_2\text{O}$: $M_r = 1901.05$, monoclinic, space group $P2_1/c$, $a = 15.440(4)$, $b = 33.765(5)$, $c = 20.485(2)$ Å, $\beta = 94.64(2)^\circ$, $V = 10644.5(33)$ Å³, $Z = 4$, $T = 150(2)$ K, $\rho_{\text{calcd}} = 1.186$ g cm⁻³, $\mu = 0.508$ mm⁻¹, blue/green prisms $0.30 \times 0.20 \times 0.15$ mm³, Enraf-Nonius DIP2000 image plate diffractometer, radiation $\text{MoK}\alpha$ ($\lambda = 0.71073$ Å), ω scans, range $1.45 < \theta < 20$; measured reflections: 4436; independent reflections: 4436 ($R_{\text{int}} = 0.0000$), R values ($I < 2\sigma I$): $R1 = 0.1159$, $wR2 = 0.3212$, all data: $R1 = 0.1628$, $wR2 = 0.3711$. Structure refinement: full-matrix-block least-squares on F^2 , max/min residual electron density $1.255/-0.393$ e Å⁻³. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-139644. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [10] A search of the Cambridge Structural Database showed that the average C=C bond lengths in cumulenes are 1.34 and 1.25 Å for the $\text{sp}^2\text{-sp}^1$ and $\text{sp}^1\text{-sp}^1$ bonds, respectively; D. A. Fletcher, R. F. McMeeking, D. Parkin, *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 746–749; F. H. Allen, O. Kennard, *Chem. Design Autom. News* **1993**, *8*, 31–37.
- [11] E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, pp. 544–550; G. Märkl, M. Hafner, P. Kreitmeier, C. Stadler, J. Daub, H. Nöth, M. Schmidt, G. Gescheidt, *Helv. Chim. Acta* **1997**, *80*, 2456–2476; J.-H. Fuhrhop, E. Baumgartner, H. Bauer, *J. Am. Chem. Soc.* **1981**, *103*, 5854–5861.
- [12] S. Yamaguchi, T. Hanafusa, T. Tanaka, M. Sawada, K. Kondo, M. Irie, H. Tatemitsu, Y. Sakata, S. Misumi, *Tetrahedron Lett.* **1986**, *27*, 2411–2414.
- [13] N. Yoshida, H. Shimidzu, A. Osuka, *Chem. Lett.* **1998**, 55–56; A. Nakano, H. Shimidzu, A. Osuka, *Tetrahedron Lett.* **1998**, *39*, 9489–9492.
- [14] No $^{12}\text{C}\text{-H}_\text{D}$ to $^{13}\text{C}\text{-H}_\text{D}$ NOE was observed in **6**, which is explained by the larger $\text{H}_\text{D}\text{-H}_\text{D}$ distance in **6** (calculated: 2.9 Å for D_2 isomer; 2.6 Å for C_{2h} isomer) compared to that in **3** (crystallographic: 2.47 and 2.41 Å; calculated: 1.9 Å). The ^1H and ^{13}C NMR spectra of **6** were assigned by assuming that the most shielded β -pyrrolic proton is H_D .
- [15] Molecular mechanics calculations were carried out using the CAChe 4.1 software from Oxford Molecular Ltd. with augmented MM2 parameters; MM3 gave similar results.

Fully Reversible Cyclic Dimerization of Diphosphanylketenimines Promoted by Intramolecular C–H⋯N Hydrogen Bonds**

Javier Ruiz,* Fernando Marquínez, Víctor Riera, Marilín Vivanco, Santiago García-Granda, and M. Rosario Díaz

Ketenimines are important functional groups that show enhanced reactivity in nucleophilic addition and cycloaddition reactions. Furthermore, compounds containing this moiety are useful starting materials which have been applied in heterocycle chemistry.^[1] We recently reported the synthesis of the diphosphanylketenimines $(\text{PPh}_2)_2\text{C}=\text{C}=\text{NR}$ (**1a**: R = Ph, **1b**: R = *t*Bu) by metal-assisted coupling of a transient diphosphanylcumene with isocyanides (Scheme 1).^[2] As these



Scheme 1. Metal-assisted synthesis of diphosphanylketenimines **1**. In the case of R = Ph or *p*-tolyl, reversible dimerization takes place to form **2**. **1a**: R = Ph; **1b**: R = *t*Bu; **1c**: R = *p*-tolyl; **1d**: R = *xylyl*; **2a**: R = Ph, X = H; **2c**: R = *p*-tolyl, X = Me.

molecules combine the properties of diphosphanes with those of ketenimines, it was anticipated that new reactivity patterns could arise from them. Here we report a unique cyclic dimerization of *N*-aryldiphosphanylketenimines involving a new type of [2 + 3] cycloaddition reaction to give an azaphospha five-membered heterocycle featuring a phosphorus-ylide functionality. This dimerization is reversible under mild conditions and appears to be promoted by nonconventional CH⋯N hydrogen bonds.

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The *N*-phenylketenimine **1a** forms colorless solutions in different organic solvents which are stable for weeks. However, when a solution of **1a** in hexane is very slowly concentrated to dryness under vacuum, and the process of adding and removing hexane is repeated at least three times, an orange solid corresponding to the unsymmetrical dimer **2a** is formed (Scheme 1). This dimerization is fully reversible, and refluxing a solution of **2a** in toluene for 10 min leads to the quantitative formation of monomer **1a**. By contrast, the *N*-*tert*-butylketenimine **1b** did not dimerize at all, showing the strong influence of the substituent at nitrogen in this process. It has been described that ketenimines tend to dimerize, but only upon prolonged thermolysis at high temperatures, and usually by [2+2] cycloaddition reactions to give azetidines;^[3] related processes such as base-induced dimerization^[4] and anodic oxidation^[5] of ketenimines to give heterocyclic dimers and trimers are also known. Therefore the present reaction is a rare example of ketenimine dimerization occurring during crystallization.

The ³¹P NMR spectrum of **2a** revealed the presence of four strongly coupled signals corresponding to the four inequivalent phosphorus atoms of the molecule (see the Experimental Section). The structure of **2a** was definitively established by X-ray crystallography^[6] (Figure 1). The five-membered heterocycle C(1)–P(1)–C(3)–C(2)–N(1) is essentially planar. The short C(2)–C(3) (1.40(2) Å) and C(3)–P(1) bonds (1.70(2) Å) suggest that the negative charge at the ylide carbon atom C(3) is strongly shared with the neighboring atoms. An important feature of the structure of **2a** is the presence of two intramolecular CH⋯N hydrogen bonds. Thus, a phenyl ring at phosphorus atom P(2) acts as a hydrogen-bond donor

through an *ortho* proton toward the exocyclic nitrogen atom N(2), with a rather short H(2)⋯N(2) contact of 2.44(2) Å (C–H⋯N 141(1)°). A much longer contact exists between an *ortho* proton of the phenyl group at N(2) and the endocyclic nitrogen atom N(1) (H(1)⋯N(1) 2.94(2) Å) with a very closed C–H⋯N angle of 90(1)°. These two parameters appear to indicate that this last interaction cannot be considered as a real hydrogen bond, when taking into account the cut-off of 2.7 Å based on van der Waals criteria and the preference for a linear geometry.^[7] However, it has been shown that the electrostatic component of the hydrogen bond also operates beyond the van der Waals separation, and that directionality is blurred if other factors such as steric restrictions comes into play.^[8] Additionally, a structural feature in favor of a hydrogen-bonding interaction is that the H(1)–N(1) vector is approximately directed toward the sp³ lone pair of N(1).^[9, 8a] Moreover, some cooperativity^[8a] could exist, as the two hydrogen bonds are interconnected (the N(2)Ph group acts simultaneously as hydrogen-bond donor and acceptor), resulting in an enhancement of the total bond energy.

To assess the influence of the substituent at nitrogen in this dimerization process, the new *N*-aryldiphosphanylketenimines **1c** (R = 4-methylphenyl = *p*-tolyl) and **1d** (R = 2,6-dimethylphenyl = *xylyl*) were prepared following the same procedure used for the synthesis of **1a** (Scheme 1). The *N*-*p*-tolylketenimine **1c** tends to dimerize under the same conditions as **1a** to give **2c**, whereas the *N*-*xylyl*ketenimine **1d**, which lacks *ortho* hydrogen atoms for the substituent at N, does not dimerize at all. This result suggests that not only the H(2)⋯N(2) hydrogen bond but also the very weak interaction H(1)⋯N(1) could have some influence in the dimerization of **1a** and **1c**, although other factors such as steric hindrance of the R substituent at nitrogen and packing forces in the crystal must also be considered.

The dimerization of diphosphanylketenimines described here is noteworthy because: 1) it takes place under mild conditions (room temperature), 2) it is reversible, 3) it involves a new type of [2+3] cycloaddition, and 4) it appears to be promoted by intramolecular nonconventional CH⋯N hydrogen bonds. Furthermore, the involvement of the phosphanyl substituent in the annelation process suggests an enhancement of the synthetic potential of **1a–d** with respect to normal ketenimines. This is clearly shown in the reaction of **1a** with two equivalents of dimethyl acetylenedicarboxylate, which takes place instantaneously at room temperature to give **3a** (Scheme 2, see the Experimental Section).^[10] This unique bicyclic compound contains two fused five-membered phosphaheterocycles resulting from two successive [2+3] cycloaddition reactions involving both phosphorus atoms of the original ketenimine **1a**.^[11] Very likely this reaction proceeds through the formation of the intermediate species **1a**, although it was not detected in the reaction mixture (even at low temperature), suggesting an enhancement of the reactivity after the first [2+3] cycloaddition process. Other preliminary studies show that **1a–d** react at room temperature not only with electron-poor alkynes but also with heterocumulenes such as isocyanates and isothiocyanates.

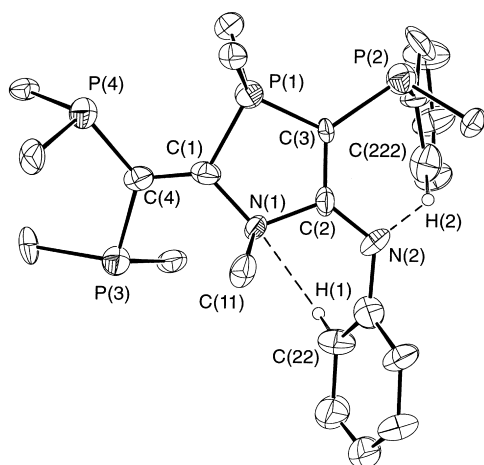
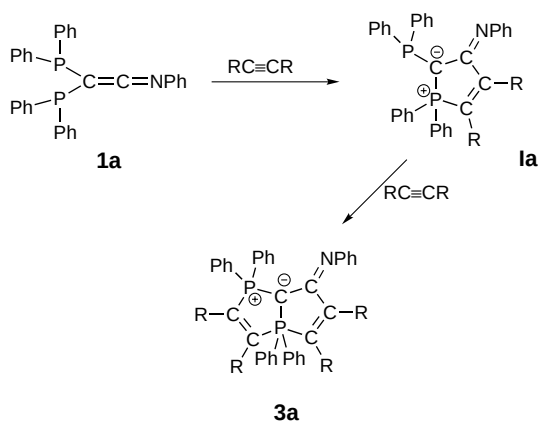


Figure 1. Molecular structure of **2a**. Hydrogen atoms and phenyl groups, except those involved in hydrogen bonds, are omitted for clarity (ellipsoids at the 30% probability level). Selected bond lengths [Å] and angles [°]: N(1)–C(1) 1.45(2), N(1)–C(2) 1.45(2), P(1)–C(1) 1.80(2), P(1)–C(3) 1.70(2), C(2)–C(3) 1.40(2), N(1)–C(11) 1.45(2), C(1)–C(4) 1.38(2), C(2)–N(2) 1.27(2), C(3)–P(2) 1.83(2), C(4)–P(3) 1.88(2), C(4)–P(4) 1.77(2), N(1)⋯H(1) 2.94(2), N(2)⋯H(2) 2.44(2), C(22)⋯N(1) 3.09(3), C(22)⋯N(2) 3.22(3); C(22)–H(1)⋯N(1) 90(1), C(22)–H(2)⋯N(2) 141(1), C(1)–P(1)–C(3) 91(1), C(1)–N(1)–C(2) 111(1), C(4)–C(1)–N(1) 126(2), C(4)–C(1)–P(1) 125(2), N(1)–C(1)–P(1) 108(4), N(1)–C(2)–N(2) 120(2), N(2)–C(2)–C(3) 129(2), N(1)–C(2)–C(3) 112(2), C(2)–C(3)–P(1) 114(1), C(2)–C(3)–P(2) 124(4), P(1)–C(3)–P(2) 122(1), C(1)–C(4)–P(4) 114(1), C(1)–C(4)–P(3) 115(1), P(3)–C(4)–P(4) 129(1).



Scheme 2. Two [2+3] cycloaddition reactions of **1a** with dimethyl acetylenedicarboxylate provide **3a**. $\text{R} = \text{CO}_2\text{Me}$.

Experimental Section

2a: A solution of **1a** (0.1 g, 0.2 mmol) in hexane (10 mL) was very slowly evaporated to dryness under vacuum. Hexane (10 mL) was added to the residue, giving an orange precipitate corresponding to the dimer **2a**; the monomer **1a** remained in solution. The solution was filtered off and then retreated with hexane to afford a new fraction of **2a**. The process was repeated three times; yield 70 % (0.07 g). The yield can be improved by repeating the process as many times as possible. Crystals suitable for X-ray diffraction were grown from a solution in CH_2Cl_2 /hexane. Elemental analysis calcd for $\text{C}_{64}\text{H}_{50}\text{N}_2\text{P}_4$: C 79.16, H 5.19, N 2.88; found: C 78.84, H 5.27, N 2.78; ^1H NMR (300 MHz, CD_2Cl_2): $\delta = 7.90$ (4H, dd, $^3J_{\text{PH}} = 13$, $^3J_{\text{HH}} = 7$ Hz), 6.4–7.4 (44H, m), 5.85 (2H, d, $^3J_{\text{HH}} = 7$ Hz); $^{31}\text{P}\{^1\text{H}\}$ NMR (121.5 MHz, CD_2Cl_2): $\delta = 28.5$ (ddd, $^3J_{\text{PP}} = 52$, $^3J_{\text{PP}} = 24$, $^3J_{\text{PP}} = 6$ Hz), 14.3 (dd, $^3J_{\text{PP}} = 24$, $^3J_{\text{PP}} = 4$ Hz), 6.8 (dd, $^3J_{\text{PP}} = 4$, $^3J_{\text{PP}} = 6$ Hz), –22.8 (d, $^2J_{\text{PP}} = 52$ Hz).

2b: Compound **2b** was prepared analogous to **2a**. Elemental analysis calcd for $\text{C}_{60}\text{H}_{54}\text{N}_2\text{P}_4$: C 79.35, H 5.45, N 2.80; found: C 79.26, H 5.32, N 2.75; ^1H NMR (300 MHz, CD_2Cl_2): $\delta = 7.85$ (4H, dd, $^3J_{\text{PH}} = 13$, $^3J_{\text{HH}} = 7$ Hz), 6.4–7.4 (42H, m), 5.84 (2H, d, $^3J_{\text{HH}} = 7$ Hz), 2.35 (3H, s), 2.12 (3H, s); $^{31}\text{P}\{^1\text{H}\}$ NMR (121.5 MHz, CD_2Cl_2): $\delta = 28.6$ (m), 13.7 (m), 6.5 (s, br), –23.8 (m).

3a: To a solution of **1a** (0.05 g, 0.1 mmol) in CH_2Cl_2 (10 mL) was added dimethyl acetylenedicarboxylate (0.025 mL, 0.2 mmol). The color changed to red instantly. After 5 min of stirring at room temperature the solvent was evaporated to dryness, and the brown residue recrystallized from CH_2Cl_2 /hexane to afford red crystals; yield 85 % (0.065 g). Elemental analysis calcd for $\text{C}_{44}\text{H}_{37}\text{NO}_8\text{P}_2$: C 68.65, H 4.84, N 1.81; found: C 68.43, H 4.80, N 1.83; ^1H NMR (300 MHz, CD_2Cl_2): $\delta = 7.99$ (4H, dd, $^3J_{\text{PH}} = 14$, $^3J_{\text{HH}} = 7$ Hz), 6.8–7.8 (19H, m), 6.52 (2H, d, $^3J_{\text{HH}} = 7$ Hz), 3.50 (3H, s), 3.35 (3H, s), 3.27 (3H, s), 2.89 (3H, s); $^{31}\text{P}\{^1\text{H}\}$ NMR (121.5 MHz, CD_2Cl_2): $\delta = 8.97$ (d, $^2J_{\text{PP}} = 71$ Hz), –81.40 (d, $^2J_{\text{PP}} = 71$ Hz); ^{13}C NMR (75.5 MHz, CD_2Cl_2): $\delta = 199.43$ (dd, $J_{\text{PC}} = 32$, 22 Hz), 170.30 (dd, $J_{\text{PC}} = 25$, 4 Hz), 170.05 (dd, $J_{\text{PC}} = 14$, 10 Hz), 169.15 (dd, $J_{\text{PC}} = 3$, 1 Hz), 166.51 (dd, $J_{\text{PC}} = 6$, 2 Hz), 162.41 (dd, $J_{\text{PC}} = 16$, 2 Hz), 159.52 (dd, $J_{\text{PC}} = 23$, 13 Hz), 151.86 (d, $J_{\text{PC}} = 5$ Hz), 131.28 (dd, $J_{\text{PC}} = 12$, 7 Hz), 117.2 (dd, $J_{\text{PC}} = 93$, 6 Hz), 52.60 (s, $1 \times \text{CH}_3$), 51.57 (s, $3 \times \text{CH}_3$); signals for phenyl groups not given.

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- [1] “The Chemistry of Ketenes, Allenes and Related Compounds”: M. W. Barker, W. E. McHenry in *The Chemistry of Functional Groups, Part 2* (Ed.: S. Patai), Interscience, New York, **1980**, chap. 17, pp. 701–720; G. Tennant in *Comprehensive Organic Chemistry. The Synthesis and Reactions of Organic Compounds, Vol. 2* (Eds.: D. Barton, W. D. Ollis, I. O. Sutherland), Pergamon, Oxford, **1979**, pp. 521–527.
- [2] J. Ruiz, V. Riera, M. Vivanco, M. Lanfranchi, A. Tiripicchio, *Organometallics* **1998**, *17*, 3835–3837.
- [3] M. W. Barker, J. D. Rosamond, *J. Heterocycl. Chem.* **1972**, *9*, 1147–1148; M. W. Barker, J. D. Rosamond, *J. Heterocycl. Chem.* **1972**, *9*, 1419–1421.

- [4] R. Gertzmann, R. Fröhlich, M. Grehl, E. Würthwein, *Tetrahedron* **1995**, *51*, 9031–9044.
- [5] J. Y. Becker, E. Shakkour, J. A. R. P. Sarma, *J. Chem. Soc. Chem. Commun.* **1990**, 1016–1017; J. Y. Becker, E. Shakkour, J. A. R. P. Sarma, *J. Org. Chem.* **1992**, *57*, 3716–3720.
- [6] Crystal data for **2a** ($\text{C}_{64}\text{H}_{50}\text{N}_2\text{P}_4$): $M_r = 970.94$, orthorhombic, space group $P2_12_12_1$, $a = 10.609(7)$, $b = 13.36(1)$, $c = 36.06(2)$ Å, $V = 5111(7)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.262$ g cm^{–3}, $F(000) = 2032$, $\text{MoK}\alpha$ radiation ($\lambda = 0.71073$ Å), $\mu(\text{MoK}\alpha) = 0.191$ mm^{–1}; crystal dimensions $0.20 \times 0.20 \times 0.13$. Diffraction data were collected on a Enraf-Nonius at 293 K. The structure was solved with direct methods and refined using full-matrix least squares on F^2 with all non-hydrogen atoms anisotropically defined. The hydrogen atoms were placed in calculated positions, isotropically refined with common thermal parameters, and allowed to ride on their parent carbon atoms. For 5590 unique reflections and 632 parameters, $R = 0.063$ and $wR2 = 0.109$. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-136772. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [7] a) R. Taylor, O. Kennard, *J. Am. Chem. Soc.* **1982**, *104*, 5063–5070; b) G. R. Desiraju, *Acc. Chem. Res.* **1996**, *29*, 441–449.
- [8] a) T. Steiner, *Chem. Commun.* **1997**, 727–734; b) T. Steiner, B. Lutz, J. van der Maas, A. M. M. Schreurs, J. Kroon, M. Tamm, *Chem. Commun.* **1998**, 171–172.
- [9] The H(1) atom, together with C(1), C(2), and C(11), completes a virtual distorted tetrahedron around N(1): C(1)–N(1)–H(1) 132°, C(2)–N(1)–H(1) 107°, C(11)–N(1)–H(1) 77°, C(1)–N(1)–C(2) 111°, C(1)–N(1)–C(11) 111°, C(2)–N(1)–C(11) 115°.
- [10] A preliminary X-ray diffraction study of **3a** confirms the proposed bicyclic structure. Furthermore, **3a** has been fully spectroscopically characterized (see the Experimental Section).
- [11] For other cycloaddition reactions involving phosphorus atoms, see for instance G. Veneziani, R. Réau, F. Dahan, G. Bertrand, *J. Org. Chem.* **1994**, *59*, 5927–5929; J. Grobe, D. L. Van, B. Broschek, M. Hegemann, B. Lüth, G. Becker, M. Böhringer, E. U. Würthwein, *J. Organomet. Chem.* **1997**, *529*, 177–187, and references therein.

Dianionic Homoleptic Biphosphinine Complexes of Group 4 Metals**

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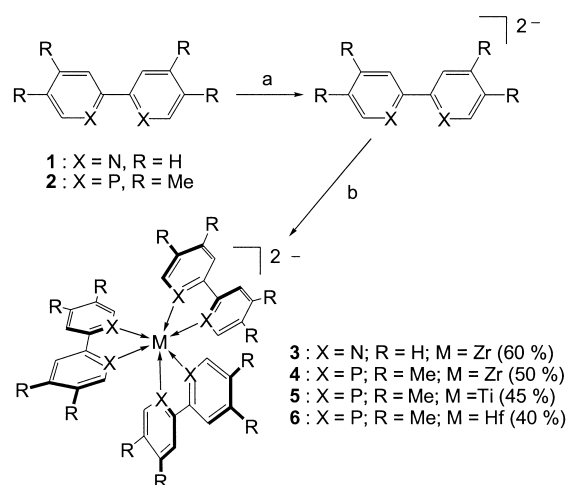
The stabilization of anionic transition metal centers is generally associated with the use of ancillary ligands possessing strong π -acceptor properties. For the most part, it was achieved with molecules such as CO ,^[1] isocyanides,^[2] ethylene,^[3] and arenes^[4] which display a suitable synergistic effect between σ -donor and π -acceptor capabilities, and in rare cases, by using tailored tertiary phosphanes.^[5] However, only little is known about the stabilizing effects provided by

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heterocycle-based ligands. Herzog et al. reported on the synthesis of some homoleptic neutral and anionic complexes of 2,2'-bipyridine (bpy),^[6] and more recently it was shown that bipyridine radical anions efficiently stabilize lanthanide complexes.^[7]

Several years ago, we proposed that 2,2'-biphosphinines could efficiently stabilize reduced metal centers.^[8] This proposal was based on preliminary studies which showed that these ligands display a lower σ -donor but a better π -acceptor capability than their nitrogen counterparts. Although electrochemical reduction led to the formation of stable reduced species, anionic complexes remained to be isolated and structurally characterized. Here we report on the synthesis of complexes of the Group 4 metal dianions, carbonyl derivatives of which have attracted much attention.^[9] To facilitate structural comparisons between biphosphinine and bipyridine complexes, we synthesized $[\text{Zr}(\text{bpy})_3]^{2-}$, which was discovered by Herzog et al. but whose X-ray structure remained unknown.^[10] Both complexes were synthesized by the same experimental procedure (Scheme 1), which involves the reaction of the dianion of bipyridine **1**^[11] or biphosphinine **2** (tmbp)^[12] with MCl_4 ($\text{M} = \text{Ti}, \text{Zr}, \text{Hf}$) in THF or dimethoxyethane (DME) at low temperature (see Experimental Section).



Scheme 1. Syntheses of **3–6**: a) 2Li, THF or DME, 25 °C, 2 h. b) $\text{MCl}_4 \cdot n\text{THF}$, –78 to 25 °C, 15 min.

Complexes **3–6** were isolated as dark colored, extremely air-sensitive solids and were characterized by NMR spectroscopy (^{31}P , ^1H , ^{13}C). The crystal structure analysis of the $[\text{Li}_2(\text{thf})_{8.5}]^{2+}$ salt of **3** showed that the coordination geometry around zirconium is between octahedral and trigonal prismatic ($\theta = 33.4^\circ$, where θ is the angle between the medians of the triangles formed by three nitrogen atoms; $\theta = 60^\circ$ for a regular octahedron). Most interestingly, while the N–Zr bond length lies in the usual range, the C–C and C–N bond lengths differ strongly from those of the free ligand. The alternation of short and long C–C distances clearly shows that the aromaticity of each pyridine unit is totally disrupted. This phenomenon is also evidenced by the shortening of the C–C bridge, which now has double-bond character (1.36(1) Å).^[13] Conversely, the internal C=N bonds are lengthened (from

1.346(2) Å in free bipyridine to 1.435(7) Å in **3**) and approach the usual values recorded for C–N single bonds. These data, which indicate electron transfer into the symmetrical LUMO of the ligand, are very similar to those of a bipyridine dianion whose structure was reported recently.^[11]

Structure determinations for **4–6** were also carried out. The structure of **4** is shown in Figure 1.^[14] In the three complexes, $[\text{Li}_3(\text{dme})_6\text{Cl}]\text{-4}$, $[\text{Li}_3(\text{thf})_9\text{Cl}]\text{-5}$, and $[\text{Li}_3(\text{thf})_9\text{Cl}]\text{-6}$ the overall

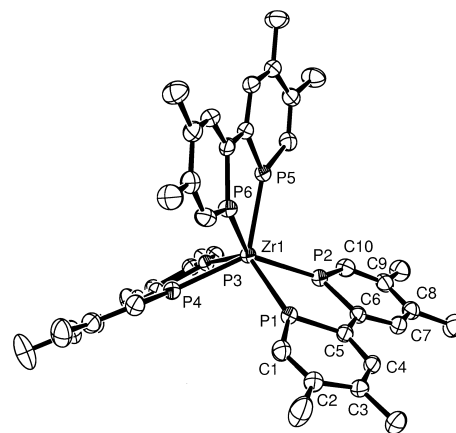
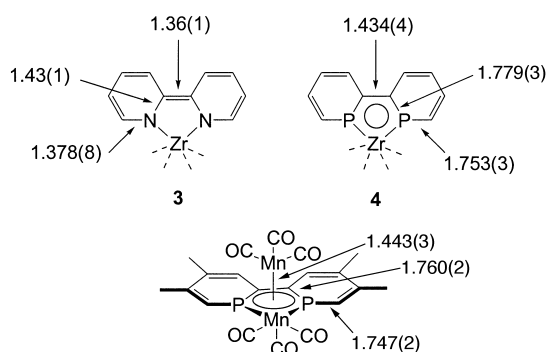


Figure 1. Structure of **4** in the crystal (50 % probability ellipsoids; hydrogen atoms omitted for clarity). Selected bond lengths [Å] and angles [°] of one biphosphinine unit: P1–Zr1 2.5441(8), P2–Zr1 2.563(1), P3–Zr1 2.551(1), P4–Zr1 2.5248(8), P5–Zr1 2.5486(8), P6–Zr1 2.532(1), P1–C1 1.753(3), C1–C2 1.362(4), C2–C3 1.428(5), C3–C4 1.368(4), C4–C5 1.414(4), C5–P1 1.779(3), C5–C6 1.434(4), C6–P2 1.779(3), P2–C10 1.752(3), C10–C9 1.380(5), C9–C8 1.429(5), C8–C7 1.370(5), C7–C6 1.422(4); P1–Zr–P2 69.91(3), C5–P1–C1 100.4(2).

geometry around the metal center is trigonal prismatic ($\theta = 3^\circ$ for **4**, 0° for **5** and **6**). A comparison between their Zr–P bond lengths and those of other complexes is difficult because of the lack of data. In **4** these distances (2.5248(8)–2.563(1) Å) are shorter than in the only other known phosphinine zirconium complex, the η^2 -phosphabenzynes zirconocene complex (av P–Zr 2.69 Å).^[15] As in **3**, disruption of the aromaticity is observed in each phosphinine subunit. However, the C–C bridge and the internal C=P bond lengths exhibit smaller variations than in **3**. With lengths of 1.428(4)–1.438(4) and 1.774(3)–1.785(3) Å, respectively, these two bonds are intermediate between single and double bonds. The same phenomenon is observed in **5** and **6**.

From these data, it is clear that the –2 oxidation state of Zr is only formal. Interestingly, the two ligands seem to accommodate the release of electron density from the metal center in different ways. Whereas **3** can be described as the coordination of three bipyridine dianions to a Zr^{IV} center, **4** has a delocalized character that makes the assignment of a precise oxidation state difficult (Scheme 2).^[16] This assumption is reinforced by the striking analogy between the bond lengths in **4** and those of the complex $[\text{Mn}_2(\text{tmbp})(\text{CO})_6]$, in which a $[\text{Mn}(\text{CO})_3]^+$ fragment is η^5 -coordinated to a delocalized $[\text{P}_2\text{C}_2\text{Mn}(\text{CO})_3]^-$ unit.^[17]

A second important point is the overall geometry of **3** and **4**. If the two ligands display the same geometrical constraints



Scheme 2. Bonding modes in complexes **3** and **4** in comparison with those of a Mn complex of tmbp. Bond lengths in Ångström.

(bulk and bite angles are nearly identical), only electronic factors can explain the observed differences. According to Hoffmann's prediction,^[18] ML₆ complexes of chelating ligands can adopt a trigonal-prismatic geometry if the ligands possess a low-lying symmetrical LUMO (the shape of this orbital is similar in biphosphinine and bipyridine). This was verified previously for [W(tmbp)₃], which, like **4**–**6**, displays a strong preference for this geometry ($\theta = 15^\circ$).^[19] At this stage and without any further theoretical data it is difficult to fully rationalize why complex **3** adopts an intermediate geometry.

In conclusion, we have shown that biphosphinine dianions, like their nitrogen counterparts, can be used as efficient precursors of previously unknown homoleptic dianionic Group 4 metal complexes. Additionally, the first examples of Ti and Hf η^1 -phosphinine complexes were also synthesized. Extension of this work to other metals is currently underway.

Experimental Section

All manipulations were conducted by using Schlenk and glove-box techniques under an argon atmosphere. Complexes **3**–**6** were prepared by the same methodology. To a solution of the ligand (123 mg, 0.50 mmol) in dry THF or DME (10 mL) was added an excess (about 10 equiv) of lithium in pieces. The excess lithium was removed after 2 h, and the resulting solution of **1** or **2** was cooled to -78°C . Solid ZrCl₄·2 THF, TiCl₄·2 THF, or HfCl₄ (0.16 mmol) was then added, and the resulting solution was stirred at -78°C for 5 min then slowly warmed to room temperature. The compounds separated from the solution over ca. 12 h as small deep purple to black crystals, which were collected by filtration in the glove box. Complexes **3**–**6** are very sensitive towards air and moisture.

4: (114 mg, 50%); m.p. > 220 °C (decomp); ³¹P NMR (C₄D₈O/DME 1/9, 81 MHz, 25 °C): δ = 226.30; ¹H NMR (C₄D₈O/DME 1/9, 400 MHz, 25 °C): δ = 2.32, 2.48 (s, 18H, Me), 8.02 (brs, 6H, H³, H³), 8.84 (brs, 6H, H⁶, H⁶); ¹³C NMR (C₄D₈O/DME 1/9, 100 MHz, 25 °C): δ = 23.35 and 24.80 (s, Me), 121.15 (s, C⁴, C⁴ or C⁵, C⁵), 125.30 (brs, C³, C³), 131.85 (s, C⁵, C⁵ or C⁴, C⁴), 139.30 (brs, C², C²), 146.10 (brs, C⁶, C⁶); C,H analysis calcd for C₆₆H₁₀₈ClLi₃O₁₂P₆Zr (1426.9): C 55.56, H 7.63; found: C 55.80, H 7.70.

5: (107 mg, 45%); m.p. > 220 °C (decomp); ³¹P NMR (C₄D₈O/DME 1/9, 81 MHz, 25 °C): δ = 252.95; ¹H NMR (C₄D₈O/DME 1/9, 400 MHz, 25 °C): 2.33 and 2.56 (s, 18H, Me), 7.98 (brs, 6H, H³, H³), 9.28 (brs, 6H, H⁶, H⁶); ¹³C NMR (C₄D₈O/DME 1/9, 100 MHz, 25 °C): δ = 23.05 and 23.45 (s, Me), 120.15 (s, C⁴, C⁴ or C⁵, C⁵), 124.95 (brs, C³, C³), 132.35 (s, C⁵, C⁵ or C⁴, C⁴), 141.60 (brs, C², C²), 146.05 (brs, C⁶, C⁶); C,H analysis calcd for C₇₈H₁₂₀ClLi₃O₉P₆Ti (1491.8): C 62.80, H 8.11; found: C 62.40, H 8.17.

6: (104 mg, 40%); m.p. > 220 °C (decomp); ³¹P NMR (C₄D₈O/DME 1/9, 81 MHz, 25 °C): δ = 214.90; ¹H NMR (C₄D₈O/DME 1/9, 400 MHz, 25 °C): δ = 2.34 and 2.50 (s, 18H, Me), 8.06 (brs, 6H, H³, H³), 8.81 (brs, 6H, H⁶, H⁶). The poor solubility of **6** precluded recording a ¹³C NMR spectrum;

C,H analysis calcd for C₇₈H₁₂₀ClHfLi₃O₉P₆ (1622.4): C 57.74, H 7.46; found: C 57.85, H 7.42.

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- [1] H. Willner, F. Aubke, *Angew. Chem.* **1997**, *109*, 2506–2530; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2402–2425.
- [2] P. A. Leach, S. J. Geib, J. A. Corella II, G. F. Warnock, N. J. Cooper, *J. Am. Chem. Soc.* **1994**, *116*, 8566–8574.
- [3] K. Jonas, *Angew. Chem.* **1985**, *97*, 292–307; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 295–311.
- [4] See for example: a) M. Jang, J. E. Ellis, *Angew. Chem.* **1994**, *106*, 2036–2038; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1973–1975; b) J. E. Ellis, D. W. Blackburn, P. Yuen, M. Jang, *J. Am. Chem. Soc.* **1993**, *115*, 11616–11617.
- [5] a) H. Schönberg, S. Boulmaâz, M. Wörle, L. Liesum, A. Schweiger, H. Grützmaier, *Angew. Chem.* **1998**, *110*, 1492–1494; *Angew. Chem. Int. Ed.* **1998**, *37*, 1423–1426; b) S. Boulmaâz, M. Mlaka, S. Loss, H. Schönberg, S. Deblon, M. Wörle, R. Nesper, H. Grützmaier, *Chem. Commun.* **1998**, 2623–2624.
- [6] a) S. Herzog, R. Taube, *Z. Chem.* **1962**, *2*, 208–214; see also: b) W. R. McWhinnie, J. D. Miller, *Adv. Inorg. Chem. Radiochem.* **1969**, *12*, 135–215; c) J. Quirk, G. Wilkinson, *Polyhedron* **1982**, *1*, 209–211.
- [7] I. Fedushkin, T. V. Petrovskaya, F. Girgsdies, R. D. Köhn, M. N. Bochkarev, H. Schumann, *Angew. Chem.* **1999**, *111*, 2407–2409; *Angew. Chem. Int. Ed.* **1999**, *38*, 2262–2264.
- [8] F. Mathey, P. Le Floch, *Chem. Ber.* **1996**, *129*, 263–268.
- [9] a) K. M. Chi, S. R. Frerichs, S. B. Philson, J. E. Ellis, *Angew. Chem.* **1987**, *99*, 1203–1204; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1190–1191; b) J. E. Ellis, K.-M. Chi, *J. Am. Chem. Soc.* **1990**, *112*, 6022–6025.
- [10] S. Herzog, H. Zühlke, *Z. Chem.* **1966**, *6*, 382–383.
- [11] H. Bock, J.-M. Lehn, J. Pauls, S. Holl, V. Krenzel, *Angew. Chem.* **1999**, *111*, 1004–1008; *Angew. Chem. Int. Ed.* **1999**, *38*, 952–955.
- [12] P. Rosa, L. Ricard, F. Mathey, P. Le Floch, *Organometallics* **1999**, *18*, 3348–3352.
- [13] Shortening of the C–C bridge in bipyridine complexes has already been observed: M. H. Chisholm, J. C. Huffman, I. P. Rothwell, P. G. Bradley, N. Kress, W. H. Woodruff, *J. Am. Chem. Soc.* **1981**, *103*, 4945–4947.
- [14] X-ray structure data: Nonius Kappa CCD diffractometer, ϕ and ω scans, MoK α radiation ($\lambda = 0.71073$ Å), graphite monochromator, $T = 150$ K, structure solution with maxus,^[20] refinement against F^2 (SHELXL97^[21]) with anisotropic thermal parameters for all non-hydrogen atoms; calculated hydrogen positions with riding isotropic thermal parameters. Data collection for **4**: crystal dimensions $0.16 \times 0.16 \times 0.14$ mm, triclinic, space group $P\bar{1}$, $a = 15.0930(4)$, $b = 22.6420(7)$, $c = 23.7110(7)$ Å, $\alpha = 76.592(2)^\circ$, $\beta = 74.626(2)^\circ$, $\gamma = 89.957(2)^\circ$, $V = 7584.0(4)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.250$ g cm⁻³, $\mu = 0.360$ cm⁻¹, $F(000) = 3024$, $\theta_{\text{max}} = 26.40^\circ$, h, k, l ranges: 0 to 18, -28 to 28 , -28 to 29 ; 30043 reflections collected, of which 30043 were unique ($R_{\text{int}} = 0.05$); 21458 reflections with $I > 2\sigma(I)$ and 1694 parameters refined; GOF(F^2) 1.032, final R indices: $R_1 = 0.0480$, $wR_2 = 0.1350$, max./min. residual electron density 0.849(0.091)/ $-0.582(0.091)$ e Å⁻³. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-136105 (**3**), -136106 (**4**), -136107 (**5**), and -136108 (**6**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [15] P. Rosa, P. Le Floch, L. Ricard, F. Mathey, *J. Am. Chem. Soc.* **1997**, *119*, 9417–9423.
- [16] S. Greulich, W. Kaim, A. F. Stange, H. Stoll, J. Fiedler, S. Zalis, *Inorg. Chem.* **1996**, *35*, 3998–4002.
- [17] P. Le Floch, N. Maigrot, L. Ricard, C. Charrier, F. Mathey, *Inorg. Chem.* **1995**, *34*, 5070–5072.
- [18] R. Hoffmann, J. M. Howell, A. R. Rossi, *J. Am. Chem. Soc.* **1976**, *98*, 2484–2492.

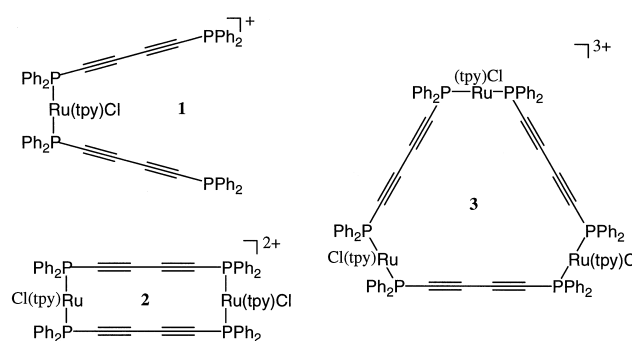
- [19] P. Rosa, L. Ricard, P. Le Floch, F. Mathey, G. Sini, O. Eisenstein, *Inorg. Chem.* **1998**, 37, 3154–3158.
 [20] S. Mackay, C. J. Gilmore, C. Edwards, M. Tremayne, N. Stuart, K. Shankland, maXus, University of Glasgow, Scotland, UK, Nonius BV, Delft, The Netherlands and MacScience Co. Ltd., Yokohama, Japan, **1998**.
 [21] G. M. Sheldrick, SHELXL-97, Universität Göttingen, Germany, **1997**.

Investigation of Electronic Communication and Guest Inclusion Using Photoluminescent Macrocyclic Receptors with Ru^{II} Centers and Ph₂P–C≡C–C≡C–PPh₂ Spacers**

Dengfeng Xu and Bo Hong*

The study of photoluminescent and redox-active supramolecular systems has attracted recent attention as a consequence of a rapid growing interest in designing artificial arrays for applications in photonic and electronic devices.^[1, 2] Systems with various designs have been studied using the assembly of active (for example, metal-based chromophores and redox-active moieties) and passive components (for example, rigid or flexible spacers) as a basis.^[1–7] Such systems are constructed to gain a practical insight into parameters and factors that govern processes, such as signal transportation, by electron/energy transfer, electronic and excitonic interaction, as well as guest inclusion and selective molecular sensing.

We have become interested in investigating the photoluminescent and redox-active macrocyclic inorganic cyclophanes for their dual capability in molecular recognition through host–guest interaction and electronic communication between multiple redox centers within a macrocyclic platform. Monomeric model complex [RuCl(tpy)(‘C₄P₂’)](PF₆) (**1**), rectangular dimer [[RuCl(tpy)(‘C₄P₂’)]₂](PF₆)₂ (**2**), and triangular macrocycle [[RuCl(tpy)(‘C₄P₂’)]₃](PF₆)₃ (**3**) have been prepared from [RuCl(tpy)]-based moieties (tpy = 2,2':6',2''-terpyridine) and rigid ditopic phosphane spacer Ph₂P–C≡C–C≡C–PPh₂ (‘C₄P₂’), in order to study the redox interaction and guest inclusion by electrochemical and optical methods. The short alkyne bridge affords the desired structural rigidity to provide controllable cavity sizes in the systems. The Ru^{II}-based moieties with polypyridine and polyphosphane ligands also have well-behaved reversible redox properties and photoluminescence characteristics. Such properties are extremely important for the design and applications of new spectral and electrochemical sensory agents for organic guests based on organometallic and coordination receptor systems.^[4]



Herein, the synthesis, chromatographic separation, and characterization of **1–3** are reported. Other properties have also been studied, including the ground-state absorption, excited-state emission, redox characteristics, and guest inclusion using anisole and 1,4-dimethoxybenzene as guest molecules. Specifically, the emission intensities of hosts **2** and **3** increase steadily upon addition of guest molecules, and the binding constants of both guest molecules increase significantly when the trimeric system **3** with the larger cavity is used. In addition, the redox interaction between the metal-based corner units is also investigated using cyclic and square-wave voltammetry. The best electronic communication is observed between the two terminal Ru^{II} centers in the doubly bridged complex **2**.

The spacer ‘C₄P₂’ is prepared from Me₃Si–C≡C–C≡C–SiMe₃ by treatment with 2.5 equivalents of MeLi·LiBr and then 2.5 equivalents of ClPPh₂. Complexes **1–3** are obtained upon reaction of ‘C₄P₂’ and [RuCl₃(tpy)] (3/1 ratio, with an excess NH₄PF₆) in refluxing THF/ethylene glycol mixture (4/1), in yields of 15, 30, and 12%, respectively, after purification. When a 1/1 ratio of ‘C₄P₂’ and [RuCl₃(tpy)] is used only **2** and **3** (35 and 15% yield, respectively, after chromatographic separation) are obtained.

The identification of all new complexes was accomplished using ³¹P{¹H} NMR spectroscopy and electrospray mass spectrometry (ESI-MS). The ³¹P{¹H} NMR measurements show two peaks at δ = +13.6 (corresponding to the coordinated PPh₂ group in ‘C₄P₂’) and δ = –29.7 (free PPh₂ group in ‘C₄P₂’) for **1**. Only one peak at δ = +23.1 for **2** and δ = +19.3 for **3** are observed, both corresponding to the coordinated PPh₂ units in the ‘C₄P₂’ spacer. ESI-MS measurements offered a straightforward identification of **1–3**, giving fragments with single or double positive charges. A representative ESI mass spectrum of **3** is shown in Figure 1, as well as the comparison of the observed and simulated isotope distributions for the doubly charged fragment at m/z 1255.5 [3 – 2PF₆]²⁺.

Cyclic voltammetry (CV) and square-wave voltammetry (SWV) can be used to probe the redox characteristics and electronic communication between the Ru^{II} termini in the macrocyclic molecular systems and the corresponding model complex. Only a single redox peak at +1.06 V (1e[–], ΔE_p = 81 mV) for **1** or +1.30 V (3e[–], ΔE_p = 96 mV) for **3** is observed for the Ru^{II}/Ru^{III} redox couples, while two consecutive one-electron redox waves (1:1 ratio, peak separation ΔE⁰ = E₂⁰ – E₁⁰ = 100 mV) are found at +1.03 V (1e[–], ΔE_p = 84 mV) and +1.13 V (1e[–], ΔE_p = 85 mV) for **2** (Figure 2). All redox

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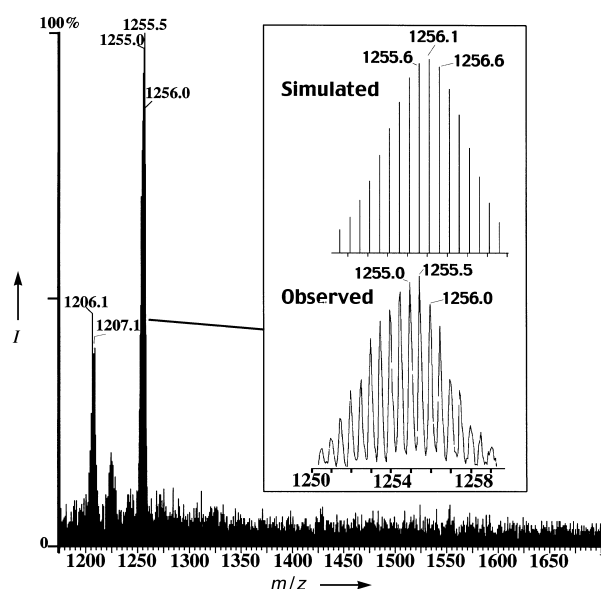


Figure 1. ESI-MS spectrum of **3**. Inset is a comparison of the simulated and observed peaks for $[3 - 2\text{PF}_6]^{2+}$. The peak at m/z 1206.1 is assigned to $[3 - 2\text{RuCl}(\text{tpy}) - \text{C}_4\text{P}_2 - 3\text{PF}_6]^+$.

potentials are referenced to the saturated calomel electrode (SCE). Such difference between the redox behavior of **1–3** can be ascribed to the different electronic communication in these complexes. All three Ru^{II} centers in **3**, bridged by single C_4P_2 spacer, are in electronic isolation and oxidize simultaneously (Scheme 1 b), while the two Ru^{II} centers in **2**, bridged by two C_4P_2 spacers, communicate much better and give consecutive oxidation peaks (Scheme 1 a). As a comparison, a separate study on the redox properties of the linear molecule $[(\text{bpy})_2\text{ClRu}(\text{C}_4\text{P}_2)\text{RuCl}(\text{bpy})_2](\text{PF}_6)_2$ (**4**) ($\text{bpy} = 2,2'$ -bipyridine) also reveals no significant redox interaction between the two Ru^{II} termini bridged by a single C_4P_2 spacer and gives two overlapping one-electron redox waves at +1.06 V (versus SCE).^[8]

For the complexes that can undergo multistep charge transfer [Eq. (1)] the equilibrium constant (K_c) for the comproportionation reaction is defined as shown in Equation (2), with $\Delta E^0 = E_2^0 - E_1^0$ in mV and at 298 K.^[9]

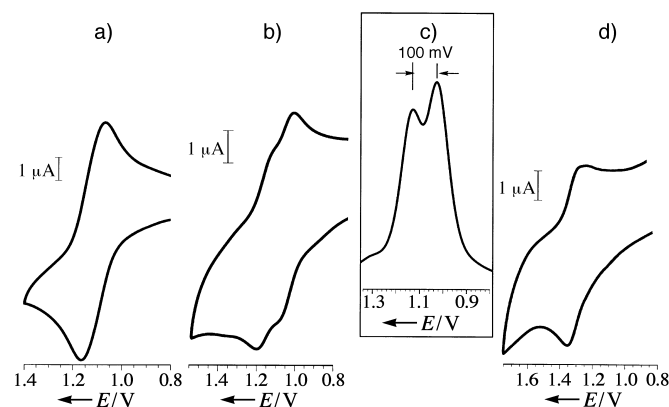
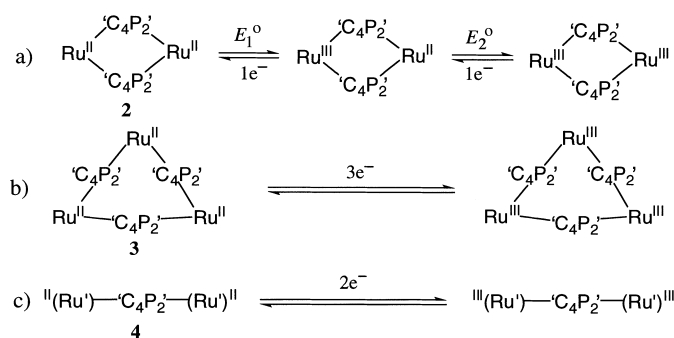


Figure 2. Cyclic voltammograms of **4** (a), **2** (b), and **3** (d) and square-wave voltammogram of **2** (c) obtained at 22 °C in spectrograde acetonitrile, platinum disk (1.0 mm diameter) working electrode, platinum-wire counter electrode, and Ag/AgCl reference electrode. 0.1 M $n\text{Bu}_4\text{NPF}_6$ was used as the electrolyte.



Scheme 1. Description of the redox behavior of **2–4**: $\text{Ru} = [\text{RuCl}(\text{tpy})]$, $\text{Ru}' = [\text{RuCl}(\text{bpy})_2]$, and $\text{C}_4\text{P}_2 = \text{Ph}_2\text{P}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{PPh}_2$.

$$\text{A} \xrightleftharpoons[1e^-, E_1^0]{1e^-, E_2^0} \text{B} \xrightleftharpoons[1e^-, E_2^0]{1e^-, E_1^0} \text{C} \quad (1)$$

$$K_c = [\text{B}]^2/[\text{A}][\text{C}] = \exp[(E_2^0 - E_1^0)/25.69] \quad (2)$$

Here $\Delta E^0 = E_2^0 - E_1^0$ can be estimated from $\Delta E_{1/2}$ measured by CV and SWV studies. A quantitative measure of the energetics for the comproportionation reactions can be achieved from $\Delta E_{1/2}$, and K_c values range from 4 in the uncoupled Robin and Day class I systems to 10^{13} in strongly coupled class III systems.^[10] The K_c value is calculated as approximately 50 for **2** (Robin and Day class II system), and about 4 for both **3** and **4** (class I). Presumably the two C_4P_2 spacers between the two Ru^{II} -based termini facilitate the interaction and hence give a better electronic communication in complex **2** than that observed in **3** and **4**, where only one C_4P_2 spacer is available to mediate the interaction between pairs of Ru^{II} centers.

The electronic absorption spectra show two intense absorption peaks between 300 and 800 nm, with a chromophore summation effect observed. These two peaks are at 330 nm ($\epsilon = 12370 \text{ cm}^{-1}\text{M}^{-1}$, ligand centered (LC), L is tpy) and 450 nm ($\epsilon = 3590 \text{ cm}^{-1}\text{M}^{-1}$, metal to ligand charge transfer (MLCT)) in **1**. Correspondingly, two peaks at 331 nm ($\epsilon = 17490 \text{ cm}^{-1}\text{M}^{-1}$, LC) and 452 nm ($\epsilon = 6530 \text{ cm}^{-1}\text{M}^{-1}$, MLCT) are observed for **2**, and two peaks at 329 nm ($\epsilon = 27015 \text{ cm}^{-1}\text{M}^{-1}$, LC) and 473 nm ($\epsilon = 9615 \text{ cm}^{-1}\text{M}^{-1}$, MLCT) are obtained for **3**. Comparison of the extinction coefficients of the absorptions in **1–3** shows that there is an increase in the extinction coefficients that is proportional to the number of Ru^{II} centers, although the lower energy peak (MLCT band) in **3** is shifted slightly to a lower energy (Figure 3). Such an increase in the extinction coefficient reflects a chromophore summation effect, and has been previously observed in systems with multiple metal-based chromophores such as metallogendrimers.^[11]

A study of the steady-state emission at room temperature shows lumi-

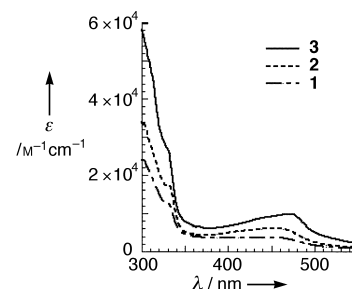


Figure 3. Electronic absorption spectra of **1–3** recorded at 22 °C in spectrograde acetonitrile.

nescence at 635, 565, and 550 nm for **1**, **2**, and **3**, respectively, upon excitation at 470 nm. Here, a trend of blue-shifted emissions is observed from monomeric **1** to dimeric **2** and then trimeric **3**. When the guest molecules anisole and 1,4-dimethoxybenzene are added into the solution of the macrocyclic inorganic receptors **2** and **3** in CH₃CN, the intensity of the host emission increases steadily (Figure 4). Presumably, the enhanced luminescence effect may derive from altered paths for quenching the excited state of the host molecules upon guest encapsulation. In addition, there is no significant change in the line shape of the host emission during the host–guest study, which implies that the guest affects the luminescence efficiency indirectly.^[4a]

We can fit this change of emission intensity as a function of the guest concentration to Equation (3),^[4a] to obtain the host–guest binding constant.

$$I = I_0 + \Delta I K_b c / (1 + K_b c) \quad (3)$$

Here I_0 and ΔI are the initial intensity of the emission and the extrapolated maximum change in intensity of the host molecule, respectively, and c is the concentration of guest. The values of the binding constant K_b thus obtained are compared in Table 1. A direct relationship between the cavity size and the host–guest binding constant is observed. The binding constants for both guests anisole and 1,4-dimethoxybenzene in the dimeric macrocycle **2** with the smaller cavity size (cavity dimension $7.2 \times 5.0 \text{ \AA}^2$ from molecular modeling study, Table 1) are rather small (between 220 – 250 M^{-1}). However, the binding constants for both guest molecules increase signifi-

Table 1. Binding constants of anisole and 1,4-dimethoxybenzene with macrocycles **2** and **3**.

Complex	K_b , anisole [M^{-1}]	K_b , 1,4-dimethoxy- benzene [M^{-1}]	Cavity dimension ^[a] [$\text{\AA}$]
2	220	250	7.2 (Ru to Ru) 5.0 (P to P)
3	2370	1390	8.9 (Ru to Ru)

[a] Cavity dimension is determined from molecular modeling calculations. In the rectangular dimer **2** the Ru to Ru and P to P (on the same Ru center) distances are used to define the edges. In the triangular complex **3** the average Ru–Ru distance is used to define the edge of the triangle.

cantly when the cavity size is increased by using the trimeric **3**. Specifically, the K_b value of anisole (2370 M^{-1}) is larger than that of 1,4-dimethoxybenzene (1390 M^{-1}), which suggests a better fit of the smaller guest molecule anisole in the cavity of **3**. Such a correlation between the cavity size and the host–guest binding constant may suggest the inclusion of guest molecules within the host cavities.^[6d]

In conclusion, the new type of photoluminescent and redox-active macrocyclic dimer and trimer with Ru^{II} and ditopic phosphane spacers ‘C₄P₂’ were successfully synthesized and characterized. A chromophore summation effect is found in the electronic absorption of the macrocyclic species. A better electronic communication is observed between the two Ru^{II} centers bridged by two ‘C₄P₂’ spacers, while the triangular macrocycle **3** displays similar electrochemical behavior to the linear dimeric species **4**. By using the rigid polyphosphane spacer ‘C₄P₂’, the cavity sizes in different molecules can also be controlled for selective molecular sensing.

Experimental Section

All reactions are carried out in a N₂ atmosphere unless otherwise noted. ‘C₄P₂’: A solution of 1.5 M MeLi·LiBr (2.5 equiv) in diethyl ether was added dropwise to a solution of Me₃Si–C≡C–C≡C–SiMe₃ (1 equiv) in THF/diethyl ether (1/1) at 0 °C. The reaction was slowly warmed up to room temperature. After 24 h, the resulting slurry of an off-white solid was cooled to 0 °C and treated with ClPPh₂ (2.5 equiv). Acetic acid was then added to quench the reaction. The solution was then neutralized with aqueous NaHCO₃ solution, and the water layer was extracted with diethyl ether (3 × 75 mL). The combined organic phase was dried over anhydrous Na₂SO₄, and the solvent was removed to give a dark brown solid. This solid was washed with acetone (1–2 mL) to give a light-yellow solid. It was then recrystallized from ethanol/chloroform (1/1). Yield: 51%. ³¹P{¹H} NMR (202 MHz, CDCl₃): $\delta = -29.2$; CI-MS: m/z : 418 [M^+], 341 [$M^+ - \text{Ph}$], 233 [$M^+ - \text{PPh}_2$].

General preparation of **1–3**: A suspension of [RuCl₃(tpy)] and ‘C₄P₂’ (1/3 ratio) in 50 mL THF/ethylene glycol (4/1) was heated to reflux for 2 d. The dark brown solution was cooled down to room temperature. The THF was removed under reduced pressure, and the residue was dropped into a saturated aqueous KPF₆ solution (50 mL). The resulting brown precipitate was collected by vacuum filtration, washed with water (3 × 10 mL) and diethyl ether (3 × 10 mL), and dried in vacuo. The solid was purified by column chromatography, using basic alumina and an acetonitrile/toluene eluent. The first portion is **1** (15% yield) when 1/2 acetonitrile/toluene is used, the second portion is **2** (30% yield) when 2/3 acetonitrile/toluene is used, and the third portion is **3** (12% yield) when a 2/1 acetonitrile/toluene eluent is used. ³¹P{¹H} NMR (CD₃CN, 202 MHz): **1**: $\delta = +13.6$, -29.7 ; **2**: $\delta = +23.1$; **3**: $\delta = +19.3$; ESI-MS: **1**: m/z : 1206.2 [**1**–PF₆]⁺, 788.1 [**1**–‘C₄P₂’–PF₆]⁺; **2**: m/z : 1720.8 [**2**–PF₆]⁺, 787.9 [**2**–2PF₆]²⁺; **3**: m/z : 1255.5 [**3**–2PF₆]²⁺, 1206.1 [**3**–2RuCl(tpy)–‘C₄P₂’–3PF₆]⁺, 788.0 [**3**–RuCl(tpy)(‘C₄P₂’)–3PF₆]²⁺.

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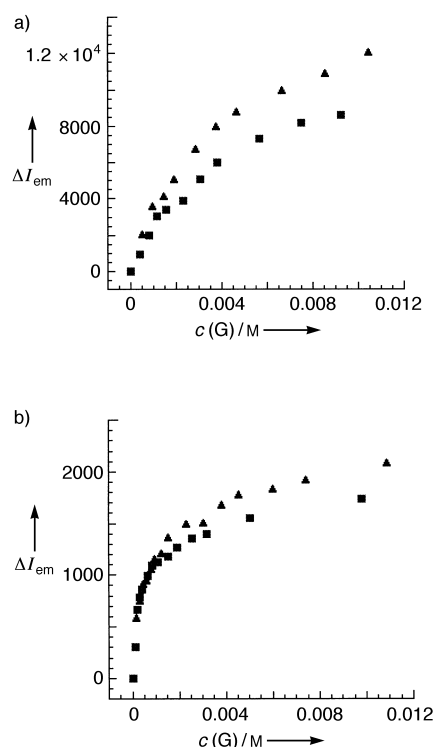


Figure 4. Change of emission intensity of host molecules **2** (a) and **3** (b) as a function of guest concentration recorded at 22 °C in spectrograde acetonitrile, concentration of host molecule: $4.0 \times 10^{-4} \text{ M}$ for **2** and $1.0 \times 10^{-4} \text{ M}$ for **3**. Total solution volume change upon addition of guests is < 5%. ■ = anisole, ▲ = 1,4-dimethoxybenzene.

- [1] J.-M. Lehn, *Supramolecular Chemistry: Concept and Perspectives*, VCH, Weinheim, 1995.
- [2] a) V. Balzani, F. Scandola, *Supramolecular Photochemistry*, Harwood, Chichester, 1991; b) V. Balzani, A. Juris, M. Venturi, S. Campagna, S. Serroni, *Chem. Rev.* **1996**, 96, 759–833.
- [3] P. J. Stang, B. Olenyuk, *Acc. Chem. Res.* **1997**, 30, 502–518.
- [4] a) R. V. Slone, K. D. Benkstein, S. Bélanger, J. T. Hupp, I. A. Guzei, A. L. Rheingold, *Coord. Chem. Rev.* **1998**, 171, 221–243; b) S. M. Woessner, J. B. Helms, J. F. Houllis, B. P. Sullivan, *Inorg. Chem.* **1999**, 38, 4380–4381; c) M. Fujita, J. Yazaki, K. Ogura, *J. Am. Chem. Soc.* **1990**, 112, 5645–5647.
- [5] a) A. Harriman, R. Ziessel, *Chem. Commun.* **1996**, 1707–1716; b) V. Grosshenny, A. Harriman, M. Hissler, R. Ziessel, *J. Chem. Soc. Faraday Trans.* **1996**, 92, 2223–2238.
- [6] a) B. Hong, J. V. Ortega, *Angew. Chem.* **1998**, 110, 2210–2214; *Angew. Chem. Int. Ed.* **1998**, 37, 2131–2134; b) B. Hong, *Comments Inorg. Chem.* **1999**, 20, 177–207; c) J. V. Ortega, B. Hong, S. Ghosal, J. C. Hemminger, B. Breedlove, C. P. Kubiak, *Inorg. Chem.* **1999**, 38, 5102–5112; d) D. Xu, H. J. Murfee, W. E. van der Veer, B. Hong, *J. Organomet. Chem.* **2000**, 596, 53–63.
- [7] P. D. Beer, F. Szemes, V. Balzani, C. M. Salá, M. G. B. Drew, S. W. Dent, M. Maestri, *J. Am. Chem. Soc.* **1997**, 119, 11864–11875.
- [8] **4** is prepared by combining 'C₄P₂' and [RuCl₂(bpy)₂] (1/2.2, with excess NH₄PF₆) in THF/ethylene glycol (2/1, 30 mL). After refluxing the mixture for 48 h, removing the THF, and adding the residue into saturated aqueous KPF₆ solution (50 mL), the yellow solid is collected by filtration (yield 85%, after column chromatography using basic alumina and acetonitrile/toluene as eluent). Analysis of this complex by CV and SWV shows one metal-based redox peak at +1.06 V. D. Xu, B. Hong, unpublished results.
- [9] a) D. E. Richardson, H. Taube, *Inorg. Chem.* **1981**, 20, 1278–1285; b) T. Ito, T. Hamaguchi, H. Nagino, T. Yamaguchi, J. Washington, C. P. Kubiak, *Science* **1997**, 277, 660–663.
- [10] a) C. Creutz, *Prog. Inorg. Chem.* **1983**, 30, 1–73; b) M. B. Robin, P. Day, *Adv. Inorg. Chem. Radiochem.* **1967**, 10, 247–270.
- [11] a) V. Balzani, S. Campagna, G. Denti, A. Juris, S. Serroni, M. Venturi, *Acc. Chem. Res.* **1998**, 31, 26–34; b) G. D. Storrier, K. Takada, H. D. Abruña, *Langmuir* **1999**, 15, 872–884.

The Absolute Configuration and Asymmetric Total Synthesis of the CP Molecules (CP-263,114 and CP-225,917, Phomoidrides B and A)**

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The stunning molecular structures^[1] coupled with the promising biological activities of the CP compounds (pho-

moidrides,^[2] **1a** and **b**; Figure 1) have inspired numerous studies directed at their total synthesis.^[3] In 1999 we reported the first total synthesis of these molecules in their racemic form.^[4] Their absolute configuration, however, remained unknown despite the many attempts to prepare and analyze, through X-ray crystallography, a suitably crystalline derivative. After securing the overall structure and configuration at

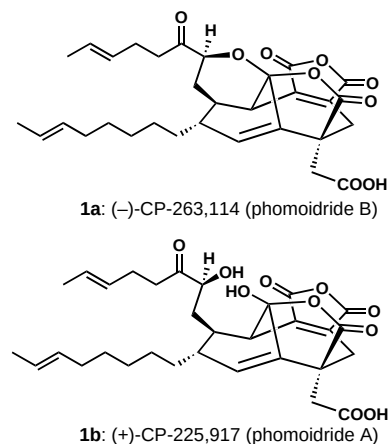
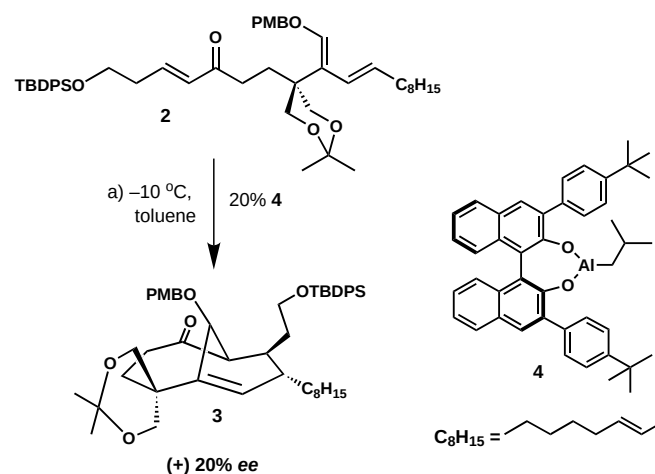


Figure 1. Absolute configuration of the CP compounds **1** and **2**.

C7^[5] of the CP molecules, admirably deduced by Kaneko and co-workers solely from NMR spectroscopic data, we were enticed by the challenge of establishing their absolute configuration. Such knowledge would provide an opportunity to explore modifications of our racemic synthesis and pave the way to optically active CP analogues and mimics, as well as guide future asymmetric total syntheses of these challenging target molecules. Here we report the absolute configuration of the CP molecules as unambiguously determined through chemical synthesis.

Our reported total synthesis of the CP compounds^[4] involved a type-II intramolecular Diels–Alder reaction^[6] as a key step to generate the core skeleton of their molecules. Logic dictated that this step (Scheme 1), which converts the



Scheme 1. Enantioselective Diels–Alder reaction of prochiral triene **2** to form the known CP precursor **3** (20% ee). PMB = *p*-methoxybenzyl; TBDPS = *tert*-butyldiphenylsilyl.

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prochiral triene **2** into the CP core **3**, would be a most appropriate stage for the introduction of asymmetry. Unfortunately, however, several reagent-based enantioselective approaches commencing with the known precursor **2**^[4] failed to deliver appreciable levels of enantiocontrol. A study of this intramolecular process with a number of optically active catalysts led to Lewis acid **4**^[7] as the optimum inducer of asymmetry, albeit delivering the CP core **3** in only 20% *ee*. We attributed the low levels of enantioselectivity in this reaction to the monodentate nature of the dienophile moiety and the flexibility of the aluminum–oxygen coordination bond of the initially formed complex between the substrate and the Lewis acid.

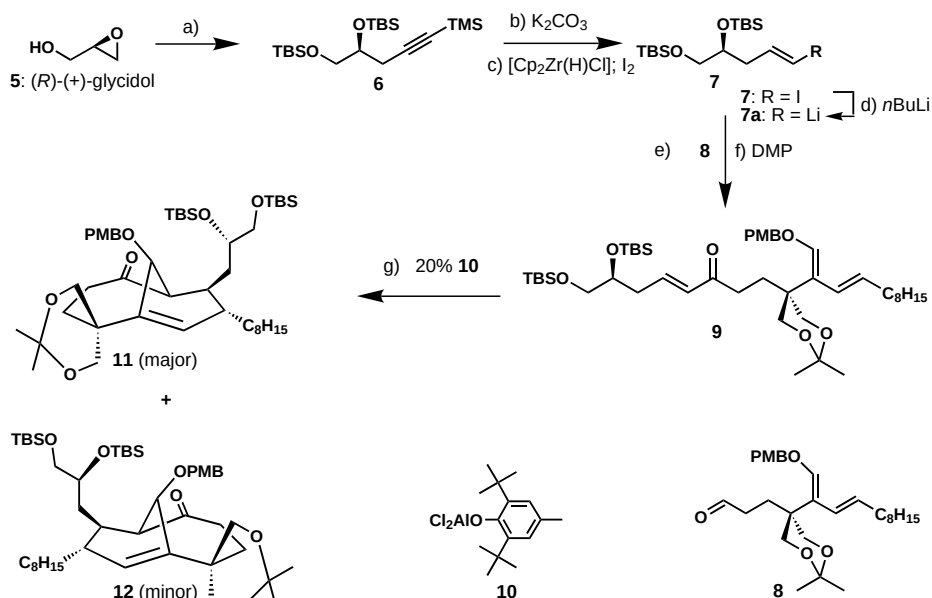
We then turned our attention to a strategy predicated on a substrate-based control of the diastereoselectivity of the intramolecular cycloaddition. Specifically, we envisaged modifying the triene **2** so as to introduce a bulky chiral moiety capable of influencing the facial selectivity of the intramolecular Diels–Alder reaction. Our goal was a smooth reentry into the previously chartered^[4] path to the CP molecules to complete the asymmetric total synthesis without additional steps.

The construction of the designed asymmetric Diels–Alder precursor **9** is outlined in Scheme 2. Thus, the *R* enantiomer of glycidol (**5**) was arbitrarily chosen and converted, in one step (epoxide opening with TMS-acetylide, followed by quenching with TBSOTf) to the *tri*-silyl acetylene **6**. Selective removal of the pendant TMS group, followed by exposure to the Schwartz reagent [Cp₂Zr(H)Cl]^[8] and addition of I₂ led to the vinyl iodide **7** in 54% overall yield from **5**. Metal–halogen exchange (*n*BuLi) facilitated the union of the vinyl iodide **7** with the aldehyde **8**^[4] furnishing, after oxidation with DMP,^[9] enone **9** via the lithio derivative **7a**.

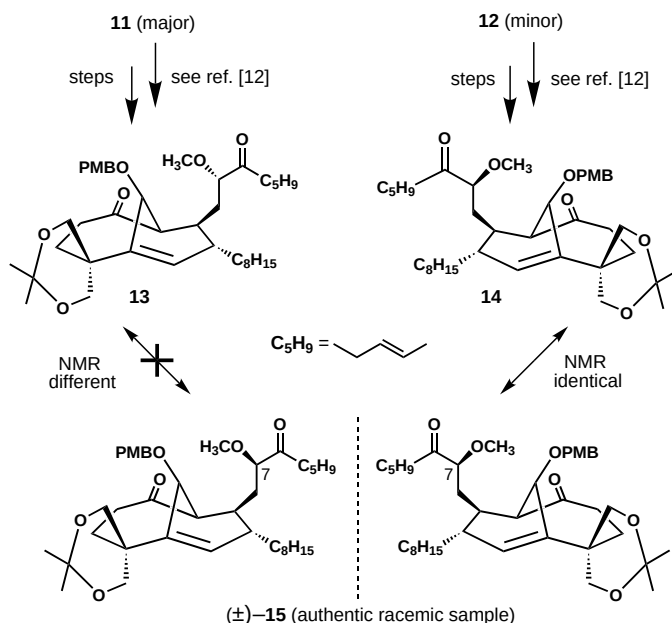
Initial Lewis acid induced cyclizations with **9** led to disappointingly low diastereomeric excesses. For instance, exposure of **9** to 20 mol% Et₂AlCl in CH₂Cl₂ at –10 °C afforded **11** and **12** as a 2:1 mixture (Scheme 2). Extensive experimentation, whose details will have to await the full account of this work, led us to catalyst **10**^[10] (Scheme 2). Thus, in the presence of 20 mol% of **10** at –80 °C in toluene, cycloaddition ensued to produce a 5.7:1 mixture (70% *de*) of **11** and **12** in 88% combined yield.

The relative configuration of these two diastereomers (**11** and **12**) was deduced by NMR correlation of downstream intermediates **13** and **14** with an authentic racemic sample **15**^[11] (Scheme 3).^[12]

In preparation for reentry into the previously developed route for the synthesis of the CP compounds, **11** and **12** were

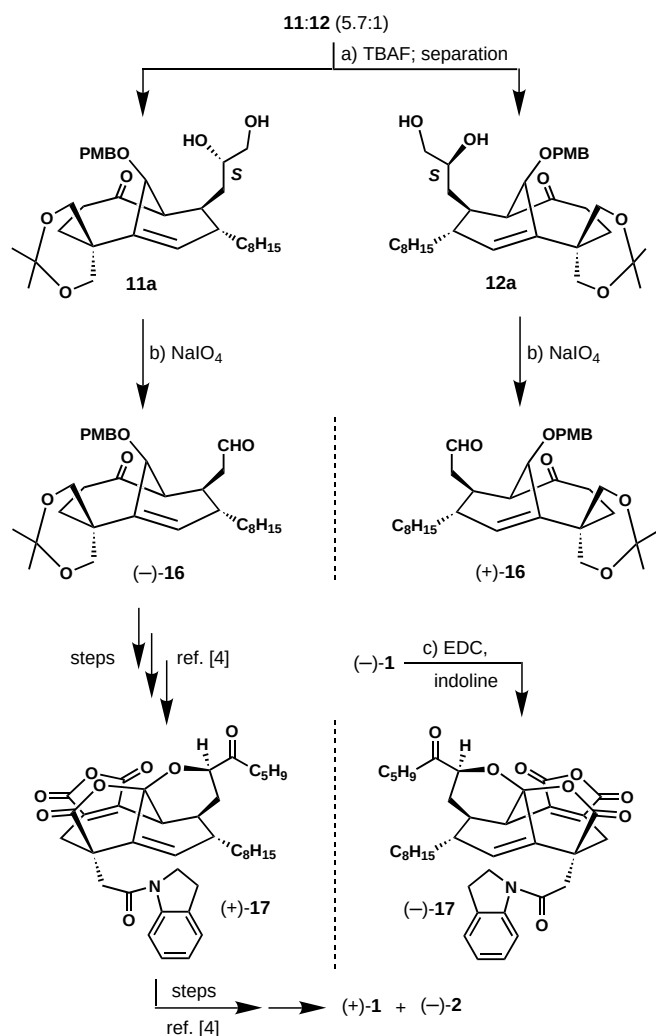


Scheme 2. Diastereoselective Diels–Alder reaction of triene **9** to form the CP precursors **11** and **12**. a) 1. TMSCH₂CH (1.5 equiv), *n*BuLi (1.4 equiv), THF, –78 °C, added to a solution of (*R*)-glycidol (**5**; 1.0 equiv), *n*BuLi (1.1 equiv), THF, –78 → 25 °C, 12 h; 2. TBSOTf (3.0 equiv), 2,6-lutidine (1.0 equiv), 0 → 25 °C; b) K₂CO₃ (2.0 equiv), MeOH, 25 °C, 12 h; c) [Cp₂Zr(H)Cl] (1.2 equiv), benzene, 25 °C, then I₂ (1.0 equiv), CCl₄, 25 °C, 54% overall yield (from **5**); d) **7** (1.5 equiv), *n*BuLi (1.5 equiv), THF, –78 °C, 30 min; e) **7a** added to **8** in THF, –78 °C, 30 min, 90%; f) DMP (1.3 equiv), NaHCO₃ (5.0 equiv), CH₂Cl₂, 25 °C, 10 min, 70%; g) **10** (0.2 equiv), toluene, –80 °C, 1 h, 88% (**11**:**12** = 5.7:1). DMP = Dess–Martin periodinane; TBS = *tert*-butyldimethylsilyl; Tf = trifluoromethanesulfonyl; TMS = *tert*-butyltrimethylsilyl.



Scheme 3. Determination of the relative configuration of intermediates **11** and **12** by NMR spectroscopic correlation with the authentic racemic sample **15** (relative configuration at C7 as depicted).

converted to the enantiomeric aldehydes (–)-**16** and (+)-**16**, respectively, (Scheme 4). Desilylation with TBAF followed by flash column chromatography (silica gel, hexanes/EtOAc (2:1)) of the diol mixture **11a**/**12a** allowed almost complete separation of the two diastereomers **11a** (major) and **12a** (minor) (Table 1). Sodium periodate induced oxidative cleav-



Scheme 4. Unambiguous proof of the absolute configuration of the CP compounds (-)-1 and (+)-2 and of the indoline derivative (-)-17 by asymmetric total synthesis of (+)-17, (+)-1, and (-)-2 from intermediate 11. a) TBAF (4.0 equiv), THF, 0 $\rightarrow$ 25 $^{\circ}\text{C}$, 2 h, >95%; c) EDC (3.0 equiv), DMAP (3.0 equiv), indoline (1.5 equiv), CH_2Cl_2 , 25 $^{\circ}\text{C}$, 2.5 h; then 1N HCl, 25 $^{\circ}\text{C}$, 5 min, 92%. EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; DMAP = 4-(dimethylamino)pyridine; TBAF = tetra-*n*-butylammonium fluoride.

age of diols 11a and 12a resulted in the formation of aldehydes (-)-16 and (+)-16, respectively. Based on the diastereomeric enrichment of 11a (ca. 15:1), the enantiomeric excess of (-)-16 was calculated to be ca. 87%.

The optically enriched aldehyde (-)-16, whose racemic form is a known intermediate in the total synthesis of racemic 1 and 2, was then efficiently converted to the indoline (-)-17 as previously described^[4] (Scheme 4). Due to the ease of purification and handling and the amplified optical rotation of the indoline derivatives, we made comparisons at this stage, and therefore, the naturally derived indoline (-)-17 was obtained by coupling 1^[13] with indoline in the presence of EDC (92%). Synthetic 17, derived from the major Diels–Alder product 11, was found to be identical to natural 17 according to NMR spectroscopy, TLC (three different solvent systems), HPLC, and IR spectroscopy. The optical rotation, however, was opposite in magnitude ($[\alpha]_{\text{D}}^{23} = +56.2$; $c = 0.08$;

Table 1. Selected physical and spectroscopic data of compounds 11a and 12a.

11a: $[\alpha]_{\text{D}}^{23} = -50.6$ ($c = 1.3$, CHCl_3); IR (neat): $\tilde{\nu} = 3388, 2991, 2928, 2857, 1692, 1613, 1514, 1452, 1376, 1303, 1252, 1199, 1160, 1094, 1035, 968, 875, 840 \text{ cm}^{-1}$; ^1H NMR (CDCl_3 , 500 MHz): $\delta = 7.19$ (d, $J = 8.8 \text{ Hz}$, 2H), 6.85 (d, $J = 8.8 \text{ Hz}$, 2H), 5.44–5.35 (m, 3H), 4.49 (d, $J = 11.0 \text{ Hz}$, 1H), 4.37 (d, $J = 11.0 \text{ Hz}$, 1H), 4.30 (dd, $J = 11.4, 1.1 \text{ Hz}$, 1H), 4.14 (q, $J = 2.2 \text{ Hz}$, 1H), 4.06 (d, $J = 11.8 \text{ Hz}$, 1H), 4.02 (d, $J = 11.4 \text{ Hz}$, 1H), 3.90–3.85 (m, 1H), 3.79 (s, 3H), 3.56 (dd, $J = 11.4, 3.3 \text{ Hz}$, 1H), 3.51 (dd, $J = 11.4, 1.5 \text{ Hz}$, 1H), 3.44 (dd, $J = 11.4, 7.3 \text{ Hz}$, 1H), 3.03 (s, 1H), 2.81 (s, 1H), 2.59 (s, 1H), 2.39 (q, $J = 7.3 \text{ Hz}$, 1H), 2.33 (t, $J = 13.2 \text{ Hz}$, 1H), 2.21 (td, $J = 13.2, 1.9 \text{ Hz}$, 1H), 2.02–1.93 (m, 3H), 1.69–1.54 (m, 6H), 1.43 (s, 3H), 1.39 (s, 3H), 1.48–1.11 (m, 9H); ^{13}C NMR (CDCl_3 , 125 MHz): $\delta = 216.4, 159.4, 142.3, 131.4, 129.7, 129.5, 129.4, 124.8, 114.0, 97.9, 77.3, 71.6, 69.4, 67.3, 67.1, 64.5, 58.3, 55.3, 41.9, 41.8, 40.6, 38.9, 36.4, 32.7, 32.6, 29.6, 29.3, 28.7, 27.9, 27.0, 20.5, 18.0$; HR-MS (MALDI): calcd for $\text{C}_{34}\text{H}_{50}\text{O}_7\text{Na}^+$ [$M + \text{Na}^+$]: 593.3449, found: 593.3457

12a: $[\alpha]_{\text{D}}^{23} = +43.4$ ($c = 2.0$, CHCl_3); IR (neat): $\tilde{\nu} = 3413, 2991, 2928, 2857, 1685, 1613, 1514, 1452, 1375, 1303, 1251, 1199, 1165, 1092, 1035, 968, 875, 839 \text{ cm}^{-1}$; ^1H NMR (CDCl_3 , 500 MHz): $\delta = 7.20$ (d, $J = 8.4 \text{ Hz}$, 2H), 6.87 (d, $J = 8.8 \text{ Hz}$, 2H), 5.45–5.38 (m, 2H), 5.37–5.33 (m, 1H), 4.54 (d, $J = 11.0 \text{ Hz}$, 1H), 4.36 (d, $J = 11.0 \text{ Hz}$, 1H), 4.31 (dd, $J = 11.4, 1.4 \text{ Hz}$, 1H), 4.13 (q, $J = 1.9 \text{ Hz}$, 1H), 4.07 (d, $J = 11.8 \text{ Hz}$, 1H), 4.03 (d, $J = 11.8 \text{ Hz}$, 1H), 3.80 (s, 3H), 3.78–3.72 (m, 2H), 3.61–3.56 (m, 1H), 3.51 (dd, $J = 11.4, 1.5 \text{ Hz}$, 1H), 3.46–3.40 (m, 1H), 3.30 (s, 1H), 2.43 (q, $J = 7.0 \text{ Hz}$, 1H), 2.38 (t, $J = 13.6 \text{ Hz}$, 1H), 2.24–2.18 (m, 2H), 2.03–1.94 (m, 3H), 1.71–1.52 (m, 6H), 1.43 (s, 3H), 1.40 (s, 3H), 1.48–1.13 (m, 9H); ^{13}C NMR (CDCl_3 , 125 MHz): $\delta = 217.3, 159.4, 142.3, 131.4, 129.6, 129.4, 128.7, 124.9, 114.0, 97.9, 77.1, 72.4, 71.5, 67.4, 66.5, 64.9, 58.5, 55.3, 45.2, 41.8, 40.6, 38.9, 37.0, 32.9, 32.6, 29.6, 29.3, 28.4, 27.9, 26.9, 20.6, 18.0$; HR-MS (MALDI): calcd for $\text{C}_{34}\text{H}_{50}\text{O}_7\text{Na}^+$ [$M + \text{Na}^+$]: 593.3449, found: 593.3471

CH_2Cl_2) to that of the naturally derived 17 ($[\alpha]_{\text{D}}^{23} = -80.0$; $c = 0.1$; CH_2Cl_2). Circular dichroism (CD) spectroscopy verified the identity of synthetic 17 as the enantiomer of natural 17 by virtue of the pronounced antipodal Cotton effect observed (Figure 2).^[14] Synthetic 17 was also processed to give *ent*-1 and

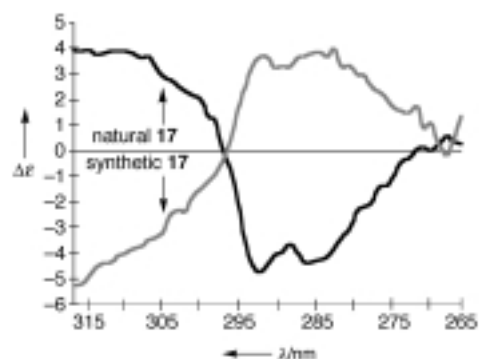


Figure 2. CD spectra (CH_2Cl_2 , 25 $^{\circ}\text{C}$, ca. 0.5 mm) of synthetic 17 (grey line) and natural 17 (black line).

ent-2. Therefore, the absolute configuration of the CP molecules can be confidently assigned as depicted in Figure 1. The asymmetric synthesis of *ent*-1 and *ent*-2 can obviously be employed to procure the natural enantiomers of the the CP molecules simply by utilizing (*S*)-glycidol.

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- [1] T. T. Dabrah, T. Kaneko, W. Massefski, Jr., E. B. Whipple, *J. Am. Chem. Soc.* **1997**, *119*, 1594; T. T. Dabrah, H. J. Harwood, Jr., L. H. Huang, N. D. Jankovich, T. Kaneko, J.-C. Li, S. Lindsey, P. M. Moshier, T. A. Subashi, M. Therrien, P. C. Watts, *J. Antibiot.* **1997**, *50*, 1.

- [2] D. Hepworth, *Chem. Ind. (London)* **2000**, 2, 59.
- [3] a) K. C. Nicolaou, M. W. Härter, L. Boulton, B. Jandeleit, *Angew. Chem.* **1997**, 109, 1243; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1194; b) K. C. Nicolaou, M. H. D. Postema, N. D. Miller, G. Yang, *Angew. Chem.* **1997**, 109, 2922; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 2821; c) H. M. L. Davies, R. Calvo, G. Ahmed, *Tetrahedron Lett.* **1997**, 38, 1737; d) P. W. M. Sgarbi, D. L. J. Clive, *Chem. Commun.* **1997**, 2157; e) A. Armstrong, T. J. Critchley, A. A. Mortlock, *Synlett* **1998**, 552; f) O. Kwon, D.-S. Su, D. Meng, W. Deng, D. C. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, 110, 1978; *Angew. Chem. Int. Ed.* **1998**, 37, 1877; g) O. Kwon, D.-S. Su, D. Meng, W. Deng, D. C. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, 110, 1981; *Angew. Chem. Int. Ed.* **1998**, 37, 1880; h) N. Waizumi, T. Itoh, T. Fukuyama, *Tetrahedron Lett.* **1998**, 39, 6015; i) C. Chen, M. E. Layton, M. D. Shair, *J. Am. Chem. Soc.* **1998**, 120, 10784; j) A. J. Frontier, S. J. Danishefsky, G. A. Koppel, D. Meng, *Tetrahedron* **1998**, 54, 12721; k) M. M. Bio, J. L. Leighton, *J. Am. Chem. Soc.* **1999**, 121, 890; l) K. C. Nicolaou, P. S. Baran, R. Jautelat, Y. He, K. C. Fong, H.-S. Choi, W. H. Yoon, Y.-L. Zhong, *Angew. Chem.* **1999**, 111, 532; *Angew. Chem. Int. Ed.* **1999**, 38, 549; m) D. L. J. Clive, S. Sun, X. He, J. Zhang, V. Gagliardini, *Tetrahedron Lett.* **1999**, 40, 4605; n) D. Meng, S. J. Danishefsky, *Angew. Chem.* **1999**, 111, 1582; *Angew. Chem. Int. Ed.* **1999**, 38, 1485; o) T. Yoshimitsu, M. Yanagiya, H. Nagaoka, *Tetrahedron Lett.* **1999**, 40, 5215; p) D. Meng, Q. Tan, S. J. Danishefsky, *Angew. Chem.* **1999**, 111, 3393; *Angew. Chem. Int. Ed.* **1999**, 38, 3197; q) G. A. Sulikowski, F. Agnelli, R. M. Corbett, *J. Org. Chem.* **2000**, 65, 337; r) For biosynthetic explorations, see: P. Spencer, F. Agnelli, H. J. Williams, N. P. Keller, G. A. Sulikowski, *J. Am. Chem. Soc.* **2000**, 122, 420; s) For reviews, see ref. [2] and U. Diederichsen, *Nachr. Chem. Tech. Lab.* **1999**, 47, 1423 and E. M. Carreira, *Angew. Chem.* **2000**, 112, 1473; *Angew. Chem. Int. Ed.* **2000**, 39, 1415.
- [4] Part 1: K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, H.-S. Choi, W. H. Yoon, Y. He, K. C. Fong, *Angew. Chem.* **1999**, 111, 1774; *Angew. Chem. Int. Ed.* **1999**, 38, 1669; Part 2: K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, K. C. Fong, Y. He, W. H. Yoon, H.-S. Choi, *Angew. Chem.* **1999**, 111, 1781; *Angew. Chem. Int. Ed.* **1999**, 38, 1676.
- [5] A synthesis of an epimer of **1** at C7 has been reported by Danishefsky and coworkers, see ref. [3p].
- [6] T. G. Lease, K. J. Shea, *J. Am. Chem. Soc.* **1993**, 115, 2248.
- [7] Catalyst **4** was prepared from the corresponding (S)-diol and Al(*i*Bu₃) (1.0 equiv, 1.0 M in hexanes) by stirring the two compounds in toluene at ambient temperature for 30 min. The diol was prepared according to the methods of K. B. Simonsen, K. V. Gothelf, K. A. Jørgensen, *J. Org. Chem.* **1998**, 63, 7536 and P. Wipf and J.-K. Jung (see J.-K. Jung, Ph.D. Thesis, University of Pittsburgh, **1999**).
- [8] J. Schwartz, J. Labinger, *Angew. Chem.* **1976**, 88, 402–409; *Angew. Chem. Int. Ed. Engl.* **1976**, 15, 333–340.
- [9] a) D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, 48, 4155–4156; b) D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, 113, 7277–7278; c) S. D. Meyer, S. L. Schreiber, *J. Org. Chem.* **1994**, 59, 7549–7552.
- [10] Catalyst **10** was prepared by adding EtAlCl₂ (1 M in hexanes) to a solution of the corresponding phenol (1.1 equiv) in toluene at 0 °C and stirring the mixture at ambient temperature for 30 min.
- [11] Racemic **15** was prepared from the corresponding α -hydroxy dithiane^[4] by stirring with NaH (1.1 equiv) and MeI (1.1 equiv) at 0 °C (80% yield) followed by dithiane deprotection with PhI(O-COCF₃)₂ in aqueous methanol (71%).
- [12] Compounds **13** and **14** were prepared from **11** and **12** in 29 and 22% overall yield, respectively, by the following seven-step sequence: 1) TBAF (3.0 equiv), THF, 0 → 25 °C; 2) TBSCl (1 equiv), imidazole (1 equiv), CH₂Cl₂, 0 → 25 °C; 3) NaH (1.1 equiv), MeI (1.1 equiv), THF, 0 °C; 4) TBAF (1.5 equiv), THF, 25 °C; 5) DMP (1.2 equiv), CH₂Cl₂, 25 °C; 6) C₅H₉MgBr (1.1 equiv), THF, 0 °C; 7) DMP (1.2 equiv), CH₂Cl₂, 25 °C.
- [13] A sample of natural **1** was kindly provided to us by Dr. T. Kaneko, Pfizer (Groton, CT).
- [14] A similar effect was observed for (–)-**16** and (+)-**16**.

An Iron Wheel on a Tin Drum: A Novel Assembly of a Hexaferrocene Unit on a Tin – Oxygen Cluster**

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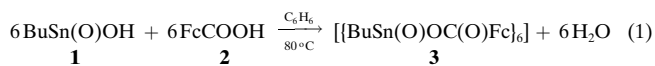
Dedicated to Professor Herbert W. Roesky on the occasion of his 65th birthday and to Professor Samaresh Mitra on the occasion of his 60th birthday

Multimetallic and multimetalocene assemblies have been attracting attention in recent years for a number of reasons.^[1] Recently, the syntheses of a dodecanickel carbosilane dendrimer^[2] and a hexametallic cartwheel-type molecule^[3] have been reported. These species have been considered as promising materials in homogeneous catalysis because the higher molecular weight multimetallic catalyst can be separated from the reaction medium by nanomembrane filtration.^[4] Similarly syntheses of compounds containing a regular arrangement of metallocenes in general and ferrocenes in particular have been pursued with the objective of generating materials possessing useful electric, optical, chemical, and magnetic properties. These include redox-active materials for modification of electrodes^[5] or materials which can function as multielectron redox systems^[6] or biosensors.^[7]

Among the synthetic approaches to prepare macromolecules containing ferrocenes, the most successful strategy has been the ring-opening polymerization of main group element-bridged [1]ferrocenophanes by thermal or anionic methods.^[8] Some of these polymers possess interesting electrochemical properties indicative of interaction between iron atoms in the solid state.^[9] In contrast to the linear polymers, syntheses of assemblies containing ferrocene units in a cyclic arrangement have been carried out by utilizing various strategies. Astruc and co-workers have pioneered the synthesis of molecular trees where a $[\text{Fe}(\text{C}_5\text{Me}_5)]$ moiety functions as the core of the tree and the branches can be suitably grafted by alkylation reactions.^[10] A heptanuclear complex has been assembled by this approach. Jutzi and co-workers have synthesized decalylferrocene and have been able to construct dendrimer-like structures.^[11] However, both these examples involve multistep synthesis, and X-ray structures of these multiferrrocene assemblies have not been reported.

In a radically different approach to the assembly of multiferrrocene compounds, we have decided to utilize reac-

tions known in organotin chemistry for this purpose.^[12] We report here the preparation of a robust, thermally stable hexaferrocene assembly in quantitative yield by the reaction of two commercially available reactants. The reaction of *n*-butylstannonic acid (**1**) with ferrocene monocarboxylic acid (**2**) in a 1:1 stoichiometry in benzene afforded a dark red compound identified as **3** [Eq. (1); Fc = ferrocenyl].^[13] This



product is obtained in nearly quantitative yield, in contrast to the poor to modest yields usually reported for many multiferrrocene compounds. Compound **3**, which contains six ferrocene units, is thermally quite stable and does not undergo any change till 244 °C.

The molecular structure of **3** is shown in Figure 1.^[14] The structure shows a giant-wheel arrangement of the six ferrocene units with a drum-like stannoxane central core serving as

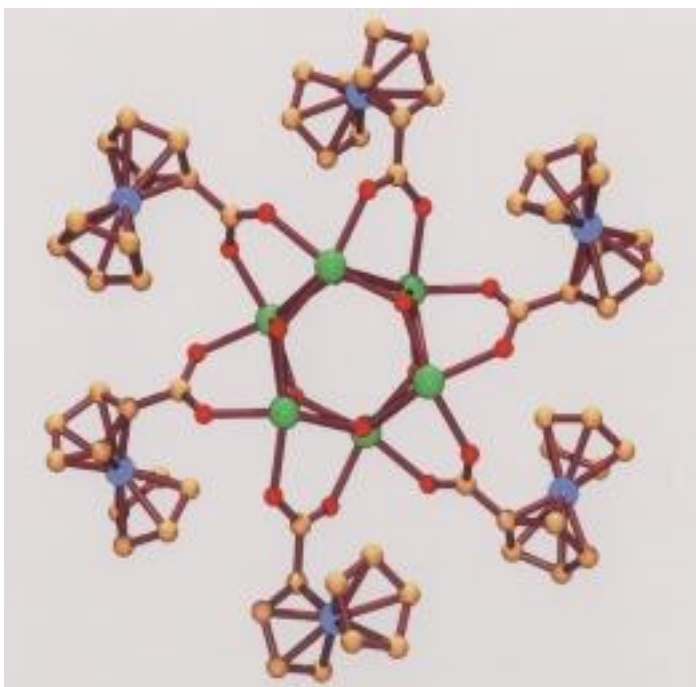


Figure 1. Pov Ray diagram of **3**. The *n*-butyl groups on tin have been omitted for clarity. Red: oxygen, green: tin, yellow: carbon, cyan: iron.

the structural support for the hexaferrocene assembly. This represents a structural arrangement distinct and unique from the ones observed by others. Most of the nonpolymer ferrocene assemblies involve the linkage of ferrocene units in the form of branches that emanate from a central tree or a core. Unlike these examples, in the present case there is a distinct cyclic connectivity of the six ferrocene units established through the linking tin atoms (Figure 2). In this respect compound **3** is reminiscent of the recently described assembly containing seven ferrocene units doubly bridged to each other by silyl bridges.^[15] The structure of **3** shows that the ferrocene carboxylate moiety is involved in binding to two alternate tin atoms of a distannoxane unit. There is a slight asymmetry in

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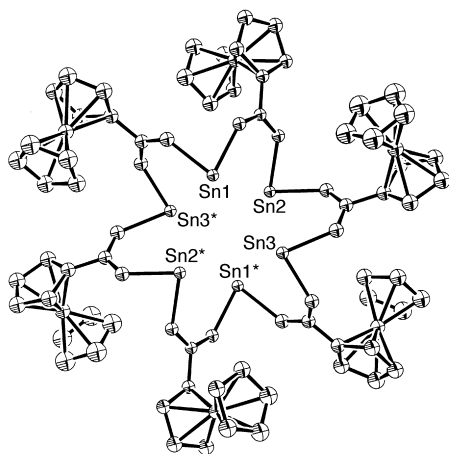


Figure 2. ORTEP diagram of **3** showing the connectivity of the six ferrocene molecules through the tin atoms (the central stannoxane core has been omitted for clarity).

the Sn–O bond lengths arising from the carboxylate linkage (Figure 3). However, the two C–O distances within a ferrocene carboxylate moiety are exactly similar. The central

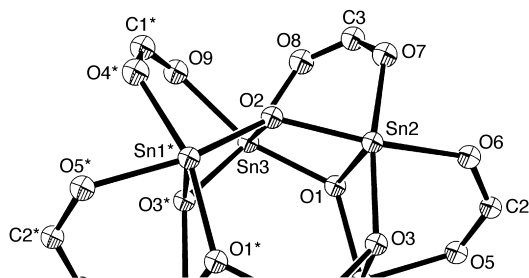


Figure 3. ORTEP diagram of **3** showing the central stannoxane core along with the connecting carboxylate units (the ferrocenyl groups and the *n*-butyl groups have been omitted for clarity). Two crystallographically independent molecules are present in the unit cell. Selected bond lengths [Å] and angles [°] for one of the two molecules: Sn1–O3 2.106(4), Sn1–O1 2.100(4), Sn1–O2* 2.079(3), Sn1–O5 2.142(3), Sn1–O4 2.161(4), C1–O4 1.271(7), C1–O9* 1.270(7); O1–Sn1–O2* 105.20(13), Sn1–O1–Sn3 132.20(17), Sn1–O3–Sn2 100.35(15), O1–Sn1–O3 77.80(14).

stannoxane cluster in **3** is made up of two hexameric Sn_3O_3 rings, each present in a puckered chair-like conformation. These rings are joined to each other to afford six Sn_2O_2 distannoxane units as the side faces of the cluster. This represents the signature structural feature of stannoxane clusters.^[12]

Cyclic voltammetric studies on compound **3** show a single quasireversible peak with an $E_{1/2}$ value of 0.73 V (vs. SCE, Figure 4).^[16] This suggests that all six ferrocene units are oxidized at the same potential. A comparison of the $E_{1/2}$ values with those of some multiferrocene assemblies is shown in Table 1. A noteworthy feature

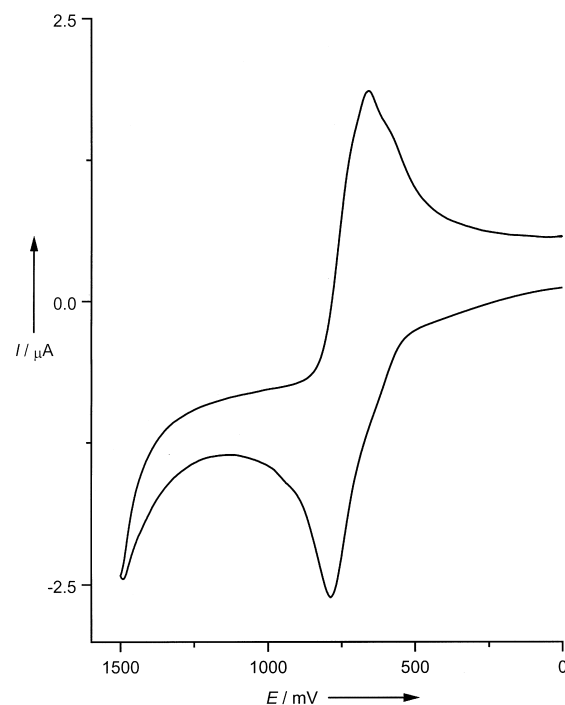


Figure 4. Cyclic voltammogram of **3** at 25 mV s^{-1} . The potential E is given versus the saturated calomel electrode (SCE).

of the electrochemical behavior of **3** is the reversibility of the voltammogram even after several cycles. Experimentally this has been verified up to ten cycles. This indicates that the cluster is robust and does not decompose upon oxidation. This situation is in contrast to the one reported by Shriver and co-workers, who observed spontaneous decomposition of the ferrocenyl-attached molybdenum cluster $\text{Na}_2[\text{Mo}_6\text{Cl}_8\{\text{O}_2\text{C}(\text{C}_5\text{H}_4\text{Fe}(\text{C}_5\text{H}_5))_6\}] \cdot \text{CH}_3\text{OH}$ upon oxidation.^[17]

Experimental Section

3: A mixture of **1** (0.21 g, 1.0 mmol) and **2** (0.23 g, 1.0 mmol) in benzene (120 mL) was heated under reflux for 6 h using a Dean–Stark apparatus to remove by azeotropic distillation the water formed in the reaction. The reaction mixture was filtered and the solvent removed from the filtrate in vacuo to afford a red-orange powder, which was found to be analytically pure and was shown to be **3**. Diamond-shaped, shining crystals of **3** were obtained in chloroform at 5 °C. Yield: 0.38 g (91.0%); m.p. 244 °C (decomp.).

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- [1] G. R. Newkome, E. He, C. N. Moorefield, *Chem. Rev.* **1999**, 99, 1689.
- [2] J. W. J. Knapen, A. W. Van der Made, J. C. de Wilde, P. W. N. M. van Leeuwen, P. Wijkens, D. M. Grove, G. van Koten, *Nature* **1994**, 372, 659.
- [3] H. P. Dijkstra, P. Steenwinkel, D. M. Grove, M. Lutz, A. L. Spek, G. van Koten, *Angew. Chem.* **1999**, 111, 2322; *Angew. Chem. Int. Ed.* **1999**, 38, 2186.

Table 1. CV data for **3** and some other multiferrocene derivatives.

Compound	$E_{1/2}$ [V] ^[a]	ΔE_p [mV]	Ref.
$[(\text{C}_5\text{H}_5)\text{FeC}_6\{(\text{CH}_2)_5(\text{C}_5\text{H}_4)\text{Fe}(\text{C}_5\text{H}_5)\}_6]\text{PF}_6$	+0.44	55	[10]
$[\text{C}_6\{(\text{CH}_2)_5(\text{C}_5\text{H}_4)\text{Fe}(\text{C}_5\text{H}_5)\}_6]$	+0.45	–	[10]
$[(\text{C}_5\text{H}_5)\text{FeC}_6\{(\text{CH}_2)_2\text{C}_6\text{H}_4\text{OC}(\text{O})(\text{C}_5\text{H}_4)\text{Fe}(\text{C}_5\text{H}_5)\}_6]\text{PF}_6$	+0.78	60	[18]
$[\text{C}_6\{(\text{CH}_2)_2\text{C}_6\text{H}_4\text{OC}(\text{O})(\text{C}_5\text{H}_4)\text{Fe}(\text{C}_5\text{H}_5)\}_6]$	+0.88	120	[18]
$[\text{BuSn}(\text{O})\text{OC}(\text{O})(\text{C}_5\text{H}_4)\text{Fe}(\text{C}_5\text{H}_5)]_6$ (3)	+0.72	128	this work

[a] Versus SCE.

- [4] U. Kragl, C. Dreisbach, *Angew. Chem.* **1996**, *108*, 684–685; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 642–664.
- [5] a) J. B. Flanagan, S. Margel, A. J. Bard, F. C. Anson, *J. Am. Chem. Soc.* **1978**, *100*, 4248; b) F. G. Bordwell, G. D. Cooper, H. Mortina, *J. Am. Chem. Soc.* **1957**, *79*, 376; c) T. W. Smith, J. E. Kunder, D. Wychick, *J. Polym. Sci.* **1976**, *14*, 2433.
- [6] a) D. Astruc, *New. J. Chem.* **1992**, *16*, 305; b) D. Astruc, *Top. Curr. Chem.* **1991**, *160*, 47.
- [7] a) H. A. U. Hill, D. J. Page, N. J. Walton, *J. Electroanal. Chem.* **1987**, *217*, 141; b) Y. Degani, A. Heller, *J. Phys. Chem.* **1987**, *91*, 1285.
- [8] R. Rulkens, A. J. Lough, I. Mannes, *J. Am. Chem. Soc.* **1994**, *116*, 797.
- [9] I. Mannes, *Adv. Mater.* **1994**, *6*, 62.
- [10] J.-L. Fillaut, J. Lineares, D. Astruc, *Angew. Chem.* **1994**, *106*, 2540; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2460.
- [11] P. Jutzi, C. Batz, B. Neumann, H.-G. Stämmler, *Angew. Chem.* **1996**, *108*, 2272; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2118.
- [12] a) V. Chandrasekhar, C. G. Schmid, S. D. Burton, J. M. Holmes, R. O. Day, R. R. Holmes, *Inorg. Chem.* **1987**, *26*, 1050; b) R. R. Holmes, C. G. Schmid, V. Chandrasekhar, R. O. Day, J. M. Holmes, *J. Am. Chem. Soc.* **1987**, *109*, 1408; c) K. C. Kumaraswamy, S. Nagabrahmanandachari, *Phosphorus sulfur silicon Retat. Elem.* **1992**, *65*, 9.
- [13] **3**: ¹H NMR (200 MHz, CDCl₃): δ = 1.00 (t, J = 7.2 Hz, 3 H; CH₃), 1.54 (m, 4 H; CH₂CH₂), 1.91 (m, 2 H; SnCH₂), 4.14 (s, 5 H; C₅H₅), 4.30 (m, 2 H; C₅H₄), 4.82 (m, 2 H; C₅H₄); ¹¹⁹Sn NMR (150 MHz, CDCl₃): δ = −486.6 (s).
- [14] Crystallographic data for **3** (C₉₀H₁₀₈Fe₆O₁₈Sn₆·2.57 CHCl₃): crystal dimensions 0.51 × 0.48 × 0.40 mm, *M_r* = 2831.96, triclinic, space group *P* $\bar{1}$; *a* = 15.786(8), *b* = 16.178(8), *c* = 24.508(12) Å, α = 91.27(2), β = 107.328(18), γ = 117.499(14)°; *Z* = 2, ρ_{calcd} = 1.807 Mg m^{−3}, *F*(000) = 2794; absorption coefficient 2.480 mm^{−1}. Intensity data were collected using a Bruker SMART ccd area detector^[19] mounted on a Bruker P4 goniometer using graphite-monochromated MoK α radiation (λ = 0.71073 Å). The sample was cooled to 138(2) K. The intensity data, which nominally covered 1.5 hemispheres of reciprocal space, were measured as a series of ϕ oscillation frames, each 0.4° for 30 s per frame. The detector was operated in the 512 × 512 mode and was positioned 5.00 cm from the sample. Coverage of unique data was 97.9% complete to 25.00° in θ. Cell parameters were determined from a nonlinear least-squares fit of 8192 peaks in the range 3.0 < θ < 25.0°. The first 50 frames were repeated at the end of data collection and yielded 698 peaks showing a variation of −0.04% during the data collection. A total of 69782 data were measured in the range 1.50 < θ < 28.34°. The data were corrected for absorption by the empirical method^[20] giving minimum and maximum transmission factors of 0.296 and 0.353. The data were merged to form a set of 24235 independent data with *R*(int) = 0.0483. The triclinic space group *P* $\bar{1}$ was determined by statistical tests and verified by subsequent refinement. The structure was solved by direct methods and refined by full-matrix least-squares methods on *F*².^[21] The hydrogen atom positions were initially determined by geometry and refined by a riding model. Non-hydrogen atoms were refined with anisotropic displacement parameters. A total of 1414 parameters were refined against 776 restraints and 24235 data to give *wR*(*F*²) = 0.1107 and *S* = 1.080 for *w* = 1/[σ²(*F*²) + (0.0364 *P*)² + 25.7453 *P*], where *P* = [*F_o*² + 2*F_c*²]/3. The final *R*(*F*) was 0.0431 for the 18440 observed data (*F* > 4σ(*F*)). The largest shift was 0.046 in the final refinement cycle. The final difference map had maxima and minima of 2.122 and −1.415 e Å^{−3}. Three of the *n*-butyl groups are disordered and each is modeled in two orientations. Three solvent sites are found in the regions of the disordered butyl groups, which are also disordered. A fourth disordered solvent site is located away from the main species and is modeled in three orientations. Restraints were applied to the positional and displacement parameters of the disordered atoms. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137558. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [15] B. Grossmann, J. Heinze, E. Herdtweck, F. H. Kohler, H. Noth, H. Schwenk, M. Spiegler, W. Wachter, B. Weber, *Angew. Chem.* **1997**, *109*, 384; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 387.
- [16] Cyclic voltammetric and differential pulse voltammetric studies were performed on a PAR model 273A polarographic analyzer utilizing the three-electrode configuration of a Pt (Beckman) working electrode, a Pt mesh counterelectrode, and a commercially available saturated calomel electrode as the reference electrode interfaced with the computer. Half-wave potentials were measured as the average of the cathodic and the anodic peak potentials. The voltammograms were recorded in dichloromethane containing 0.1 M tetrabutylammonium hexafluorophosphate as the supporting electrolyte, and the potential was scanned from −1.5 to +1.5 V at scan rates from 25 to 200 mV s^{−1}.
- [17] N. Prokopuk, D. F. Shriver, *Inorg. Chem.* **1997**, *36*, 5609.
- [18] J.-L. Fillaut, D. Astruc, *J. Chem. Soc. Chem. Commun.* **1993**, 1320.
- [19] a) Data Collection: SMART Software Reference Manual Bruker-AXS, 6300 Enterprise Dr., Madison, WI 53719-1173, USA, **1994**; b) Data Reduction: SAINT Software Reference Manual Bruker-AXS, 6300 Enterprise Dr., Madison, WI 53719-1173, USA, **1995**.
- [20] G. M. Sheldrick, SADABS, Universität Göttingen, Germany, **1996**.
- [21] a) G. M. Sheldrick, SHELXTL, Version 5 Reference Manual, Bruker-AXS, 6300 Enterprise Dr., Madison, WI 53719-1173, USA, **1994**; b) *International Tables for Crystallography*, Vol. C, Tables 6.1.1.4, 4.2.6.8, and 4.2.4.2, Kluwer, Boston, **1995**.

Molecular Signal Transduction through Conformational Transmission of a Perhydroanthracene Transducer**

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Signal transduction plays an important role in biological systems.^[1] While many processes in biological signal transduction^[2, 3] use the diffusion of a “second messenger” to the effector site, a molecular transducer linking the receptor and the effector site offers the advantages of a predictable signal distance and signal direction.^[4] Herein we show how conformational transmission^[5, 6] can be used to design a synthetic transducer.^[7] Novel biconformational perhydroanthracene derivatives^[8] were synthesized and successfully used as signal transducers in which conformational transmission occurs via a triple ring flip.^[9]

Molecular signal transduction via conformational transmission leads, upon a signal stimulus, to a conformational change at the receptor site (Figure 1a). This motion is transmitted by the transducer to the effector site and a second conformational change results in a measurable effect. A good transducer should show a two-state conformational behavior: Binding of the molecular signal should lead to a switching from one conformational state to the other.^[10–13] The

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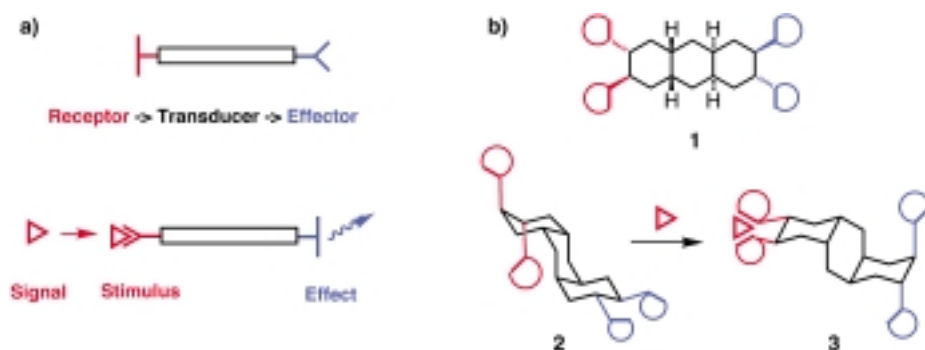


Figure 1. Molecular signal transduction via conformational transmission. a) The signal causes a conformational change at the receptor site, which is carried to the effector site. b) General structure of a tetrasubstituted perhydroanthracene transducer **1** with two receptor sites (red) and two effector sites (blue). The all-chair conformer **2** is converted, upon binding of the signal compound in a chelation mode enforces the conformational switching **2** → **3**. In conformer **3** the receptor substituents have exchanged to equatorial positions and the effector substituents are now at axial positions. Recently, we have shown by X-ray crystal structural analysis that a covalently induced triple ring flip **2** → **3** is possible.^[9]

two all-chair conformers of biconformational^[14, 15] *cis-anti-cis*-perhydroanthracene interconvert by a triple ring flip.^[8] A tetra-substituted^[9] *cis-anti-cis*-perhydroanthracene of type **1** with two receptor substituents (red) and two effector substituents (blue) was chosen as a transducer (Figure 1b). In conformer **2** the receptor substituents are positioned axially and the effector substituents equatorially. Binding of the signal compound in a chelation mode enforces the conformational switching **2** → **3**. In conformer **3** the receptor substituents have exchanged to equatorial positions and the effector substituents are now at axial positions. Recently, we have shown by X-ray crystal structural analysis that a covalently induced triple ring flip **2** → **3** is possible.^[9]

In compound **4**, two 2,2'-bipyridine groups,^[16] fixed by an ether linkage to the perhydroanthracene, were chosen as receptor substituents (Figure 2). Pyrene groups were selected

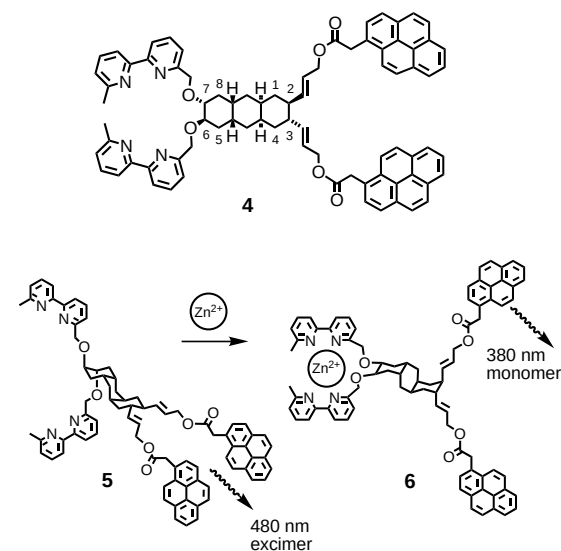


Figure 2. Structure of the perhydroanthracene transducer **4** featuring a bipyridine receptor and a pyrene effector. In solution, compound **4** has the conformation **5** (axial bipyridine substituents, equatorial pyrene side-chains). Upon complexation of a zinc(II) ion, a triple ring flip of the transducer occurs and compound **4** has the conformation **6**. The former equatorial pyrene groups (excimer fluorescence at 480 nm) have switched to axial positions (monomer fluorescence around 380 nm).

as effectors as two pyrene substituents in the equatorial position form an excimer.^[17] This excimer exhibits a fluorescence signal at 480 nm. In contrast, two pyrenes positioned axially are sufficiently remote to give only a monomer fluorescence signal at 380 nm.

Different compounds with equatorially and axially located pyrene effectors were synthesized and analyzed spectroscopically. The choice of the linker between the perhydroanthracene transducer and the pyrene effector was found to be crucial. An *E* olefin spacer and an ester group is suited to suppress, to a large extent, excimer formation in

the bisaxial conformer. The synthesis of **4** and the reference compounds were based on earlier work^[9] (synthetic details and spectroscopic characterization of **4** are given in the Supplementary Information).

NMR spectroscopy of uncomplexed **4** revealed conformer **5** as the only detectable species. The only proton signal of the perhydroanthracene skeleton of **5** with three large coupling constants of $J = 12$ Hz is now H(1)_β. The two equatorial protons of **5** H(6) and H(7) show three small ^3J coupling constants of 3–4 Hz. The emission fluorescence spectrum of uncomplexed **4** displayed the expected weak pyrene monomer band at around 380 nm and the strong excimer band at 480 nm (Figure 3). Zinc(II) ions and bis-2,2'-bipyridyl ether ligands

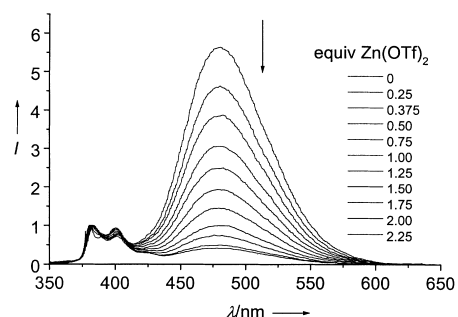
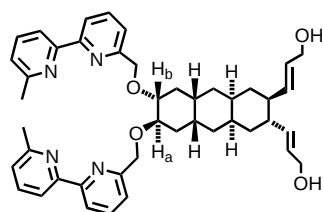


Figure 3. Photoresponse of **4** to the signal (presence of zinc(II)). Fluorescence spectrum^[20] of **4** in acetonitrile/chloroform (1/1) as a function of added zinc(II) triflate ($\lambda_{\text{ex}} = 343$ nm, $c = 5.6 \cdot 10^{-6}$ mol L⁻¹, $T = 298$ K). Fluorescence spectra were normalized to the monomer peak ($\lambda_{\text{em}} = 380$ nm).

form a chelating complex with the zinc(II) ion coordinated to two bidentate bipyridyl groups and two monodentate ether oxygen atoms.^[16] An NMR analysis of the zinc(II) complex of **4** indicated conformational switching^[18] from **5** to **6**: The only proton signal of the perhydroanthracene skeleton of **6** with three large coupling constants of $J = 12$ Hz is H_α-C(8) and the two axial protons of **6** H-C(6) and H-C(7) show two large and one small ^3J coupling constants. Addition of zinc(II) ions to compound **4** resulted in a distinct decrease of the excimer band at 480 nm relative to the pyrene monomer fluorescence band at 380 nm (Figure 3).

The zinc signal causes a conformational axial to equatorial change at the receptor site, which is transduced via a triple ring flip of the perhydroanthracene moiety to the pyrene effector site, where the induced equatorial to axial flip yields the observed fluorescence photosignal. A UV/Vis titration^[19] was used to determine the receptor binding energy necessary to induce the triple ring flip. To avoid spectral overlap due to the pyrene chromophore, compound **7** was studied as a model



7

system (Figure 4a). From a UV/Vis titration of **7** with zinc(II) triflate (Figure 4b) a binding energy of $\Delta G = 7.1 \text{ kcal mol}^{-1}$ in acetonitrile/chloroform for the complex $[\text{Zn}^{\text{II}}\text{-}\mathbf{7}]^{2+}(\text{OTf})_2$ was derived.^[19] The triple ring flip of **7** upon addition of zinc(II) was demonstrated by a ¹H NMR titration of **7** with zinc(II) triflate (Figure 4c).

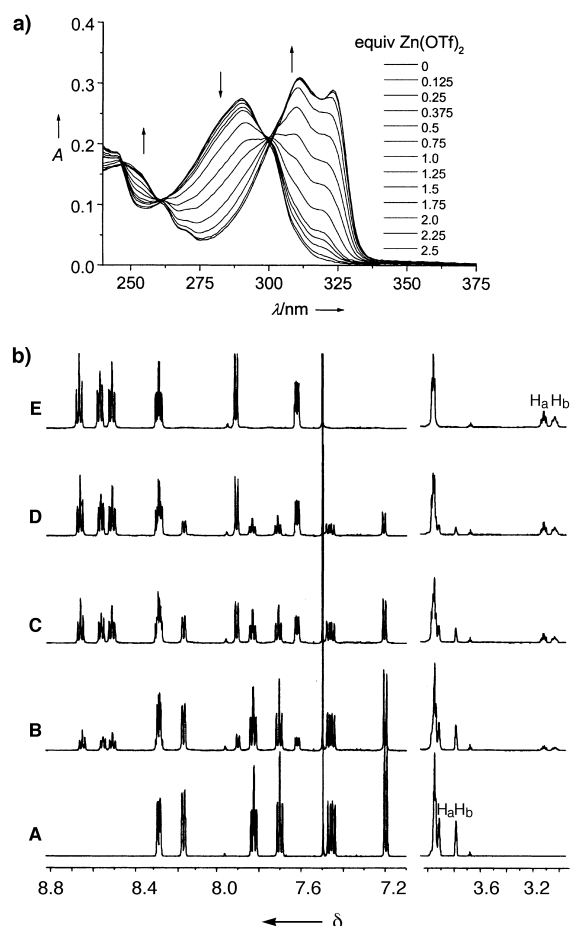


Figure 4. Analysis of the complexation-induced triple ring flip. a) UV/Vis titration of **7** with zinc(II) triflate (acetonitrile/chloroform (1/1), $c = 8.1 \times 10^{-6} \text{ mol L}^{-1}$, $T = 298 \text{ K}$). c) ¹H NMR titration of **7** with zinc(II) triflate (acetonitrile/chloroform (1/1), $c = 2.9 \times 10^{-2} \text{ mol L}^{-1}$). Zinc(II) triflate quantity: A = 0, B = 0.3, C = 0.6, D = 0.9, E = 1.1 equiv.

It is important for functional signal transduction to be reversible: The process needs both an on and off switch. Addition of chelating agents (2,2'-bipyridine or ethylenediaminetetraacetic acid) to the solution of the zinc complex **6** resulted in a distinct increase of the pyrene excimer emission relative to the monomer fluorescence. This is the expected photoresponse for the off switch (**6** → **5**).

In the above example, molecular signal transduction by conformational transmission was demonstrated with a particular receptor–effector pair. One can imagine a variety of stimuli at the receptor site such as photoisomerization or a redox process. An interesting topic of investigation will be the induced dissociation of an ion or ligand at the effector side.

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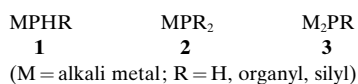
- [1] G. Krauss, *Biochemie der Regulation und Signaltransduktion*, Wiley-VCH, Weinheim, **1997**.
- [2] L. B. Ray, *Science* **1999**, *284*, 755–756.
- [3] K. Hinterding, D. Alonso-Díaz, H. Waldmann, *Angew. Chem.* **1998**, *110*, 716–780; *Angew. Chem. Int. Ed.* **1998**, *37*, 688–749.
- [4] J.-M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, **1995**.
- [5] D. H. R. Barton, *Science* **1970**, *169*, 539–544.
- [6] H. Yuasa, H. Hashimoto, *J. Am. Chem. Soc.* **1999**, *121*, 5089–5090.
- [7] R. W. Hoffmann, *Angew. Chem.* **1992**, *104*, 1147–1157; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1124–1134.
- [8] P. Vanhee, B. van de Graaf, D. Tavernier, J. M. A. Baas, *J. Org. Chem.* **1983**, *48*, 648–652.
- [9] J. Berninger, R. Krauss, H.-G. Weinig, U. Koert, B. Ziemer, K. Harms, *Eur. J. Org. Chem.* **1999**, 875–884.
- [10] R. A. Bissel, E. Córdova, A. E. Kaifer, J. F. Stoddart, *Nature* **1994**, *369*, 133–137.
- [11] L. Gobbi, P. Seiler, F. Diederich, *Angew. Chem.* **1999**, *111*, 737–740; *Angew. Chem. Int. Ed.* **1999**, *38*, 674–678.
- [12] P. R. Ashton, V. Balzani, J. Becher, A. Credi, M. C. T. Fyfe, G. Matternsteig, S. Menzer, M. B. Nielsen, F. M. Raymo, J. F. Stoddart, M. Venturi, D. J. Williams, *J. Am. Chem. Soc.* **1999**, *121*, 3951–3957.
- [13] J. N. H. Reek, H. Engelkamp, A. E. Rowan, J. A. A. W. Elemans, R. J. M. Nolte, *Chem. Eur. J.* **1998**, *4*, 716–722.
- [14] R. W. Hoffmann, I. Münster, *Liebigs Ann.* **1997**, 1143–1150.
- [15] A. G. De Oliveira Santos, W. Klute, J. Torode, V. P. W. Böhm, E. Cabrita, J. Runsink, R. W. Hoffmann, *New. J. Chem.* **1998**, 993–997.
- [16] A. Bilyk, M. M. Harding, P. Turner, T. W. Hambley, *J. Chem. Soc. Dalton Trans.* **1994**, 2783–2790.
- [17] a) I. Aoki, T. Harada, T. Sakaki, Y. Kawahara, S. Shinkai, *J. Chem. Soc. Chem. Commun.* **1992**, 1341–1345; b) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566.
- [18] M. Raban, D. L. Burch, E. R. Hortelano, D. Durocher, D. Kost, *J. Org. Chem.* **1994**, *59*, 1283–1287.
- [19] R. Foster, *Organic Charge–Transfer Complexes*, Academic Press, London, **1969**, p. 128.
- [20] H. Braatz, S. Hecht, H. Seifert, S. Helm, J. Bendig, W. Rettig, *J. Photochem. Photobiol. A* **1999**, *123*, 99–108.

**Lithium, Sodium, and Copper(I)
Supersilylphosphanediides M_2PSiR_3 :
Compounds with Novel Spherical $(M_2P)_n$
Frameworks****

Nils Wiberg,* Angelika Wörner, Dieter Fenske,
Heinrich Nöth, Jörg Knizek, and Kurt Polborn

*Dedicated to Professor Gerd Becker
on the occasion of his 60th birthday*

Among the alkali metal phosphanides and phosphanediides **1**–**3**, suitable as phosphanide transfer agents,^[1] hitherto only the—more soluble and more readily crystallizable—phosphanides **1** and **2** (ratio M:P = 1:1) have been structurally investigated in detail (see below). Herein we report on the synthesis, reactions, and structures of several phosphanediides **3** (ratio M:P = 2:1) with space-filling supersilyl groups $R = R^* = SiR_3$ ^[2] (for structurally characterized alkali metal phosphides M_3P (ratio M:P = 3:1) see ref. [3], for several supersilylphosphanides and -phosphanediides of the type **1** and **3** with the divalent metals Mg^{2+} , Ba^{2+} , and Sn^{2+} see ref. [4]).



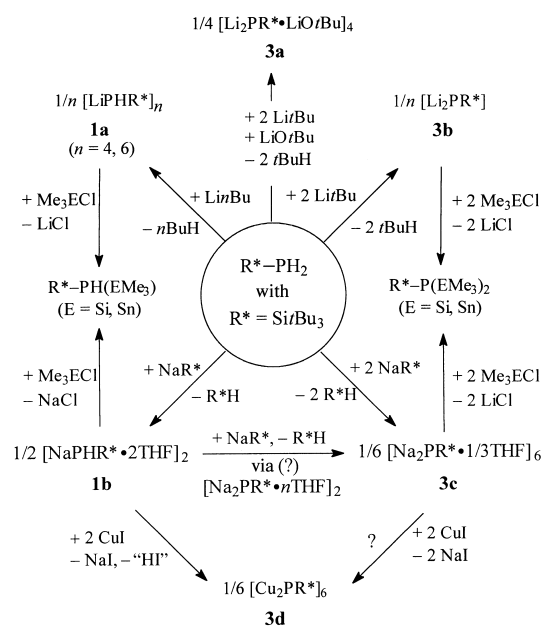
Syntheses: Donor-containing and donor-free *supersilylphosphanides* **1** are readily accessible by the reaction of alkali metal organyl or silyl compounds with supersilylphosphane R^*PH_2 in alkanes, benzene, or ethers (Scheme 1). For instance, the air- and moisture-sensitive phosphanides **1a** and **1b** are formed at room temperature by reaction of equimolar amounts of R^*PH_2 and Li^+nBu^- in *n*-pentane or NaR^* in THF, respectively (NaR^* is an ideal sodiating agent as result of its good solubility in organic media and its high basicity). Compound **1a**, which is soluble in hot benzene and toluene, crystallizes in the form of colorless parallelepipeds on cooling from the mentioned solvents. These contain—according to preliminary X-ray structure analyses^[5]—molecules of $[LiPHR^*]_4$ (from C_6H_6) and $[LiPHR^*]_6$ (from $C_6H_5CH_3$). Compound **1b** crystallizes from THF at $-25^\circ C$ as colorless needles with $[NaPHR^* \cdot 2THF]_2$ units (see Experimental Section).

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[**] Compounds of Silicon, Part 133; Supersilyl Compounds of Phosphorus, Part 6. This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. Part 132: N. Wiberg, T. Passler, S. Wagner, *J. Organomet. Chem.* **2000**, 598/2, 304; Part 5: N. Wiberg, A. Wörner, H.-W. Lerner, K. Karaghiosoff, H. Nöth, *Z. Naturforsch. B* **1998**, 53, 1004.



Scheme 1. Preparation of lithium, sodium, and copper(I) supersilylphosphanediides (NaR^* used as $tBu_3SiNa(THF)_2$).

Among the *supersilylphosphanediides* **3** (Scheme 1), the air- and moisture-sensitive compound **3a** forms by reaction of R^*PH_2 in *n*-heptane with two molar equivalents of Li^+nBu^- in the presence of $LiOtBu$, and the air- and moisture-sensitive compound **3b** forms by reaction of R^*PH_2 in toluene with two molar equivalents of Li^+nBu^- in *n*-hexane or Li^+nBu^- in *n*-pentane. Both give colorless powders which are soluble in toluene at $100^\circ C$. On slow cooling of the solutions **3a** crystallizes as colorless octahedra, which were suitable for X-ray structure analysis (see below), whereas **3b** precipitated again as a colorless powder. The reaction of **1b** with an equimolar amount of NaR^* (as THF adduct) in benzene led at $60^\circ C$ (Scheme 1)—probably via dimeric Na_2PR^* (see Experimental Section)—to the extremely air- and moisture-sensitive, pale yellow, donor-poor phosphanediide **3c**. The likewise air- and moisture-sensitive phosphanediide K_2PR^* can be obtained particularly conveniently by reaction of PCl_3 with four- to fivefold molar amounts of KR^* in benzene at room temperature. It crystallizes from benzene as orange-red parallelepipeds, which have to date proved unsuitable for X-ray structure analyses.

Reactions: Among the reactions of the supersilylphosphanides and -phosphanediides, the silylations and stannylations of **1a**, **1b**, **3b**, and **3c** with Me_3ECl ($E = Si, Sn$) are worthy of mention. These reactions lead smoothly (Scheme 1) at increased temperature (Me_3SiCl) or at room temperature (Me_3SnCl) to the compounds $R^*PH(EMe_3)$ and $R^*P(EMe_3)_2$, whose composition conversely allows conclusions about the number of alkali metal atoms in the used phosphanides and phosphanediides. A further reaction (Scheme 1) which demonstrates the suitability of the compounds obtained as phosphanide transfer agents^[1] is that of CuI and **1b** (and probably also **3c**) with metal exchange to give the air- and moisture-stable dicopper phosphanediide **3d**. This compound, which crystallizes as yellow-green prisms, could not be characterized by NMR spectroscopy because of its insolubility

in organic solvents such as THF or benzene; however, the crystals were suitable for an X-ray structure analysis (see below).

Structures: Previous structure investigations have shown that monolithium phosphanides MPR **1** and MPR₂ **2** display -M-P-M-P- chains of linked MP groups^[6,7] (e.g. polymeric LiPH₂·DME, LiPMe·DME, LiPPh₂·2THF; DME = MeOCH₂CH₂OMe; for more complex structures of lithium and sodium phosphanides see ref. [8]). As expected, the repulsive forces remain small between ions of equivalent charge in chains with alternate cationic and anionic members. The chains in question can combine to give double chains^[6,9] (ladder structures; e.g. hexameric LiP(SiMe₃)₂) or rings^[6,10–12] (e.g. 3 LiP(SiPr₃)₂·LiPH(SiPr₃); see also **1a**, **1b**); in addition, they exist in a separated form as monomers^[6,13] (e.g. LiP(SiMe₃)₂·(Me₂NCH₂CH₂)₂NMe). According to preliminary structure investigations by Driess et al.,^[8,14] the dilithium phosphanediides M₂PR **3** display *spherical frameworks* from linked M₂P groups. Evidently, the formation of such structures allows a minimization of the repulsive forces between ions of equivalent charge in dialkali metal phosphanediides. According to previous studies,^[14] additional stabilization of the spherical phosphanediides **3** can be achieved by incorporation of an anion in the center of the framework (e.g. [Li₂PSiR₃]₈·Li₂O and [Li₂PSiR₃]₁₂·Li₂O with SiR₃ = SiPr₂Me₂ and SiR₃' = SiMe₂(CMe₂iPr)) or by cation expulsion (e.g. Li₁₆P₁₀(SiPr₃)₁₀ with Li:P = 1.6:1). The phosphanediides presented herein show for the first time—probably as a result of the phosphane-bound space-filling supersilyl groups—structures of spherical (M₂P)_n frameworks without anion centers.

Figure 1 shows the comparatively symmetrical structure of [Li₂PR*·LiOrBu]₄ **3a** in the crystal (orthorhombic; molecular

point group *S*₄) together with selected bond lengths and angles.^[15] Apparently the compound is based on a spherical framework comprising P, O, and Li atoms. Each P atom is six- (Si + 5Li), each O atom five- (C + 4Li), and each Li atom three-coordinate (2P + O or P + 2O). The structure of **3a** can be described as follows: The twelve Li atoms occupy the corners of a polyhedron formed from annelated Li₅ and Li₄ rings (see Figure 2). In this polyhedron all Li₅ rings are each capped by a PR*^{2–} group and all Li₄ rings are each capped by one of the less extended OrBu[–] groups. Thus, the four P and the four O atoms occupy the corners of interpenetrating tetrahedra (Figure 1).

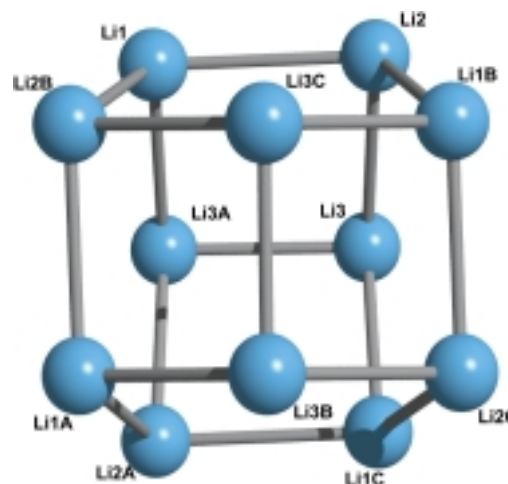


Figure 2. Positions of the Li atoms of **3a** (Figure 1) in the crystal (local symmetry *S*₄; the solid lines do not represent bonds, but only highlight the polyhedron whose corners are occupied by Li atoms).

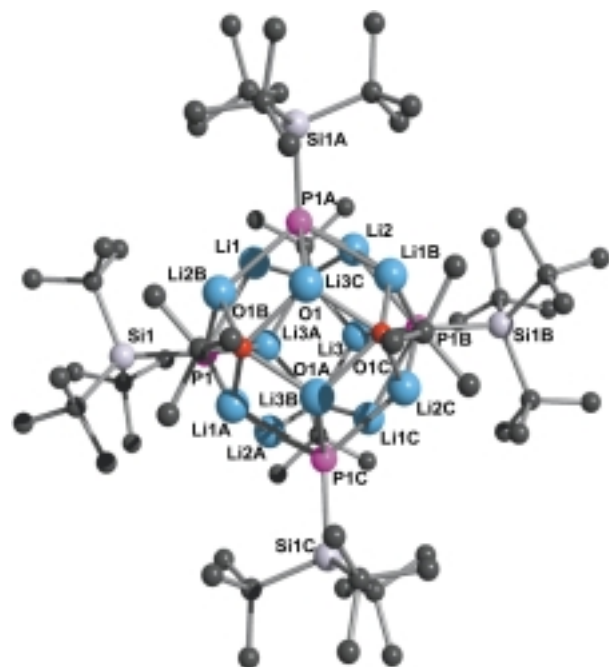


Figure 1. Structure of [Li₂PSiR₃·LiOrBu]₄ **3a** in the crystal (local symmetry *S*₄; SCHAKAL; hydrogen atoms are omitted for clarity). Selected bond lengths [Å]: P1–Si1 2.218(2), P1–Li1/Li1B/Li2A/Li2B/Li3A 2.54(1)/2.49(1)/2.54(1)/2.52(1)/2.48(1), P1A–Li2/Li3 2.54(1)/2.48(1), P1C–Li1/Li2 2.49(1)/2.52(1), O1–Li1/Li2/Li3/Li3A 2.06(1)/2.08(1)/2.00(1)/2.01(1), O1A–Li3 2.01(1), Si–C 1.96 (average).

The structure of [Na₂PR*]₆·2THF **3c** in the crystal (monoclinic, molecular point group *C*_s) is illustrated in Figure 3 together with selected bond lengths and angles.^[15] According to this Na and P form a „spherical“ atomic framework, which is dented on one side. The twelve Na atoms occupy (see Figure 4) the corners of six annelated five-membered rings (half-chair), which are each capped by a PR* group. The Na₁₂ polyhedron (Figure 4) can be derived from the Li₁₂ polyhedron (Figure 2) after replacement of Li for Na and the movement of the common atoms of a pair of annelated M₄ rings. This movement takes place in the middle of the common edges of both pairs of annelated M₄ rings (thus Li3/Li3A in Figure 2 correspond to Na1/Na6 in Figure 4). In this way the M₄ rings of the Li₁₂ polyhedron, which are evidently less suitable for PR*^{2–} caps, are converted into the M₅ rings of the Na₁₂ polyhedron. Moreover, one of the displaced M atoms (Na6 in Figure 3) migrates towards the center of the polyhedron, where it can be additionally coordinated by two P atoms (Figure 3). The four Na atoms (Na7/Na7/Na8/Na8) in the Na₅ rings adjacent to this Na atom (Na6), which links the rings, are additionally coordinated to two THF molecules (Figure 3). The other displaced M atom (Na1 in Figure 3) forms contacts with one methyl group each of two adjacent supersilyl groups (C8/C8 in Figure 3). Thus the coordination number of the P atoms is six (Si + 5Na) or seven (Si + 6Na) and that of the Na atoms is three (3P or

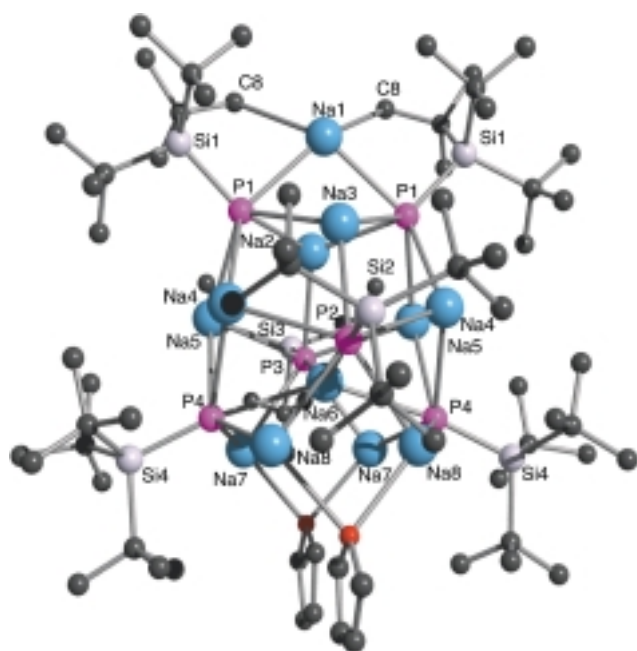


Figure 3. Structure of $[\text{Na}_2\text{PSiBu}_3]_6 \cdot 2\text{THF}$ **3c** in the crystal (local symmetry C_s ; SCHAKAL; hydrogen atoms are omitted for clarity). Selected bond lengths [Å]: P1–Na1/Na2/Na3 2.832(3)/2.898(5)/2.871(5), P2–Na3/Na4/Na6/Na8 2.839(6)/3.112(4)/3.006(7)/2.890(4), P3–Na2/Na5/Na6/Na7 2.876(5)/3.085(4)/3.021(7)/2.877(4), P4–Na4/Na5/Na6/Na7/Na8 2.942(4)/2.979(4)/2.959(2)/2.822(5)/2.820(5), P1–Si1/P2–Si2/P3–Si3/P4–Si4 2.210(3)/2.210(6)/2.223(6)/2.201(3), Na7–O1/Na8–O2 2.624(12)/2.548(9), Na1–C8 3.03(2).

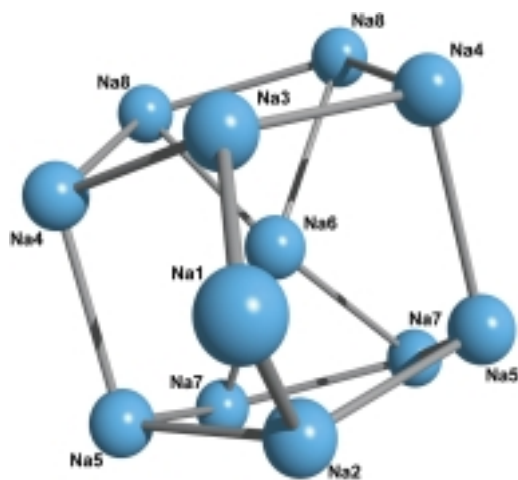


Figure 4. Positions of the Na atoms of **3c** (Figure 3) in the crystal (local symmetry C_s ; the solid lines do not represent bonds, but only highlight the polyhedron whose corners are occupied by Na atoms).

$2\text{P} + \text{O}$) or four (4P or $2\text{P} + 2\text{CH}_3$). The P atoms occupy the corners of a distorted octahedron (Figure 3).

Figure 5 displays the highly symmetrical structure of $[\text{Cu}_2\text{PR}^*]_6$ **3d** in the crystal (trigonal; molecular point group S_6) together with selected bond lengths and angles.^[15] In this case, the Cu and P atoms form a spherical atomic framework, in which each P atom is five- (Si + 4 Cu) and each Cu atom six-coordinate ($2\text{P} + 4\text{Cu}$). The twelve Cu atoms occupy the corners of a regular cuboctahedron; only the six Cu_4 rings of which bear PR^* caps. It follows that, whereas in the above-

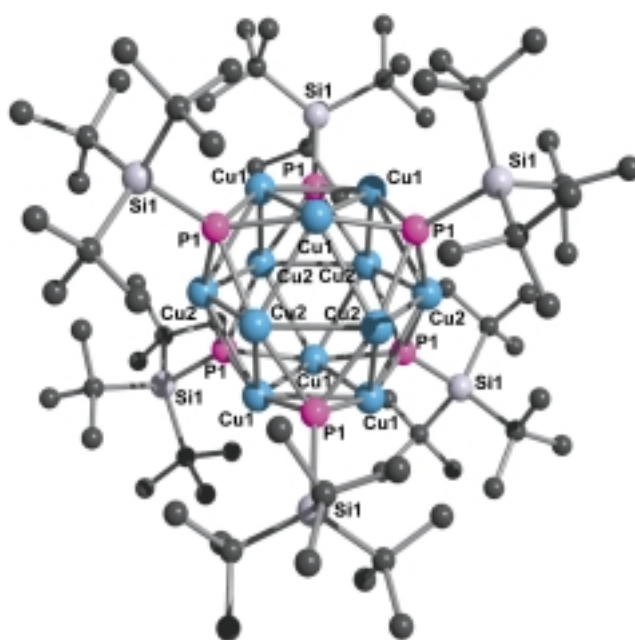


Figure 5. Structure of $[\text{tBu}_3\text{SiPCu}_2]_6$ **3d** in the crystal (local symmetry S_6 ; SCHAKAL; hydrogen atoms are omitted for clarity). Selected bond lengths [Å] and angles [°]: Cu1–Cu2 2.611(1), Cu2–P1 2.239(2), P1–Si1 2.241(3), Si–C 1.95 (average); P1–Cu2–Cu1 113.90(7), Cu2–P1–Si1 125.9(1), C–Si–C 112.2 (average).

mentioned polyhedra from alkali metal cations each face of the polyhedron is capped (to achieve a good charge balance), polyhedra from copper cations, whose ionic bonds to phosphorus are probably significantly polarized, do not require all faces to be capped. In addition, M_4 rings are capped instead of M_5 rings. The structure of **3d** resembles the structure of the cluster $[\text{Cu}_2\text{PPh} \cdot \text{PPh}_3]_6$, recently synthesized from $\text{PhP}(\text{SiMe}_3)_2$ and CuCl in the presence of PPh_3 and THF, in which the twelve Cu atoms occupy the corners of a compressed cuboctahedron and the six PPh groups cap the Cu_4 rings of the Cu_{12} polyhedron.^[16] However, the six Ph groups are not sufficient to completely surround the Cu_{12} framework, which as a result of this adds an additional six PPh_3 groups—for the stabilization of the Cu_{12} polyhedron—with the formation of CuPPh_3 building blocks. The only other neutral dicopper phosphanediide without additional donor ligands, $[\text{Cu}_2\text{PR}^*]_{12}$ with $\text{R} = \text{SiMe}_2(\text{CMe}_2\text{iPr})$, was synthesized by Driess and co-workers.^[17]

Experimental Section

1a: Over the course of three days **1a** precipitated as a colorless, fine powder from a solution of R^*PH_2 (0.770 g, 3.31 mmol)^[18] in *n*-pentane (5 mL) and *Li*nBu (3.42 mmol) in *n*-hexane (2 mL). (With regard to the reaction in Et_2O see **1b**.) According to NMR spectroscopy the resulting solution contained no starting materials but small amounts of **1a**. Compound **1a** was dissolved by heating for 5 h in benzene or toluene (3 mL). On slow cooling of the solutions, colorless cubic, air- and moisture-sensitive crystals were formed (yield ca. 60%), which according to preliminary X-ray structure analytical studies contained tetramers (from benzene) and hexamers (from toluene), respectively, of lithium supersilylphosphanide **1a**. ^1H NMR (C_6D_6 , TMS internal): $\delta = 1.31$ (br.; $\text{Si}t\text{Bu}_3$); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6 , TMS internal): $\delta = 24.25/32.30$ (br./br.; $3\text{CMe}_3/3\text{CMe}_3$); $^{29}\text{Si}\{^1\text{H}\}$ NMR (C_6D_6 , TMS external): $\delta = 34.15$ (d, br., $^1J_{\text{SiP}} = 54.83$; $\text{Si}t\text{Bu}_3$); $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6 , TMS internal): $\delta = -335.4$ ($h_{1/2} = 90.0$ Hz; LiHPR^*); ^7Li NMR (C_6D_6 , LiCl

in D₂O external): $\delta = 3.75$ ($h_{1/2} = 35.7$ Hz; LiHPR*). Notes: 1) LiHPR* (0.225 g, 0.950 mmol) and Me₃SiCl (4.0 mmol) were heated to 60 °C for 24 h in C₆D₆ (1 mL). According to NMR spectroscopy complete conversion to supersilyltrimethylsilane R*PHSiMe₃ had taken place, which, after removal of soluble components and volatile components, remained as a colorless, air- and moisture-sensitive solid. ¹H NMR (C₆D₆, TMS internal): $\delta = 0.297$ (d, $^3J_{\text{H,P}} = 4.42$ Hz; SiMe₃), 1.186 (s; SiBu₃); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = 7.55$ (d, $^2J_{\text{C,P}} = 11.6$ Hz; SiMe₃), 24.5/31.9 (d, $^2J_{\text{C,P}} = 9.1$ Hz/br.; 3 CMe₃/3 CMe₃); ²⁹Si{¹H} NMR (C₆D₆, TMS external): $\delta = 28.0$ (d, $^1J_{\text{Si,P}} = 45.8$ Hz; SiBu₃); ³¹P NMR (C₆D₆, 85 % H₃PO₄ external): $\delta = -245.58$ (d, $^1J_{\text{H,P}} = 194.8$ Hz; R*PHSiMe₃). 2) After treatment of LiHPR* (0.225 g, 0.950 mmol) in C₆D₆ (1 mL) with Me₃SnCl (2.0 mmol), a colorless precipitate (LiCl) formed immediately. According to NMR spectroscopy complete conversion to supersilyltrimethylstannane R*PHSnMe₃ had taken place, which, after removal of insoluble components and volatile components, remained as a colorless, air- and moisture-sensitive solid. ¹H NMR (C₆D₆, TMS internal): $\delta = 0.046$ (d, $^3J_{\text{H,P}} = 2.36$ Hz, Sn coupling constants 46.66, 43.02 Hz; SnMe₃), 1.160 (s; SiBu₃); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = -1.31$ (d, $^2J_{\text{C,P}} = 7.08$ Hz, Sn coupling constants 308.88, 277.99 Hz; SnMe₃), 24.30/31.18 (d/d, $^2J_{\text{C,P}} = 7.8$, $^3J_{\text{C,P}} = 2.68$ Hz; 3 CMe₃/3 CMe₃); ²⁹Si{¹H} NMR (C₆D₆, TMS external): $\delta = 28.1$ (d, $^1J_{\text{Si,P}} = 56.08$ Hz; SiBu₃); ³¹P NMR (C₆D₆, 85 % H₃PO₄ external): $\delta = -275.30$ (d, $^1J_{\text{H,P}} = 187.9$ Hz, Sn coupling constants 723.9, 694.9 Hz; R*PHSnMe₃); ¹¹⁹Sn{¹H} NMR: $\delta = 15.26$ (d, $^1J_{\text{Sn,P}} = 723.9$ Hz). 3) LiHPR* reacted with *t*Bu₂HSiF and *t*Bu₂SiF₂ to give R*PHSiH*t*Bu₂ and R*PHSiF*t*Bu₂, respectively.^[18]

1b: NaR* (2.15 mmol)^[19] in THF (5 mL) was added to R*PH₂ (0.500 g, 2.15 mmol)^[18] in THF (3 mL). After 1 h the resulting deep red solution—which according to NMR spectroscopy contained exclusively **1b** and R*H^[2]—was concentrated to 4 mL. Dark red needles of dimeric sodium supersilylphosphanide tetrahydrofuran (1/2) (**1b**) precipitated from this solution in the course of five days at -25 °C after addition of *n*-pentane (1 mL) (conversion to R*PHSiMe₃ and R*PHSnMe₃ is possible by reaction with Me₃SiCl and Me₃SnCl, see **1a**). ¹H NMR (C₆D₆, TMS internal): $\delta = 1.40$ (2 SiBu₃), 1.42/3.40 (m/m; CH₂CH₂O/CH₂CH₂O from 4 THF); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = 23.95/32.12$ (6 CMe₃/6 CMe₃), 25.67/67.78 (CH₂CH₂O/CH₂CH₂O from 4 THF); ²⁹Si{¹H} NMR (C₆D₆, TMS external): $\delta = 33.74$ (d, $^1J_{\text{P,Si}} = 56.4$ Hz; 2 SiBu₃); ³¹P NMR (C₆D₆, 85 % H₃PO₄ external): $\delta = -327.72$ (d, $^1J_{\text{P,H}} = 164.1$ Hz; NaHPR*). According to X-ray structure analysis^[2] **1b** contains a planar NaPNaP four-membered ring (Na–P 2.840(4) Å, Na–P–Na/P–Na–P 85.7(1)/94.3(1)°) with distorted tetrahedrally coordinated P (2 Na + H + R*) and Na atoms (2 P + 2 THF) as well as *trans*-oriented R* groups (P–Si 2.197(3), Na–O 2.281(7), Na–O 2.330(8) Å; O–Na–O 91.5(3)°). Note: According to NMR spectroscopy, analogue **1b** is the dimeric lithium supersilylphosphanide diethyl ether (1/1) LiHPR*·Et₂O^[18] which is accessible from R*PH₂ in Et₂O and *Li**n*Bu in *n*-hexane; **1b** only has one space-filling donor per Li atom.

3a: Li*Or*Bu-containing Li*Or*Bu (8.00 mmol) in *n*-pentane (8 mL) was added dropwise to a solution of R*PH₂ (0.851 g, 3.66 mmol)^[18] in *n*-heptane (10 mL). In the course of five days colorless **3b** formed from this mixture as an insoluble powder (see below) that was separated by decanting off the solution, which according to NMR spectroscopy contained some **3b** and a similar amount of Li*Or*Bu. After removal of all volatile components from the decanted solution, the residue was taken up in benzene (1 mL). The suspension formed was heated to 100 °C for 5 h and the resulting solution was slowly allowed to cool to room temperature over two days during which tetrameric dilithium supersilylphosphanediide–lithium-*tert*-butylate (1/1) (**3a**) crystallized as colorless octahedra (yield not calculable since the concentrations of Li*Or*Bu and **3b** unknown). The composition of **3a** was confirmed by X-ray structure analysis (see Figure 1). According to NMR spectra **3a** decomposes in solution into the components **3b** (see below) and Li*Or*Bu [¹H NMR (C₆D₆, TMS internal): $\delta = 1.22$ (s; *Or*Bu); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = 69.9/32.0$ (OCMe₃/OCMe₃)].

3b: Li*n*Bu (6.68 mmol) in *n*-hexane (4 mL) or Li*t*Bu (6.72 mmol) in *n*-pentane (4 mL) was added dropwise to *t*Bu₂SiPH₂ (0.770 g, 3.31 mmol)^[18] in toluene (8 mL) and the clear, pale yellow solutions were concentrated to 2 mL, which led to the precipitation of colorless **3b** as a fine powder in 90 % yield. According to NMR spectroscopy (exchange of the solvent by C₆D₆), the two solutions contained the same supersilyl-group-containing compound (**3b**) and were free of starting material. Colorless, microcrystalline, air- and moisture-sensitive, oligomeric dilithium supersilylphosphanediide **3b**, which is insoluble in pentane and sparingly soluble in benzene and

toluene, also precipitated as microcrystals on cooling of toluene solutions; however, these crystals were not suitable for X-ray structure analysis. ¹H NMR (C₆D₆, TMS internal): $\delta = 1.41$ (v br.); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = 24.2/33.1$ (v br./v br.; CMe₃/CMe₃); ²⁹Si{¹H} NMR (C₆D₆, TMS external): very broad signal ($h_{1/2} = 136$ Hz) with four sharp lines at $\delta = 39.59, 38.98, 38.44, 37.91$; ³¹P{¹H} NMR (C₆D₆, 85 % H₃PO₄ external): very broad signal ($h_{1/2} = 1243$ Hz) with five maxima at $\delta = -395.5, -397.0, -400.0, -404.5, -406.5$; ⁷Li NMR (C₆D₆, LiCl in D₂O external): $\delta = 6.6$ ($h_{1/2} = 130$ Hz; Li₂PR*). Notes: 1) According to NMR spectroscopy (C₆D₆), after Li₂PR* (0.230 g, 0.94 mmol) and Me₃SiCl (4.0 mmol) in C₆D₆ (1 mL) had been heated to 100 °C for three days, complete conversion had occurred to supersilylbis(trimethylsilyl)phosphane R*P(SiMe₃)₂, which, after removal of insoluble components and volatile components, remained as a colorless, air- and moisture-sensitive solid. ¹H NMR (C₆D₆, TMS internal): $\delta = 0.463$ (d, $^3J_{\text{H,P}} = 4.21$ Hz; 2 SiMe₃), 1.230 (s; SiBu₃); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = 25.0$ (d, $^2J_{\text{C,P}} = 8.83$ Hz; 3 CMe₃), 32.2 (br.; 3 CMe₃), 4.23 (d, $^2J_{\text{C,P}} = 10.45$ Hz; 2 SiMe₃); ²⁹Si{¹H} NMR (C₆D₆, TMS external): $\delta = 35.3$ (d, $^1J_{\text{Si,P}} = 50.85$; SiBu₃). 2) Li₂PR* (0.230 g, 0.94 mmol) in C₆D₆ (1 mL) was combined with Me₃SnCl (3.0 mmol). According to NMR spectroscopy (C₆D₆) this reaction led to the complete conversion to supersilylbis(trimethylstannyl)phosphane R*P(SnMe₃)₂, which, after removal of insoluble components and volatile components, remained as a colorless, air- and moisture-sensitive solid. ¹H NMR (C₆D₆, TMS internal): $\delta = 1.201$ (s; SiBu₃), 0.398 (d, $^3J_{\text{H,P}} = 2.25$ Hz; Sn coupling constants 51.24, 47.49 Hz; 2 SnMe₃); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = -1.21$ (d, $^2J_{\text{C,P}} = 7.07$ Hz; Sn coupling constants 302.68, 291.53 and 783 Hz; 2 SnMe₃), 24.57 (d, $^2J_{\text{C,P}} = 7.63$ Hz; Sn coupling constants 7.72 Hz; 3 CMe₃), 31.86 (d, $^3J_{\text{C,P}} = 3.06$ Hz; 3 CMe₃); ²⁹Si NMR: $\delta = 32.68$ (d, $^1J_{\text{Si,P}} = 78.24$ Hz; Sn coupling constants 37.4 Hz; ³¹P{¹H} NMR (C₆D₆, 85 % H₃PO₄ external): $\delta = -293.10$ (s; Sn coupling constants SiBu₃ 839.3, 799.2 Hz; R*P(SnMe₃)₂); ¹¹⁹Sn{¹H} NMR: 25.42 (d, $^1J_{\text{Sn,P}} = 839.3$, $^2J_{\text{SnSn}} = 236.5$ Hz; 2 SnMe₃).

3c: A solution of NaR* (3.10 mmol)^[19] in THF (3.5 mL) was added dropwise to R*PH₂ (0.359 g, 1.55 mmol)^[18] which led to the formation of **1b** together with R*H (see above). After this all volatile components were removed under vacuum, the remaining R*PHNa·2 THF and NaR*·2 THF (each 1.55 mmol) were dissolved in benzene (1.5 mL), and the solution was heated to 60 °C for 18 h, after which it was free from starting material (quantitative formation of the phosphanediide, which formed only very slowly from **1b** at room temperature; see note 1). After 24 h all volatile components (C₆H₆, THF, R*H) were removed under vacuum at room temperature and the residue was dissolved in benzene (1 mL). Hexameric disodium supersilylphosphanediide precipitated slowly from this solution as THF-adduct **3c** in the form of yellow crystals (yield ca. 50 %). ¹H NMR (C₆D₆, TMS internal): $\delta = 1.40$ (br.; 6 SiBu₃), 1.42/3.40 (m/m; 2 THF); ¹³C{¹H} NMR (C₆D₆, TMS internal): $\delta = 24.3/33.0$ (br./br.; 18 CMe₃/18 CMe₃); ²⁹Si NMR (C₆D₆, TMS external): $\delta = 39.2$ (br., $h_{1/2} = 130$ Hz; 6 SiBu₃); ³¹P{¹H} NMR (C₆D₆, 85 % H₃PO₄ external): $\delta = -398.0/-405.5/-409.0$ (very broad; $h_{1/2} = 350/404/404$ Hz; 6 PR*). X-ray structure analysis: see Figure 3. Notes: 1) According to the NMR spectrum, the solution formed by the reaction of the THF-adduct **1b** in benzene with NaR*·2 THF at 60 °C for 18 h initially contained Na₂PR* as a THF adduct (not isolated; transformation into R*P(SiMe₃)₂ and R*P(SnMe₃)₂ possible by reaction with Me₃SiCl and Me₃SnCl; cf. **3b**). Owing to its good solubility, the diphosphanediide could be at most a dimer and according to NMR spectroscopy it is symmetric [²⁹Si{¹H} NMR (C₆D₆, TMS external): $\delta = 40.0$ (d, $^1J_{\text{Si,P}} = 84.0$ Hz; SiBu₃); ³¹P{¹H} NMR (C₆D₆, 85 % H₃PO₄ external): $\delta = -405.0$ (s; $h_{1/2} = 40$ Hz; Na₂P(SiBu₃)₂]. Possibly, the compound is based on a framework of four Na atoms, which are coordinated to THF, localized at the corners of a square; the Na₄ rings in this case would be capped on both sides by PR* groups. 2) [K₂PR*]_x (together with H.-W. Lerner): KR* (0.131 g, 0.550 mmol)^[19] and PCl₃ (0.021 g, 0.15 mmol) were left in C₆D₆ (0.6 mL) for four weeks at room temperature. According to NMR spectroscopy the initially green solution contained R*KP–PKR* ($\delta(^{31}\text{P}) = -255.2$). In the course of the formation of K₂PR* the solution became red (after addition of Me₃SiCl or Me₃SnCl one obtained R*P(SiMe₃)₂ or R*P(SnMe₃)₂, respectively; cf. **3b**). Orange-red cubes, which were not suitable for an X-ray structure analysis, were formed from the reaction solution in the course of a few months. ¹H NMR (C₆D₆, TMS internal): $\delta = 1.387$ (br.; SiBu₃); ²⁹Si{¹H} NMR (C₆D₆, TMS external): as in the cases of other potassium salts of supersilylphosphanes^[2] no ²⁹Si NMR signals

were observed; $^{31}\text{P}\{^1\text{H}\}$ NMR (C_6D_6 , 85% H_3PO_4 external): $\delta = -319.4$ (K_2PR^*). The reaction of KR^* and PCl_3 in the molar ratio 2:1 gave quantitative amounts of $\text{R}^*\text{CIP-PCIR}^*$ ($\delta(^{31}\text{P}) = 188$).^[2]

3d: A solution of **1b** (0.199 g, 0.500 mmol) in THF (2 mL) was added to CuI (0.160 g, 0.840 mmol), and after 1 h the insoluble components were removed by centrifugation. Hexameric dicopper supersilylphosphonediide (**3d**) crystallized in the form of yellow-green prisms (yield ca. 50%) from the remaining solution in the course of five days. The compound **3d**, which was insoluble in organic media, was characterized by X-ray structure analysis (Figure 5).

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- [1] G. Fritz, H. Schäfer, W. Hölderich, *Z. Anorg. Allg. Chem.* **1974**, 407, 266; R. Appel, K. Geisler, *J. Organomet. Chem.* **1976**, 112, 61; G. Becker, O. Mundt, M. Rössler, E. Schneider, *Z. Anorg. Allg. Chem.* **1978**, 443, 42; G. Fritz, *Chem. Rev.* **2000**, in press, and references therein.
- [2] N. Wiberg, *Coord. Chem. Rev.* **1997**, 163, 217.
- [3] A. F. Wells, *Structural Inorganic Chemistry*, 5th ed, Clarendon Press, Oxford, **1984**, p. 841; H. G. von Schnering, *Chem. Rev.* **1988**, 88, 243.
- [4] M. Westerhausen, M. H. Digeser, M. Krofta, N. Wiberg, H. Nöth, J. Knizek, W. Ponikwar, T. Seifert, *Eur. J. Inorg. Chem.* **1999**, 743; M. Westerhausen, M. Krofta, N. Wiberg, J. Knizek, H. Nöth, A. Pfützner, *Z. Naturforsch. B* **1998**, 53, 1489; M. Westerhausen, M. Krofta, A. Pfützner, *Inorg. Chem.* **1999**, 38, 598.
- [5] N. Wiberg, A. Wörner, H. Nöth, H.-S. Hwang-Park, unpublished results.
- [6] R. A. Jones, S. U. Koschmieder, C. M. Nunn, *Inorg. Chem.* **1987**, 26, 3610, and references therein.
- [7] G. Becker, B. Eschbach, O. Mundt, N. Seidler, *Z. Anorg. Allg. Chem.* **1994**, 620, 1381; G. Becker, B. Eschbach, D. Käshammer, O. Mundt, *Z. Anorg. Allg. Chem.* **1994**, 620, 29, and references therein.
- [8] M. Driess, G. Huttner, N. Knopf, H. Pritzkow, L. Zsolnai, *Angew. Chem.* **1995**, 107, 354; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 316; M. Driess, *Acc. Chem. Res.* **1999**, 32, 1017.
- [9] E. Hey-Hawkins, E. Sattler, *Chem. Commun.* **1992**, 775, and references therein.
- [10] M. Westerhausen, R. Löw, W. Schwarz, *J. Organomet. Chem.* **1996**, 513, 213; M. Driess, H. Pritzkow, *Z. Anorg. Allg. Chem.* **1996**, 622, 1524, and references therein.
- [11] P. B. Hitchcock, M. F. Lappert, P. P. Power, S. Smith, *Chem. Commun.* **1984**, 1669.
- [12] G. A. Koutsantonis, P. C. Andrews, C. L. Raston, *Chem. Commun.* **1995**, 47, and references therein.
- [13] K. Niediek, B. Neumüller, *Z. Anorg. Allg. Chem.* **1993**, 619, 885.
- [14] M. Driess, S. Rell, H. Pritzkow, R. Janoschek, *Chem. Commun.* **1996**, 305; M. Driess, H. Pritzkow, S. Martin, S. Rell, D. Fenske, G. Baum, *Angew. Chem.* **1996**, 108, 1064; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 986; M. Driess, U. Hoffmanns, S. Martin, K. Merz, H. Pritzkow, *Angew. Chem.* **1999**, 111, 2906; *Angew. Chem. Int. Ed.* **1999**, 38, 2733.
- [15] Crystal structure analyses: **3a**: orthorhombic, space group $P4(2)/n$, $a = 17.9170(2)$, $c = 13.0721(2)$ Å, $Z = 9$, $V = 4196.39(9)$ Å³, 2439 independent reflections ($I > 2\sigma(I)$). All non-hydrogen atoms were refined anisotropically and the H atoms were included in calculated positions. $R1 = 0.0904$ (observed reflections), $wR2 = 0.1984$ (all data). **3c**: monoclinic, space group $P2(1)/m$, $a = 16.320(2)$, $b = 22.210(4)$, $c = 18.410(4)$ Å, $\beta = 108.46(2)^\circ$, $Z = 4$, $V = 6329.5(1)$ Å³, 10419 independent reflections ($I > 2\sigma(I)$). All non-hydrogen atoms were refined anisotropically and the H atoms were included in calculated positions. $R1 = 0.099$ (observed reflections), $wR2 = 0.265$ (all data). The cell contained twelve solvent molecules (C_6H_6). The $R1$ value could not be improved further as a result of the poor quality of the crystal and a disorder of the *t*Bu groups bound to Si as well as the cocrystallizing benzene molecules. **3d**: trigonal, space group $R\bar{3}$ (no. 148), $a = 14.742(3)$, $c = 39.586(7)$ Å, $Z = 3$, $V = 7451(2)$ Å³, 2488 independent reflections ($I > 2\sigma(I)$). All non-hydrogen atoms were refined anisotropically and the H atoms were included in calculated positions. $R1 = 0.0541$ (observed reflections), $wR2 = 0.1334$ (all data). The intensities were measured with a Siemens-P4 apparatus with a CCD area detector (**3a**), a Stoe imaging plate (**3c**), and a Nonius Mach-3

apparatus (**3d**) ($\text{MoK}\alpha$ radiation, $\lambda = 0.71073$ Å, respectively, ω scan, **3c**: $\phi = 0-120^\circ$; $\Delta\phi = 0.3^\circ$; 10 min irradiation time), $T = 173(2)$, 193(2), and 293(2) K), respectively. The structures were solved by direct methods (SHELXS-97, SHELXL-93) and refined with full matrix against F^2 . Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-138960 (**3a**), CCDC-139064 (**3c**), and CCDC-138956 (**3d**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [16] A. Eichhöfer, D. Fenske, W. Holstein, *Angew. Chem.* **1993**, 105, 257; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 242. See also D. Fenske, J. Ohmer, J. Hachgenei, K. Merzweiler, *Angew. Chem.* **1988**, 100, 1300; *Angew. Chem. Int. Ed. Engl.* **1988**, 27, 1277; H. Krautscheid, D. Fenske, G. Baum, M. Semmelmann, *Angew. Chem.* **1993**, 105, 1364; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 1303; D. Fenske, J.-C. Steck, *Angew. Chem.* **1993**, 105, 254; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 238; D. Fenske, W. Holstein, *Angew. Chem.* **1994**, 106, 1311; *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 1290.
- [17] In $[\text{Cu}_2\text{PR}]_{12}$ with $\text{R} = (\text{iPrMe}_2\text{C})\text{Me}_2\text{Si}$ the Cu atoms form a cuboctahedron, in which the middle planar Cu_6 ring is replaced by three eclipsed planar Cu_6 rings. The twelve PR groups cap the outer four Cu_4 faces as well as—alternately—the inner twelve Cu_4 faces of the Cu_{24} polyhedron: M. Driess, S. Martin, K. Merz, V. Pintchouk, H. Pritzkow, H. Grützmacher, M. Kaupp, *Angew. Chem.* **1997**, 109, 1982; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1894. With regard to a first mention of $[\text{Cu}_2\text{PR}]_6$ see ref. [2].
- [18] N. Wiberg, H. Schuster, *Chem. Ber.* **1991**, 124, 93.
- [19] N. Wiberg, K. Amelunxen, H.-W. Lerner, H. Schuster, H. Nöth, I. Krossing, M. Schmidt-Amelunxen, T. Seifert, *J. Organomet. Chem.* **1997**, 542, 1.

Confinement of $[(\text{H}_2\text{O})_2 \cap ([18]\text{Crown-6})]$ in a Disc-Shaped Cavity Lined with Six Nickel(II) Macrocycle Dimers Acting as Divergent Receptor Molecules**

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The nickel(II) complex $[\text{Ni}(\text{tmtaa})]$ (Equation (1)) and its analogue with two methyl groups on each of the phenyl rings of the ligand, $[\text{Ni}(\text{omtaa})]$, are versatile receptors for a range of globular molecules.^[1-4] A 1:1 complex between $[\text{Ni}(\text{tmtaa})]$ and fullerene is known, in which the macrocycle acts as a divergent receptor for C_{60} . A fullerene perches in each macrocycle cavity in an infinite zigzag array.^[1] A similar structure is found for C_{70} with $[\text{Ni}(\text{omtaa})]$.^[3] For smaller globular molecules, including 1,2-dicarba-closo-dodecaborane(12), tetraphosphorus trisulfide, tetraphosphorus triselenide, and cyclo-octasulfur, the $[\text{Ni}(\text{tmtaa})]$ complex self associates through aromatic faces into dimers and these dimers then act as divergent homotopic receptors with a 1:2 ratio of guest:host

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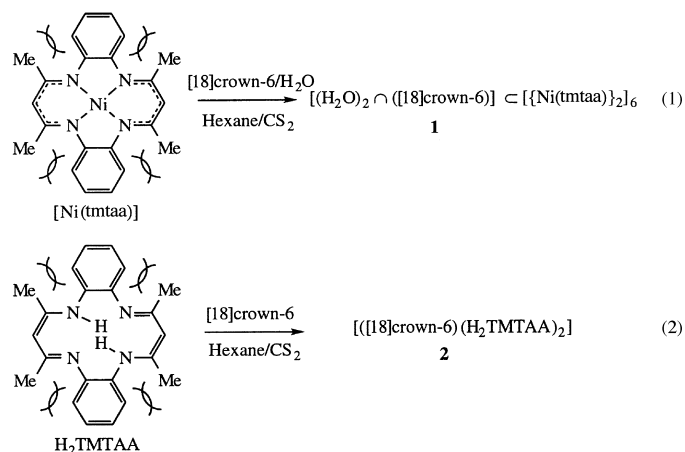
[**] This work is supported by the Australian Research Council.

species.^[1, 2] Inclusion complexes with [Ni(tmtaa)] form with the solvent toluene; the unsolvated form has been found to have at least three different structures.^[2, 5, 6]

Complementary sizes and curvatures of the interacting components seems to be a prime determinant of the supramolecular outcomes. The molecule Ni(tmtaa) is rigid and distorts into a saddle shape of C_{2v} symmetry as a consequence of the methyl groups otherwise clashing with the H-atoms on the *ortho*-carbon atoms of the phenyl rings. In some of these structures the metal macrocycle self associates through the methyl-lined faces into dimers.^[2]

While the complexation chemistry of the TMTAA²⁻ ligand to a range of metals, metalloids, and nonmetals is well developed,^[7] its supramolecular chemistry is less known,^[1, 2] and the control of the assembly process to build engineered solid-state architectures is problematic. We now report remarkable findings on the host–guest chemistry of [Ni(tmtaa)]: The extended self association of the divergent receptors around a large disc-shaped cavity which contains an [18]crown-6 unit linked via two hydrogen-bonded water-molecule bridges. Furthermore, the potential for the metal-free molecule H₂TMTAA, which also has a saddle shape, towards forming self assembled complex with [18]crown-6 is established. This complex has a different stoichiometry and supramolecular synthon interplay relative to the [Ni(tmtaa)]–crown ether complex.

The new compounds **1** and **2** were prepared by the slow evaporation of solutions of the macrocyclic complex with [18]crown-6 in CS₂/hexane with a trace of water [Eq. (1) and (2)]. Compound **1** was isolated from reactions involving 1:1 and 1:12 ratios of crown ether to macrocycle. These mole ratio



experiments demonstrate the propensity of **1** to crystallise from solution despite the weak intermolecular interactions. In contrast, **2** only formed with excess [18]crown-6. UV/Vis studies of mixtures of [Ni(tmtaa)] and [18]crown-6 in CS₂ and toluene indicate strong interaction in solution (absorption changes at submillimolar concentrations) but it was not possible to determine the nature of the complexes formed in solution or whether supermolecules of **1** are present.

Compound **1** crystallises^[8] in the space group $R\bar{3}(h)$; the supermolecules reside on the $\bar{3}$ symmetry sites with the asymmetric unit comprised of two molecules of [Ni(tmtaa)],

one sixth of a molecule of [18]crown-6, and a water molecule lying on a $\bar{3}$ symmetry axis (Figure 1). Dimers of ([Ni(tmtaa)])₂ are locked through the pendant aromatic rings of the saddle-shaped monomers, an outcome of the complementarity of curvature between the two components as well as Ni- π_{arene} interactions. The [18]crown-6 is cradled within the

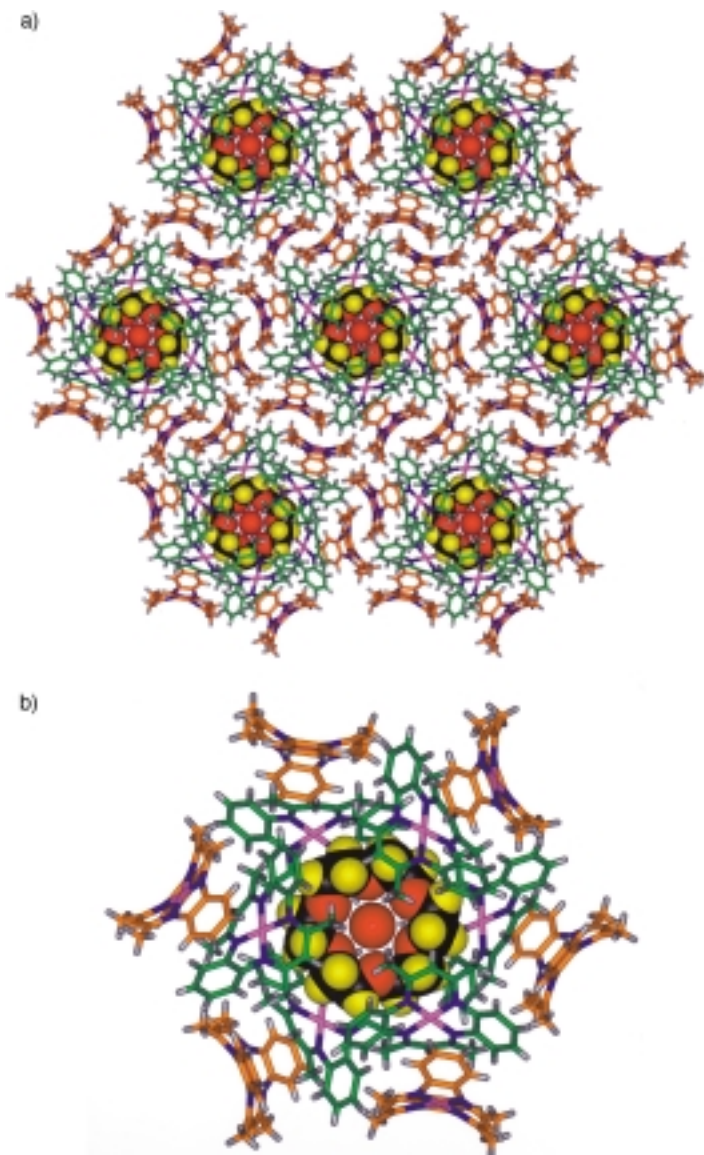


Figure 1. Crystalline structure of **1**: a) the extended structure (see Figure 2), and b) a supermolecule.

methyl-lined faces of six of these dimers. The divergent receptor prowess of the dimers^[1, 2] is borne out by one methyl-lined face directed towards the crown ether and the other towards a neighboring supermolecule dimer: A pair of methyl groups of one dimer lies in the concave cavity of another dimer and vice versa. Thus, each unit of **1** is linked to six other units, as depicted in cartoon form for the central supermolecule in Figure 2. The two interaction styles described for the [Ni(tmtaa)] complex (“locking” of pendant aromatic rings and of methyl-lined faces) have been described previously.^[2]

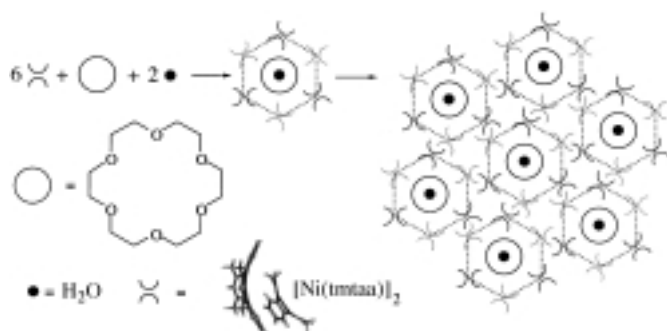


Figure 2. Simplified representation of the crystalline structure of **1**.

The closest approach of the crown ether to the [Ni(tmtaa)] molecules are the O-C_{methyl}, O-C_{phenyl}, and O-N_{imine} distances of 3.99(1), 3.96(1), and 3.92(1) Å, respectively and C-C_{methyl}, C-C_{phenyl}, and C-N_{imine} distances of 4.08(1), 3.83(1), and 3.75(1) Å, respectively. Two water molecules perch on either side of the crown ether and the oxygen atoms of the water molecules are 3.44(2) Å apart. The short 3.19(1) Å distances between each O_{H₂O} atom and three crown-ether O_{crown} atoms are consistent with hydrogen bonding. The three longer contacts for the alternate crown-ether oxygen atoms is 3.36(1) Å. The closest approach of the O_{H₂O} atom to [Ni(tmtaa)] are to methyl groups at 3.29(1) Å (Figure 1(b)).

The supermolecule **1** (Figure 1b), comprised of 15 interacting components, is held together by hydrogen bonding, π -interactions, and van der Waals interactions. The shrouding of [(H₂O)₂ ∩ ([18]crown-6)] by six dimers is arguably a shape-induced process; however, in the absence of the crown ether, crystals have been obtained with the same unit cell parameters and with a similar arrangement of [Ni(tmtaa)] dimers around the same shape and size cavity, in this case occupied by a highly disordered CS₂ molecule ((CS₂) ⊂ {[Ni(tmtaa)]₂}).^[9]

Compound **2** crystallizes^[8] in the space group *P* $\bar{1}$ with a molecule of H₂TMTAA and one half of an [18]crown-6 molecules in the asymmetric unit. Layers of (H₂TMTAA)₂ link through a mismatched overlap of methyl-lined faces, as described previously^[2] (distances: C_{methyl}-C_{methyl}, C_{methyl}-C_{imine}, C_{methyl}-C_{methine} are 3.904(3), 3.381(3), and 3.466(3) Å, respectively). These layers are separated by layers of discrete [18]crown-6 molecules (Figure 3). The H₂TMTAA layers consist of dimers (H₂TMTAA)₂ aligned with coplanar orientations of the pentanediimine fragments (about 4.0 Å apart) and result in the phenyl-lined surfaces presenting themselves to the crown ether layers on the top and bottom. The closest approaches of the crown ether to the H₂TMTAA molecule are [Å]: O-C_{phenyl} 3.360(3), O-C_{methyl} 3.509(3), C_{[18]crown-6}-C_{phenyl} 3.554(4), C_{[18]crown-6}-C_{methine} 3.683(3), C_{[18]crown-6}-C_{methyl} 4.092(3), and C_{[18]crown-6}-N 3.739(3).

The ability of [Ni(tmtaa)] and H₂TMTAA to form novel inclusion complexes suggests that other binary/ternary systems are likely. We note that in **1** the macrocycle dimers are disposed to shroud the crown ether and associated water molecules. Larger crown-ether systems may be accommodated by rotation of the macrocycle dimers relative to each other when orientated closer to the equatorial plane of the supermolecule. A similar potential exists for [Ni(omtaa)] towards

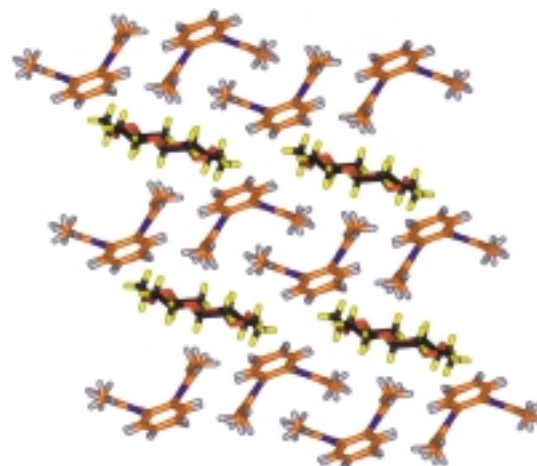


Figure 3. Packing of [18]crown-6 in **2**.

constructing new architectures, perhaps even with some predictability, upon replacing the metal centers by paramagnetic ones leading to materials with novel function.

Experimental Section

[Ni(tmtaa)] and H₂TMTAA were prepared according to published procedures.^[10] Other reagents and solvents were commercial products.

1: A CS₂ solution of [18]crown-6 (5 mg, 20 mL) was mixed with a CS₂ solution of [Ni(tmtaa)] (102 mg, 20 mL). The mixture was layered with hexane (equal volume) and allowed to evaporate slowly. The complex was collected and washed with a small amount of hexane (m.p. 242–248 °C). Elemental analysis [%]: calcd: C 64.82, H 5.68, N 13.11; found: C 64.55, H 5.76, N 13.15.

2: A toluene solution of [18]crown-6 (13 mg, 20 mL) was mixed with a toluene solution of H₂TMTAA (34 mg, 20 mL). The mixture was layered hexane (equal volume) and allowed to evaporate slowly. H₂TMTAA crystallised from solution and the collected crystals were washed with hexane. From the filtrate and hexane washings, a small amount of **2** crystallised but was difficult to purify.

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- [1] P. C. Andrews, J. L. Atwood, L. J. Barbour, P. J. Nichols, C. L. Raston, *Chem. Eur. J.* **1998**, *4*, 1384.
- [2] P. C. Andrews, P. D. Croucher, J. L. Atwood, L. J. Barbour, P. J. Nichols, N. O. Smith, B. W. Skelton, A. H. White, C. L. Raston, *J. Chem. Soc. Dalton Trans.* **1999**, 2927.
- [3] P. D. Croucher, J. M. E. Marshall, P. J. Nichols, C. L. Raston, *Chem. Commun.* **1999**, 193.
- [4] P. D. Croucher, P. J. Nichols, C. L. Raston, *J. Chem. Soc. Dalton Trans.* **1999**, 279.
- [5] Y. Wang, S.-M. Peng, Y.-L. Lee, M.-C. Chuang, C.-P. Tang, C.-J. Wang, *J. Chin. Chem. Soc. (Taipei)* **1982**, *29*, 217.
- [6] R. L. Paul, S. F. Gheller, G. A. Heath, D. C. R. Hockless, L. M. Rendina, M. Sterns, *J. Chem. Soc. Dalton Trans.* **1997**, 4143.
- [7] Z. Wang, N. Hu, K. Sakata, M. Hashimoto, *J. Chem. Soc. Dalton Trans.* **1999**, 1695, and references therein.
- [8] Crystal structure analysis for **1** and **2**: Enraf-Nonius Kappa CCD diffractometer, *T* = 123 K, crystal mounted in oil. **1**: Ni₁₂C₂₇₆H₂₉₂N₄₈O₈, *M* = 5114.06, trigonal, space group *R* $\bar{3}(h)$ (No. 148), *a* = 41.573(6), *c* = 11.646(2) Å, *V* = 17432.8(1) Å³, ρ_{calc} = 1.461 g cm⁻³, μ = 10.22 cm⁻¹ (no correction), *Z* = 3, MoK α radiation, 11302 unique reflections (*R*_{int} = 0.098), $2\theta_{\text{max}}$ = 60° (5051 observed, *I* > 3 σ (*I*), *R* = 0.064, *Rw* = 0.060). Hydrogen atoms on water molecules showing disorder over three sites were not included in the calculations. **2**: C₅₆H₇₂N₂O₆, *M* = 953.2, triclinic, space group *P* $\bar{1}$ (No. 2), *a* = 11.2248(4), *b* = 11.3120(4), *c* = 11.9329(3) Å, α = 63.367(2)°, β =

71.013(2)°, $\gamma = 72.862(2)^\circ$, $V = 1260.7(5) \text{ \AA}^3$, $\rho_{\text{calc}} = 1.255 \text{ g cm}^{-3}$, $\mu = 0.82 \text{ cm}^{-1}$ (no correction), $Z = 1$, $\text{MoK}\alpha$ radiation, 7007 unique reflections ($R_{\text{int}} = 0.043$), $2\theta_{\text{max}} = 60^\circ$ (4661 observed, $I > 3\sigma(I)$, $R = 0.063$, $R_w = 0.067$). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-136348 and -136349. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[9] Synthesis of $(\text{CS}_2)_2 \subset [\text{Ni}(\text{tmtaa})_2]_6$. A CS_2 solution of $[\text{Ni}(\text{tmtaa})_2]$ (50 mg, 20 mL) was mixed with a CS_2 solution of anthracene (18 mg, 20 mL). The mixture was layered with hexane (equal volume) and allowed to evaporate slowly (m.p. 241–245 °C). Elemental analysis [%]: calcd: C 65.09, H 5.44, N 13.75; found: C 64.98, H 5.47, N 13.68. Crystal structure analysis: Enraf-Nonius Kappa CCD diffractometer, $T = 123 \text{ K}$, crystal mounted in oil. $\text{Ni}_{12}\text{C}_{265}\text{H}_{264}\text{N}_{48}\text{S}_2$, $M = 4889.84$, $R^3(h)$ (No. 148), $a = 41.5300(17)$, $c = 11.6099(17) \text{ \AA}$, $V = 17341.3(0.8) \text{ \AA}^3$.

[10] V. L. Goedken, M. C. Weiss, *Inorganic Syntheses* **1980**, XX, 115.

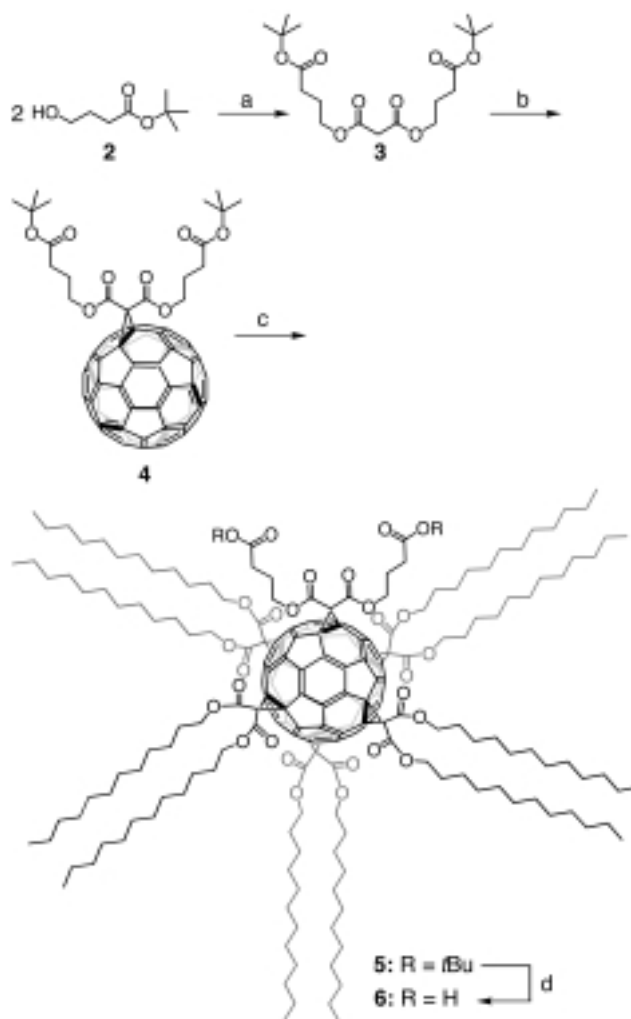
Globular Amphiphiles: Membrane-Forming Hexaadducts of C_{60} **

Michael Brettreich, Stephan Burghardt,
Christoph Böttcher, Thomas Bayerl, Sybille Bayerl,
and Andreas Hirsch*

Most of the biological membrane-forming lipids are double-chain phospho- or glycolipids with 16–22 carbon atoms per chain and which may be mono- or polyunsaturated. They perform many functions in biological systems. At the interface between biology and materials sciences, the development of artificial nanostructures as mimics of natural systems or as prototypes of novel functional aggregates is playing an important role. This article describes the synthesis and the membrane and vesicle formation of a new class of artificial lipids. They have a globular structure with the spherical C_{60} being used to define the molecular geometry. Five pairs of dodecyl chains and one pair of polyamide

dendrons are bound to C_{60} in an octahedral [1:5]-addition pattern^[1] by methylene bridges.^[2]

For a synthesis of the globular amphiphile **1** (see Scheme 2), the alcohol^[3] **2** and malonyl dichloride were allowed to react to yield malonate **3**. A nucleophilic cyclopropanation reaction^[4,5] of C_{60} with **3** leads to the monoadduct **4** (Scheme 1).



Scheme 1. Syntheses of dicarboxylic acid **6**. a) Malonyldichloride, pyridine, CH_2Cl_2 ; b) C_{60} , DBU, CBr_4 , toluene; c) 1) DMA, 2) CBr_4 , DBU, didodecyl malonate, toluene; d) TFA, toluene. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMA = dimethylantracene, TFA = trifluoroacetic acid.

The five pairs of lipophilic C_{12} chains, which are in octahedral positions relative to the first addend (Figure 1), were inserted in one step by cyclopropanation of **4** with didodecyl malonate,

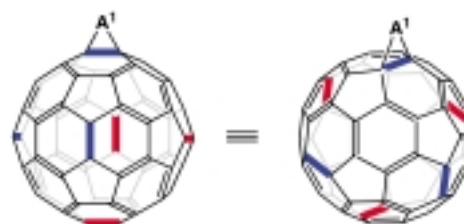


Figure 1. Octahedral positions relative to the first addend A^1 in a C_{2v} symmetrical hexaadduct of C_{60} .

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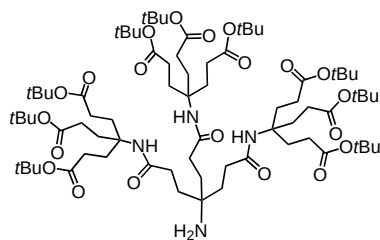
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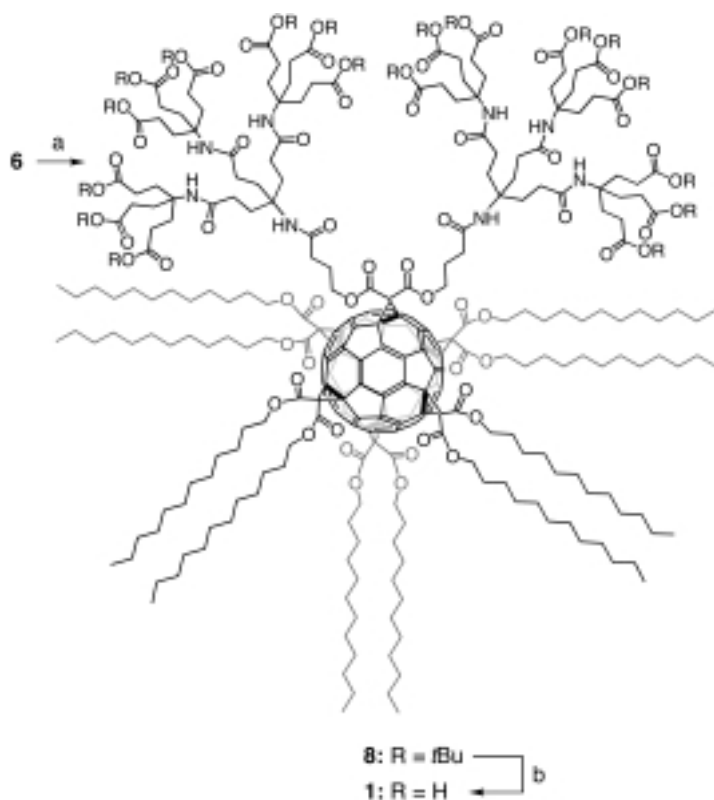
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

using reversible template activation with dimethyl anthracene (DMA).^[6–8] Chromatographic purification yielded 23 % of the mixed hexaadduct **5** as a viscous liquid. Compound **5** was deprotected with trifluoroacetic acid (TFA), and **6** was isolated in a 93 % yield as a yellow waxlike solid (Scheme 1). Amide dendron **7**,^[9] which was generated convergently, was



7

added using *N,N'*-dicyclohexylcarbodiimide and 1-hydroxybenzotriazol to give dendrimer **8** in a 30 % yield. The *tert*-butyl groups were removed by TFA and the target molecule **1** was obtained as a yellow powder in 96 % yield, with a melting point of 186 °C (Scheme 2).



Scheme 2. Syntheses of the globular amphiphile **1**. a) **7**, DCC, HOBt, THF; b) TFA, toluene. HOBt = 1-hydroxy-1*H*-benzotriazole.

Compounds **1**, **3–6**, and **8** were fully characterized by ¹H NMR, ¹³C NMR, IR, and UV/Vis spectroscopy, as well as by mass spectrometry.^[10] The spectroscopic properties are characteristic for *C*_{2v} symmetrical monoaddition compounds^[11] or *C*_{2v} symmetrical hexaaddition compounds with an octahedral [1:5]-addition pattern.^[6]

In order to visualize the structure of the globular amphiphile **1**, we first performed molecular dynamics simulations.^[12] Figure 2 shows the result of this computation. The two types

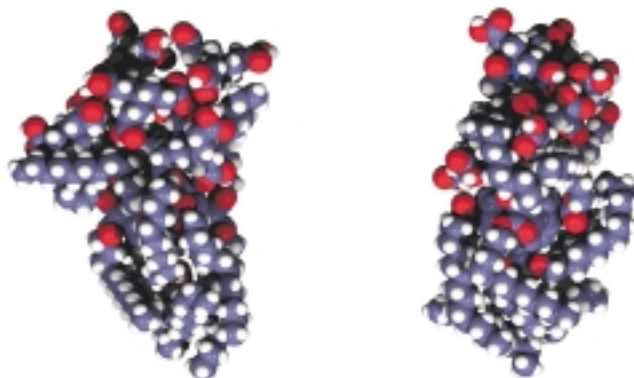


Figure 2. Molecular dynamics simulation of **1** (orthogonal views). Conditions: Heating time 5 ps, run time 15 ps, cooling time 50 ps, step size 0.001 ps, starting temperature 0 K, simulation temperature 1000 K, final temperature 0 K, in vacuum.

of addends are located in two areas which are, to a large extent, separated from each other. The densely packed hydrophilic and hydrophobic regions almost completely enclose the C₆₀ core. The average dimension of about 3.5 nm along the main polar axis is similar to that of natural phospho- or glycolipids. In contrast, the typical diameters of 1.4–2.3 nm found in directions perpendicular to this axis are considerably larger than those found for natural double-chain lipids (diameters of about 0.7 nm).

At pH 7.4 (phosphate buffer) the globular amphiphile **1** slowly disperses in water to form streaks. Large flakes appear until, after several minutes, a stable, yellow opalescent solution develops. This kind of opalescence is typical for colloidal solutions with particle sizes below the wavelength of light, that is, below 400 nm. Higher concentrations (>3 mg mL^{−1}) will, after several hours, precipitate a highly viscous and intensively light-scattering phase from the clear solution above.

Aggregation behavior of **1** was investigated by freeze-fracture and cryo-electron microscopy. Freeze-fracture electron microscopy of the yellow opalescent solution described above reveals distinctly vesicular structures. The diameters of the vesicles range from 100 to 400 nm and can, therefore, be the cause of the opalescence (Figure 3). Not a single freeze fracture shows multilamellar structures, which indicates a preference for the formation of single-layer (unilamellar) vesicles. The vesicle dimensions found are similar to those of natural lipids (such as lecithine). The propensity to form unilamellar structures seems to be assisted by the high density of negative charges (at pH 7.4) in the hydrophilic part of **1**. Cryo-electron microscopy (cryo-TEM) also reveals the vesicular structures, with this method providing an even more precise picture. It allows thin layers (100 to 200 nm) of the amorphously vitrified aqueous sample to be directly imaged and gives high-resolution projection images of the uncontrasted sample in the native environment of the solvent.^[13]

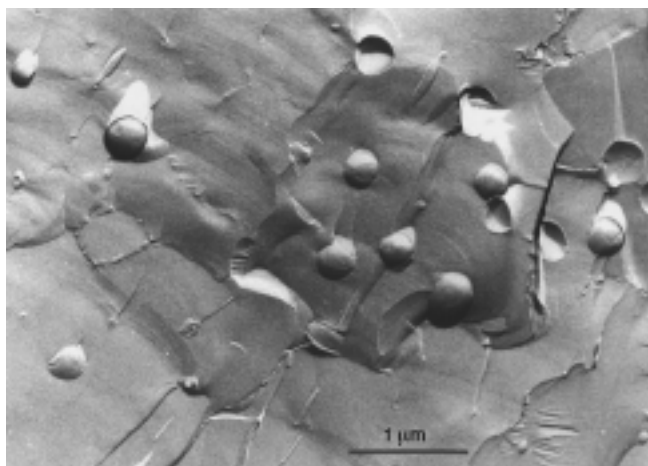


Figure 3. Freeze-fracture electron micrograph of **1** (the scale shown is 1 μm). Conditions: Solution of **1** (2 mg mL^{-1}) in phosphate buffer (pH 7.4), freezing from room temperature in liquid N_2 , further details are analogous to Ref. [6e].

The cryo-TEM investigations of **1** reveal a wide variety of aggregates, the fine structure of which, however, is invariably based upon the same structural principle—the formation of double-layer membranes. The dominant structures are vesicles of dimensions ranging between 50 and 400 nm (Figure 4). Figure 4a shows the typical projection image of a spherical vesicle with a diameter of 80 nm. The density profile of the membrane is clearly resolved and the overall thickness can be determined as about 7 nm. Since the dimensions of large vesicles ($>200\text{ nm}$) exceed the thickness of the water lamella embedding them, such vesicles are no longer of a spherical form but show deformations (Figure 4b). Figure 4b also shows that partial formation of multilayers is possible as well.

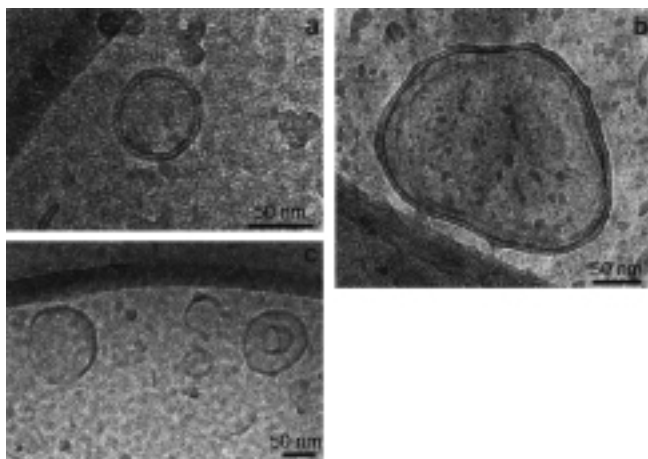


Figure 4. a) Cryo-TEM image of a vesicle of **1** (diameter 80 nm, the scale shown is 50 nm). The bilayer structure (approximate diameter 7 nm) of the membrane is clearly resolved, and the outer dark (electron-dense) regions, with a thickness of approximately 2 nm, conform well to the dimension of the hydrophilic head (compare with the model in Figure 2). The brighter inner seam with a width of approximately 3 nm is, therefore, considered to be the hydrophobic alkyl chain.^[14] b) Cryo-TEM image of a deformed vesicle of **1** (diameter 400 nm, the scale shown is 50 nm). Several regions point to a multilayered growth of the membrane. c) Short membrane fragments and incomplete vesicle structures show early stages of the growth process of the membrane structures (compare with Figure 5, the scale shown is 50 nm).

In addition to vesicles, smaller cylindrical aggregates of about 5 to 200 nm in length can often be found (Figure 5). In the lateral direction, however, they are regularly of the dimension of the double-layer membrane and show the resolution of the double-layer membrane profile. As the membrane motif remains the same through a large number of investigated structures (>100), that is, they invariably show the double-layer profile, and since the aggregates have complete freedom to orient themselves in the solution, the cross-section of the cylinders may be considered circular.

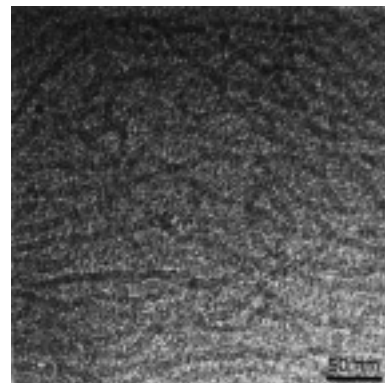


Figure 5. Cryo-TEM image of a population of cylindrical aggregates (the scale is 50 nm). The diameter (7 nm) of all aggregates, which may take on different orientations in solution, is equal and shows the resolved bilayer motif.

As a result of the high natural contrast of the electron-rich C_{60} molecules, the method also allows the smallest molecular units to be detected (Figure 6); they are spherical particles with a diameter of about 3.5 nm and, because of their dimensions, have to be considered as nonaggregated single molecules.

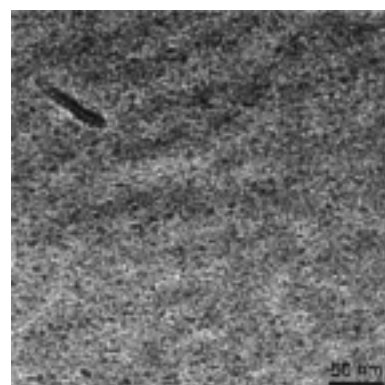


Figure 6. Cryo-TEM image of a population of spherical particles with a diameter of approximately 3.5 nm, which corresponds to the molecular dimension (the scale is 50 nm).

Light scattering tests are presently being performed, in order to investigate in more detail how the formation of aggregates is dependent upon concentration and pH value. First results show that the critical micelle concentration is very low. Aggregates of the size described above have been found up to concentrations of 3 mg L^{-1} . The amphiphile **1** has

properties, such as size and vesicle-formation concentration, which are very similar to common double-chain lipids; however, they show a propensity to form unilamellar aggregates, as well as a variety of different bilayered structures.

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- [1] A. Hirsch, I. Lamparth, T. Grösser, H. R. Karfunkel, *J. Am. Chem. Soc.* **1994**, *116*, 9385–9386.
- [2] For self-assembly of ionic monoadducts of C₆₀ and the behavior of these compounds at the air/water interface, see: a) A. M. Cassell, C. L. Asplund, J. M. Tour, *Angew. Chem.* **1999**, *111*, 2565–2568; *Angew. Chem. Int. Ed.* **1999**, *38*, 2403–2405; b) H. Murakami, M. Shirakusa, T. Sagara, N. Nakashima, *Chem. Lett.* **1999**, 815–816; c) F. Cardullo, F. Diederich, L. Echegoyen, T. Habicher, N. Jayaraman, R. M. Leblanc, J. F. Stoddart, S. Wang, *Langmuir* **1998**, *14*, 1955–1959; d) U. Jonas, F. Cardullo, P. Belik, F. Diederich, A. Gügel, E. Harth, A. Herrmann, L. Isaacs, K. Müllen, H. Ringsdorf, C. Thilgen, P. Uhlmann, A. Vasella, C. A. A. Waldruff, M. Walter, *Chem. Eur. J.* **1995**, *1*, 243–251.
- [3] H. R. Kricheldorf, J. Kaschig, *Liebigs Ann. Chem.* **1976**, 882–890.
- [4] X. Camps, A. Hirsch, *J. Chem. Soc. Perkin Trans. 1* **1997**, 1595–1596.
- [5] C. Bingel, *Chem. Ber.* **1993**, *126*, 1957–1959.
- [6] a) I. Lamparth, C. Maichle-Mössner, A. Hirsch, *Angew. Chem.* **1995**, *107*, 1755–1757; *Angew. Chem. Int. Ed.* **1995**, *34*, 1607; b) I. Lamparth, A. Herzog, A. Hirsch, *Tetrahedron* **1996**, *52*, 5065–5075; c) X. Camps, H. Schönberger, A. Hirsch, *Chem. Eur. J.* **1997**, *3*, 561–567; d) X. Camps, E. Dietel, A. Hirsch, S. Pyo, L. Echegoyen, S. Hackbarth, B. Roeder, *Chem. Eur. J.* **1999**, *5*, 2362–2373; e) M. Hetzer, S. Bayerl, X. Camps, O. Vostrowsky, A. Hirsch, T. M. Bayerl, *Adv. Mater.* **1997**, *9*, 913–917; f) M. Hetzer, H. Clausen-Schaumann, S. Bayerl, T. M. Bayerl, X. Camps, O. Vostrowsky, A. Hirsch, *Angew. Chem.* **1999**, *11*, 2103–2106; *Angew. Chem. Int. Ed.* **1999**, *38*, 1962–1965; g) A. Herzog, O. Vostrowsky, A. Hirsch, *Eur. J. Org. Chem.* **2000**, 171–180; h) F. Djojo, E. Ravanelli, O. Vostrowsky, A. Hirsch, *Eur. J. Org. Chem.* **2000**, 1051–1059.
- [7] A. Hirsch, *Top. Curr. Chem.* **1999**, *199*, 1.
- [8] F. Diederich, D. Kessinger, *Acc. Chem. Res.* **1999**, *32*, 537–545.
- [9] a) M. Brettreich, A. Hirsch, *Tetrahedron Lett.* **1998**, *39*, 2731–2734; b) M. Brettreich, A. Hirsch, *Synlett* **1998**, 1396–1398.
- [10] Complete experimental data of compounds **1**, **4**–**6**, and **8** are available in the “supporting information”. Data for **3** can be obtained from the author.
- [11] A. Hirsch in *The Chemistry of the Fullerenes*, Thieme Organic Chemistry Monograph Series, Stuttgart, **1994**.
- [12] HyperChem, Release 5, Hypercube Inc., Ontario, Canada, **1996**.
- [13] M. Adrian, J. Dubochet, J. Lepault, A. W. Dowall, *Nature* **1984**, *308*, 32.
- [14] C. Böttcher, H. Stark, M. van Heel, *Ultramicroscopy* **1996**, *62*, 133.

In Situ Monitoring of a Heterogeneous Palladium-Based Polyketone Catalyst**

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In the early 1980s a class of very active palladium catalysts was discovered which allowed the synthesis of a perfectly alternating aliphatic polyketone ($n\text{CH}_2\text{CH}_2 + n\text{CO} \rightarrow [\text{CH}_2\text{CH}_2\text{C}(\text{O})]_n$) in an efficient way.^[1–3] These catalysts consist of a Pd^{II} center, a bidentate chelating ligand, and two weakly associated anions. The origin of the perfectly alternating nature of the copolymers has received ample attention, both in experimental^[1, 3–7] and in theoretical studies.^[8–10] The mechanism for copolymer chain growth involves two sequential propagation steps: migratory CO insertion into the Pd–alkyl bond of the growing polymer chain followed by migratory ethene insertion into the resulting Pd–acyl bond. There is consensus that double CO insertion does not occur for thermodynamic reasons, while double ethene insertion is kinetically hampered due to the higher affinity of Pd^{II} centers for CO over ethene. However, thus far all experimental mechanistic studies focused on copolymerization in solution, while under actual process conditions (slurry or gas-phase), the initially single-site Pd catalyst is heterogenized and resides on the surface or in the bulk of the copolymer.

While there are several analytical techniques for the in situ study of single-site catalysts in the liquid state,^[11, 12] there is a lack of techniques to directly monitor catalysis at supported single-site catalysts.^[13] Polarization modulation reflection absorption infrared spectroscopy (PM-RAIRS) is an adaptation of the well-known RAIRS technique for studying species adsorbed at metal surfaces with submonolayer sensitivity, allowing the detection of signals normally obscured by huge gas-phase absorptions.^[14–16] We used PM-RAIRS to monitor CO/ethene copolymerization at the single-site Pd catalyst [(dppp)Pd(CH₃)(OTf)] (**1**; dppp = 1,3-bis(diphenylphosphanyl)propane; OTf = OSO₂CF₃ = triflate) in the microcrystalline state (in the absence of any solvent), and focused on two aspects of the process: single insertion steps and copolymer growth.

Single insertion steps could be observed by alternately exposing the catalyst to CO and ethene atmospheres. Starting from **1**, sequential addition and removal of CO, ethene, and CO led to the stepwise formation of the six-membered

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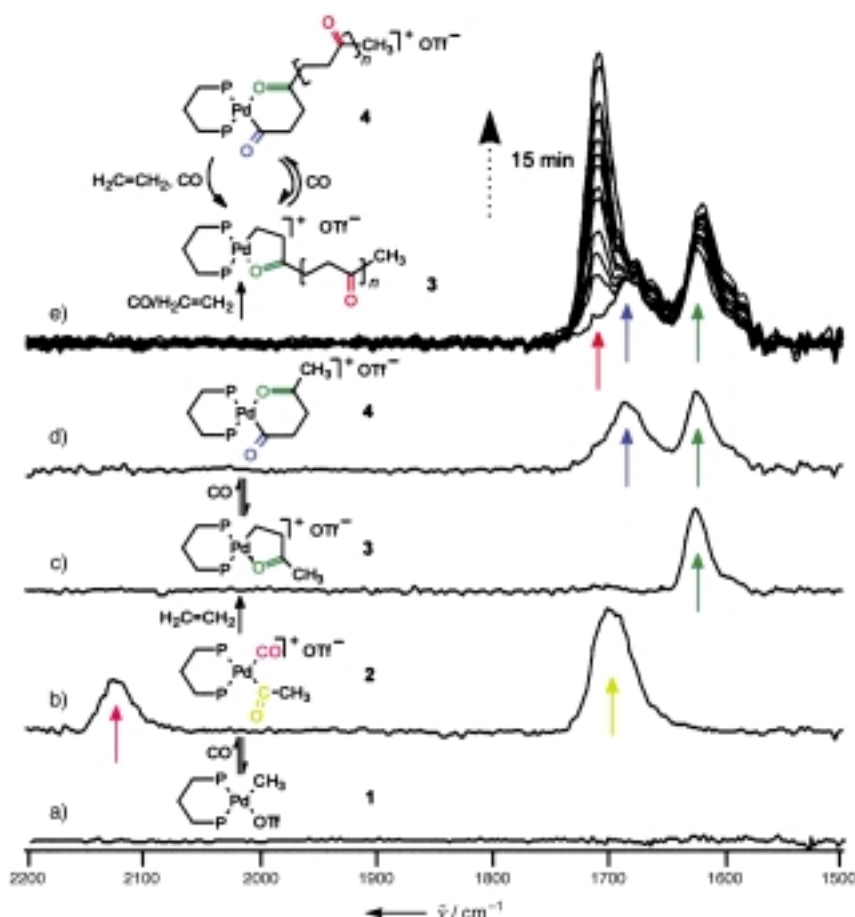


Figure 1. In situ PM-RAIR spectra of a sample of microcrystalline **1** a) at room temperature, b) under 500 mbar of CO, c) under 2 mbar of CO and 333 mbar of ethene, d) under subsequent exposure to 750 mbar CO, and e) during subsequent polymerization under 666 mbar of CO/ethene (1/1; evolution of the spectrum at 15 min intervals). For clarity the phenyl groups in dppp have been omitted.

palladacycle **4** (Figure 1 a–d). The PM-RAIR spectrum of microcrystalline **1** is featureless in the selected frequency range (Figure 1 a). Upon exposure of this species to 500 mbar of CO at room temperature, two new IR bands appear at 2125 and 1695 cm^{-1} , indicating the formation of $[(\text{dppp})\text{Pd}(\text{C}(\text{O})\text{CH}_3)(\text{CO})](\text{OTf})$ (**2**, Figure 1 b). The IR band at 2125 cm^{-1} corresponds to a cationic Pd–carbonyl moiety and the one at 1695 cm^{-1} to a Pd–acyl group, formed by CO insertion into the Pd–methyl bond.^[17] This reaction is reversible, just as in solution:^[12, 18] After removal of CO the parent complex is reformed. Upon gradual increase of the CO pressure above complex **1**, the Pd–carbonyl band initially developed somewhat faster than the Pd–acyl band, indicating the occurrence of the intermediate $[(\text{dppp})\text{Pd}(\text{CO})\text{CH}_3]^+$ in significant amounts, in line with observations in solution.^[4, 18]

After reduction of the CO pressure to 2 mbar and addition of 333 mbar of ethene the spectrum shown in Figure 1 c was recorded. The appearance of a new IR band at 1625 cm^{-1} indicates the formation of the five-membered palladacycle **3** by ethene insertion into the Pd–acyl bond and the simultaneous coordination of the (now) ketonic carbonyl group to palladium.^[19] The IR spectrum exhibits no features that can be ascribed to coordination of other ligands such as CO or triflate (see below) to the square-planar 16e Pd^{II} center.

Subsequently, the palladacycle **3** was exposed to 750 mbar of CO, resulting in the appearance of an IR band at 1690 cm^{-1} , while the band at 1625 cm^{-1} remained unaltered (Figure 1 d). This indicates the formation of **4** by CO insertion into the Pd–alkyl bond of the (opened) palladacycle **3**. Complex **4** contains both an acyl group (1690 cm^{-1}) and a chelating carbonyl moiety (1625 cm^{-1}). So, the chelating ketones of **3** and **4** cannot be distinguished from each other by IR spectroscopy. This second CO insertion is also reversible: Reduction of the CO pressure to 2 mbar leads to the gradual decrease ($t_{1/2} \approx 15$ min) of the acyl signal at 1690 cm^{-1} . The ratio between **3** and **4** proved to be related to the CO partial pressure. Surprisingly, and in contrast to the situation after the first CO insertion, exposure of **4** to 333 mbar of ethene did *not* change the spectrum, although the emergence of free ketone was expected. This suggested that ethene insertion into the Pd–acyl bond of **4** does not occur under these conditions. This was confirmed in a comparable experiment in which the even more weakly coordinating BF_4^- anion was used instead of triflate. In this case CO removal from **4** was much slower ($t_{1/2} \approx 120$ min). Exposure of this relatively stable palladacycle to 333 mbar of ethene for several hours also did not result in the insertion of ethene into the Pd–acyl bond. Only in the presence of a substantial amount of CO did ethene insertion occur (see below), indicating that in this microcrystalline catalyst insertion of ethene is CO-assisted.

The coordination of the triflate anion could be monitored during the first few consecutive steps. It is known that noncoordinated (weakly ion paired) and coordinated triflate ions have distinctly different IR absorptions.^[20] The low-frequency region of the spectra showed that triflate originally occupies the fourth coordination site in **1** (Figure 2 a), but is

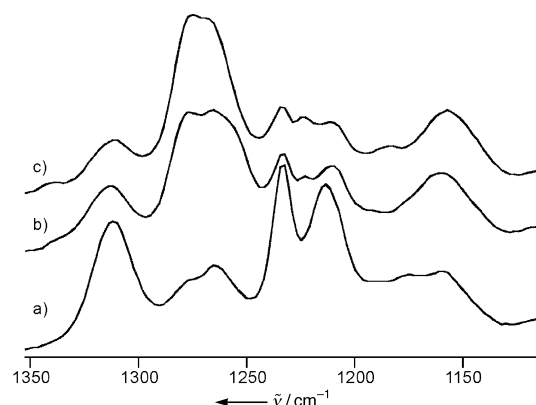
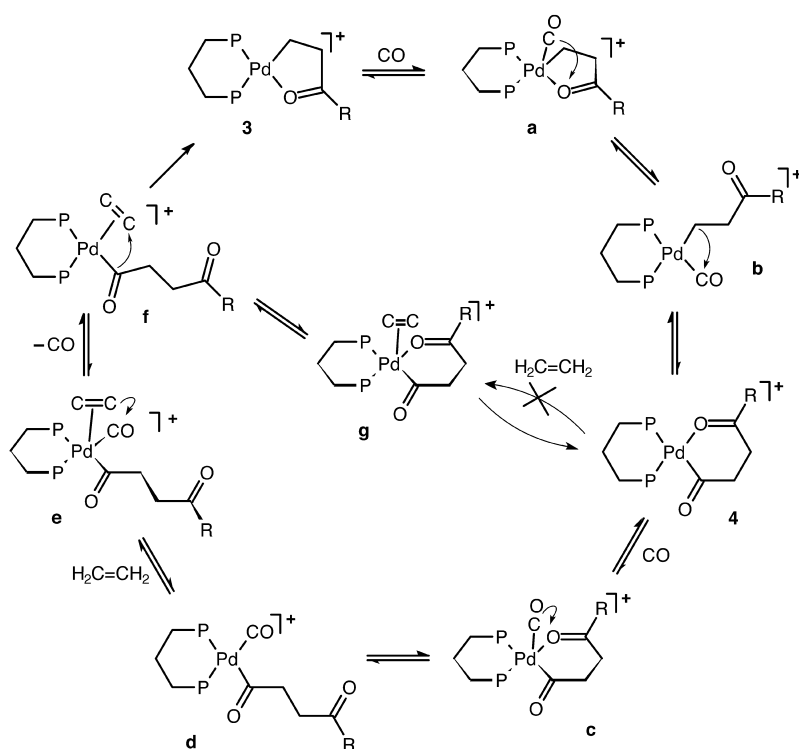


Figure 2. In situ PM-RAIR spectra of a sample of microcrystalline **1** a) at room temperature, b) under 500 mbar of CO, and c) under 2 mbar of CO and 333 mbar of ethene. Note the change from a coordinated (a) to a noncoordinated triflate anion (c).^[20] Spectra a–c represent the low-frequency regions of the spectra shown in Figure 1 a–c.

“pushed aside” during the first monomer insertions until it is essentially noncoordinated in **3** (Figure 2c). The absorption signals for coordinated triflate reduced almost to zero. This implies that, at least initially, virtually all catalyst sites, also those located in the bulk of the microcrystalline solid, reacted with CO and ethene, because RAIRS detects all molecules with equal sensitivity.

Copolymer growth could be followed in situ by exposing the Pd catalyst to a CO/ethene atmosphere (666 mbar) at room temperature, taking a mixture of **3** and **4** as a starting point. As polymerization proceeded, a signal at 1705 cm⁻¹ developed (Figure 1e), which corresponds to the carbonyl groups of the growing polyketone chain. This growth process could be followed for days, during which the growth rate decreased gradually. During polymerization, the intensities of the signals for the Pd–acyl group at 1690 cm⁻¹, visible as a shoulder of the 1705 cm⁻¹ peak, and for the chelating ketone(s) at 1625 cm⁻¹ remained constant. This implies that the total abundance of and the ratio between **3** and **4** remained constant, and that both are resting states of the catalyst. No signal was observed in the 2100 cm⁻¹ region, indicating the absence of an open-chain complex (**d** in Scheme 1), the main resting state for a Pd(phen)-based polyketone catalyst in solution (phen = 1,10-phenanthroline).^[4] Labeling studies (¹²CO/¹³CO) showed that, in contrast to the initial CO and ethene insertion steps, during copolymerization only part of the catalyst sites actually contributed to polymer growth, the degree being related to sample preparation and reaction time.

The finding that during polymer growth ethene insertion into the Pd–acyl bond of **4** is CO-assisted is one of the most remarkable findings of this study. This has never been observed during studies in solution, nor evaluated theoretically.^[8–10] It has been assumed that ethene will be able to readily displace the chelating ketone in **4** and insert subsequently to generate the five-membered palladacycle (**3**).^[2] This has been confirmed experimentally in solution.^[4] Insertion of ethene occurs by two discrete reaction steps: 1) substitution of the chelating ketone in **4** by ethene (**4**→**f**, Scheme 1) and 2) acyl migration to this ethene (**f**→**3**). The second step as such is facile in the microcrystalline state, since otherwise copolymerization would not have been achieved at all. Apparently, CO is required to enable a kinetically feasible reaction pathway for the first step. Taking into account that substitution reactions at square-planar Pd^{II} centers generally take place associatively,^[5, 21] a feasible reaction pathway for the conversion of **4** into **f** can be derived. It is proposed that substitution of the chelating ketone in **4** by ethene occurs in two consecutive steps: associative substitution of the chelating ketone by CO (**4**→**c**→**d**) followed by associative substitution of this CO by ethene (**d**→**e**→**f**). Substitution of the chelating ketone by CO is more facile than by ethene as it is thermodynamically less hindered and because an incoming



Scheme 1. Proposed catalytic propagation cycle with observed (**3**, **4**) and proposed intermediates (**a**–**f**); R represents the growing polyketone chain; the phenyl groups in dppp have been omitted.

CO ligand can approach the crowded catalytic Pd center in an end-on fashion (**4**→**c**), while an incoming ethene ligand has to approach the catalyst in a sterically more demanding side-on way (**4**→**g**). Although substitution of CO by ethene is thermodynamically unfavorable,^[4, 8, 10] the CO-coordinated Pd center in **d** is sterically less crowded than in the six-membered palladacycle **4**, thus facilitating the side-on approach of ethene required for (associative) CO displacement. In this way, a lower energy pathway becomes available for the formation of the ethene complex **f**, in which ethene can insert rapidly (**f**→**3**). Brookhart et al. showed that in solution opening of the six-membered palladacycle in [(phen)Pd{C(O)CH₂CH₂C(O)CH₃}]⁺ by ethene and its subsequent insertion into the Pd–acyl bond are facile processes.^[4] This might be related to the different type of ligand used—a phenanthroline ligand is flat, leaving more space for ethene to approach the Pd center from an apical position—or to the presence of a solvent.

In conclusion, a dedicated spectroscopic technique (PM-RAIRS) has enabled us to directly monitor CO/ethene copolymerization at a microcrystalline palladium catalyst and to elucidate some intriguing molecular aspects of this process.

Experimental Section

The PM-RAIRS experiments were performed in a home-built cell which allows studies under gas pressures up to 1 bar.^[16] Gold-coated silicon wafers (Topsil) were used as reflecting substrate. A sample of the catalyst [(dppp)Pd(CH₃)(OTf)] (**1**) was prepared by placing a droplet of [(dppp)Pd(CH₃)(CH₃CN)](OTf)^[7] in dichloromethane onto the gold-coated wafer. After drying, PM-RAIRS showed that the acetonitrile was

evaporated (Figure 2a). Atomic force microscopy (AFM) measurements showed that the majority of the sample consisted of a regular island structure of microcrystals, with an average size of about 10 nm, each containing approximately 2.5×10^3 catalyst molecules. From the island density of about 4×10^{10} islands per cm^2 , a coverage of 10^{14} molecules per cm^2 was estimated; this was supported by X-ray photoelectron spectroscopy (XPS) results (10^{14} Pd atoms per cm^2). The microcrystal size implies that roughly 35% of the catalyst molecules were located at the surface of the crystals.

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Induction of Lamellar Mesomorphic Structures in Columnar-Phase-Forming 1,3,5-Triazines through Charge-Transfer Interactions with Electron Acceptors**

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The molecular organization in thermotropic liquid-crystalline phases is predominantly associated with a rigid anisometric architecture of the constituent single molecules. Molecular self-organization may, however, also be effected by strong intermolecular forces between nonmesogenic molecules to give noncovalently bonded anisometric aggregates. The two principles can be combined by incorporating an intramolecular functionality into molecules with an anisometric shape.^[1] This combination allows the control and manipulation of the structure formed by mesogens through directed intermolecular interactions with a second complementary component. Specific manipulations of columnar mesomorphic structures, for example, may arise from formation of donor–acceptor complexes^[2] or from intermolecular hydrogen bonding^[3] between two different individual molecules.

“Open-sided” core systems that have the capacity to form columnar phases of single components and also allow the docking of a second component to a molecular recognition site located in the inner core region (side-by-side interactions) are conceivable. Structure formation should then be possible for the same molecule through associations with electron-deficient counterparts through charge-transfer (CT) interactions perpendicular to the molecular periphery (face-to-face interactions) provided that the central part of the molecule coincidentally represents an electron donor (Figure 1). To realize this concept we have synthesized 2,4,6-triaryl-amino-1,3,5-triazines with long peripheral alkoxy groups and with an electron-rich polar group in the center.^[7] The triazines substituted with six lipophilic side chains exhibit columnar mesophases in their pure state.^[8] Furthermore, hydrogen bonding with complementary alkoxy-substituted benzoic acids gives rise to the control of the two-dimensional lattice symmetries^[1, 9] and even induction of a hexagonal columnar mesophase.^[10]

We report here mesomorphic assemblies of the hexagonal columnar (Col_{hd}) phase from sixfold alkoxy-substituted 2,4,6-triaryl-amino-1,3,5-triazines **1a**, **b**,^[8] which act as electron

- [1] E. Drent, J. A. M. van Broekhoven, M. J. Doyle, *J. Organomet. Chem.* **1991**, *417*, 235–251.
- [2] E. Drent, P. H. M. Budzelaar, *Chem. Rev.* **1996**, *96*, 663–681.
- [3] A. Sen, *Acc. Chem. Res.* **1993**, *26*, 303–310.
- [4] F. C. Rix, M. Brookhart, P. S. White, *J. Am. Chem. Soc.* **1996**, *118*, 4746–4764.
- [5] K. Vrieze, J. H. Groen, J. G. P. Delis, C. J. Elsevier, P. W. N. M. van Leeuwen, *New. J. Chem.* **1997**, *21*, 807–813.
- [6] T.-W. Lai, A. Sen, *Organometallics* **1996**, *3*, 866–870.
- [7] G. P. C. M. Dekker, C. J. Elsevier, K. Vrieze, P. W. N. M. van Leeuwen, *Organometallics* **1992**, *11*, 1598–1603.
- [8] P. Margl, T. Ziegler, *J. Am. Chem. Soc.* **1996**, *118*, 7337–7344.
- [9] P. Margl, T. Ziegler, *Organometallics* **1996**, *15*, 5519–5523.
- [10] M. Svensson, T. Matsubara, K. Morakuma, *Organometallics* **1996**, *15*, 5568–5576.
- [11] I. Horváth, J. M. Millar, *Chem. Rev.* **1991**, *91*, 1339–1351.
- [12] R. Whyman, *CHEMTECH* **1991**, *21*, 414–419.
- [13] C. Sishta, R. M. Hathorn, T. J. Marks, *J. Am. Chem. Soc.* **1992**, *114*, 1112–1114.
- [14] W. G. Golden, D. D. Saperstein, M. W. Severson, J. Overend, *J. Phys. Chem.* **1984**, *88*, 574–580.
- [15] L. A. Nafie in *Advances in Applied Fourier Transform Infrared Spectroscopy* (Ed.: M. W. Mackenzie), Wiley, London, **1988**, pp. 67–104.
- [16] G. A. Beitel, A. Laskov, H. Oosterbeek, E. W. Kuipers, *J. Phys. Chem.* **1996**, *100*, 12494–12502.
- [17] P. W. N. M. van Leeuwen, C. F. Roobeek, H. van der Heijden, *J. Am. Chem. Soc.* **1994**, *116*, 12117–12118.
- [18] I. Tóth, C. J. Elsevier, *J. Am. Chem. Soc.* **1996**, *118*, 10388–10389.
- [19] F. Ozawa, T. Hayashi, H. Koide, A. J. Yamamoto, *J. Chem. Soc. Chem. Commun.* **1991**, 1469–1470.
- [20] W. Huang, R. Frech, R. A. Wheeler, *J. Phys. Chem.* **1994**, *98*, 100–110.
- [21] R. J. Cross, *Adv. Inorg. Chem.* **1989**, *34*, 219–292.

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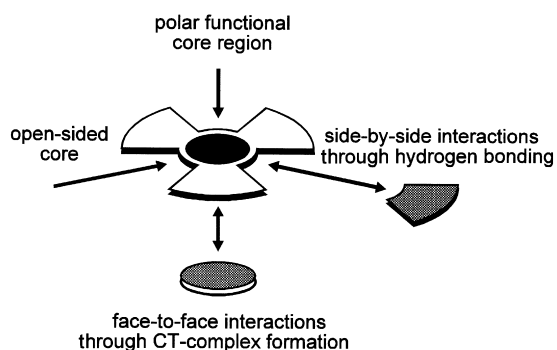
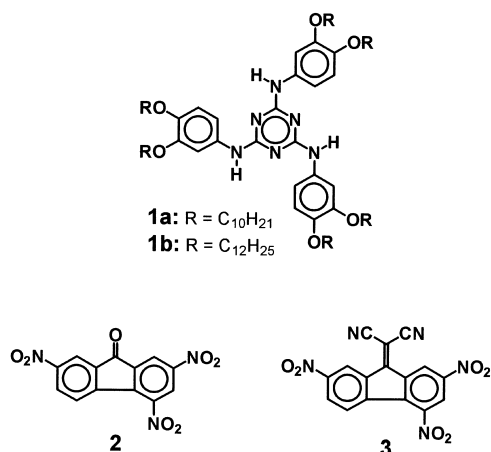


Figure 1. Aspects for controlling the structure formed by functional mesogens that are capable of forming columnar phases through non-covalent associations with complementary molecules by hydrogen bonding or through formation of a donor–acceptor complex.

donors, in mixtures with the nonliquid crystalline acceptors 2,4,7-trinitrofluoren-9-one (**2**) and 2,4,7-trinitrofluoren-9-ylidene malodinitrile (**3**).^[11] Polarizing microscopy and differential scanning calorimetry (DSC) studies reveal that the



mixtures of the melamines **1** with **2** or **3** exhibit an enantiotropic mesophase at a 1:1 molar ratio of the components (Table 1).

Table 1. Phase-transition data [°C] for equimolar mixtures of **1** with the acceptors **2** and **3** as determined by DSC; second heating with 10 K min⁻¹, transition enthalpies [kJ mol⁻¹] in parentheses.^[a]

1a/2	Cr	68.1 (21.19)	SmA	110.4 (1.91)	I
1b/2	Cr	68.6 (27.05)	SmA	115.0 (2.33)	I
1b/3	Cr	93.7 (17.72)	Col _r	147.2 (2.48)	I

[a] Cr: crystalline; SmA: smectic A; Col_r: rectangular columnar; I: isotropic.

X-ray investigations were performed to identify the types of mesophase structures displayed by the mixed systems **1/2** and **1/3** in more detail. The X-ray diagrams of the CT complexes derived from **1** and **2** show a set of reflections in the small-angle region and a diffuse scattering in the wide-angle region (Figure 2). These reflections can be indexed as (*h*00) with *h* = 1–5 (Table 2). No mixed reflections (*hkl*) were present. It is thus clear that a structure periodic in just one dimension, that

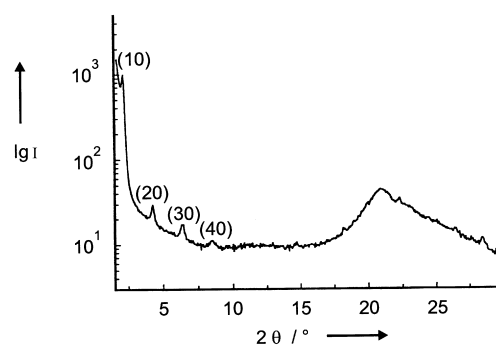


Figure 2. X-ray diffractogram of the 1:1 charge-transfer complex **1b/2**.

is, a layer structure, exists. The layer spacings amount to 36.2 Å for **1a/2** and 41.5 Å for **1b/2**. The Bragg spacings are given in Table 2. The wide-angle halo indicates that no long-range order is present within the layers. The X-ray investigations, together with the fan-shaped textures observed by polarizing microscopy, point to a smectic A-type mesophase for both **1a/2** and **1b/2**.

Table 2. X-ray data for the mesophases of the binary CT complexes **1/2** and **1/3**.

Sample	Positions of reflections [Å]						Lattice constants [Å]	
	<i>d</i> ₁₀₀	<i>d</i> ₀₁₀	<i>d</i> ₂₀₀	<i>d</i> ₃₀₀	<i>d</i> ₄₀₀	<i>d</i> ₅₀₀	<i>a</i>	<i>b</i>
1a/2	36.3	–	18.1	12.1	9.0	7.3	36.2	–
1b/2	41.5	–	20.7	13.8	10.4	–	41.5	–
1b/3	36.3	27.7	18.2	12.9	–	–	36.3	27.6

The X-ray diffraction pattern of the 1:1 mixture of **1b** and **3** differs from the scattering pattern observed for the donor–acceptor complexes **1/2** since not all the reflections can be assigned to a one-dimensional structure. This observation may be considered as an indication of the presence of at least a two-dimensional lattice. Only a diffuse halo, which is characteristic of fluid alkyl chains, is observed at larger scattering angles.

The three aryl substituents of compounds **1** are grafted on to the central 1,3,5-triazine nucleus through secondary amino groups. Since the phenyl rings can rotate around the C–N single bonds it is not only the flexible alkyl side chains but also the core region of the molecules that can adopt certain conformations, which results in a lack of inherent molecular planarity and no specific anisometric shape. Therefore, neither the hexagonal columnar mesophases formed by the triazines in their pure state nor the CT-induced lamellar phases can be explained in a conventional sense as resulting from a rigid disklike or rod-shaped molecular geometry.

The hexagonal lattice constants obtained from X-ray studies are 30.2 Å for compound **1a** and 32.8 Å for **1b**.^[8] The values are somewhat smaller than the molecular diameter of radial, two-dimensional conformers of **1a**, **b** in the most extended form, as shown in Figure 3a. We therefore conclude that the columns of the hexagonal mesophases of just the melamines **1** are composed of the polar triphenylaminotriazine cores, which preferably exhibit flat, radial conformations with a two-dimensional random distribution of the flexible side chains. This arrangement gives rise to an optimum space

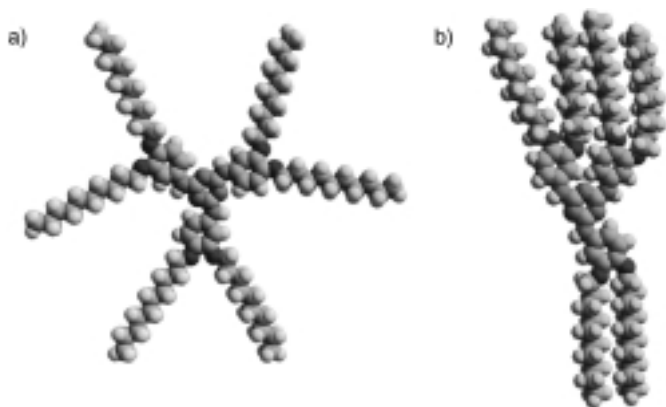


Figure 3. Two-dimensional conformers of the sixfold decyloxy-substituted triarylmelamine **1a** (Cerius 2; force field Dreiding 2). a) Radial conformation with all-*trans* alkoxy chains. b) Linear conformation with elongated chains.

filling in the outer sphere of the molecules. The main driving force for the liquid crystallinity of the triarylmelamines is thus a microsegregation^[12] of the polar part from the nonpolar part in such a way that cylindrical aggregates are formed with the polar groups of the molecules located in the center surrounded by a shell of lipophilic chains.

By taking into account that an intercalated structure with close face-to-face contacts between the donor and the acceptor is strongly preferred we are forced to the conclusion that the induction of lamellar phases originates from a two-dimensional linear conformation of the triarylmino-1,3,5-triazines (**1**), which is imposed by the CT interactions with the acceptor molecules **2**. Figure 3b shows such a conformation with extended alkyl chains for the triazine **1a**. The molecular length for linear conformers of the triazines evaluated from molecular mechanics simulations are approximately 41 Å for compound **1a** and 46 Å for **1b**. These values are slightly larger than the layer spacings determined by X-ray studies for the lamellar phases of the binary mixtures with **2** (see Table 2). Thus, the alkyl chains separating the polar cores of neighboring layers seem to interdigitate to some extent. However, the spacings would also be quite reasonable for a noninterdigitated smectic A-type layer structure for the CT complexes **1/2** with a disordered packing of the flexible alkyl groups. A structural model for the lamellar mesophase of the donor–acceptor complexes **1/2** is given in Figure 4a.

The development of a structural model for the 1:1 mixture **1b/3** that displays at least a two-dimensional, regular structure meets with great difficulties since the number of reflections present is very low. By assuming the most simplest case, a rectangular columnar phase, we find that the value of the lattice parameter *a* is similar to the layer thickness in the CT-induced smectic A phases of the binary mixtures **1/2** (see Table 2). The lattice parameter *b* would correspond, in this case, to the lateral side-by-side distance per repeat unit and we would envision a very simple molecular arrangement, namely a columnar structure on a face-centered rectangular array. The problem is, however, that such a face-centered structure would lead to systematic extinctions, which are not observed. We either have to assume that the molecules are systematically shifted, for reasons unknown to us, from the

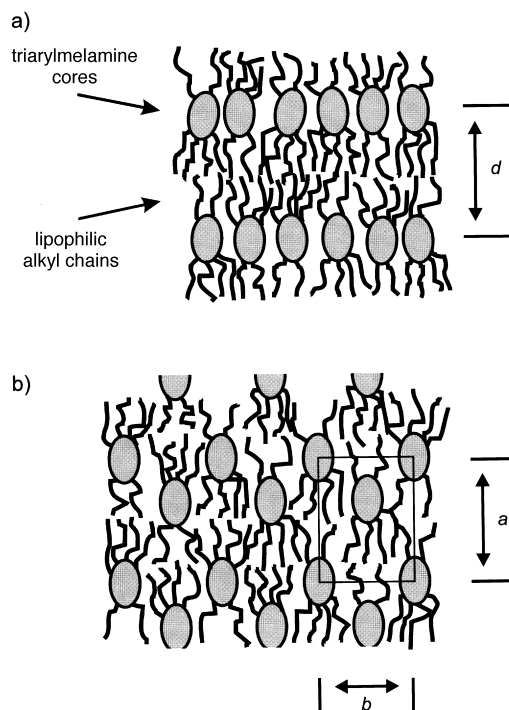


Figure 4. Schematic models for the liquid-crystalline phases of the melamines **1** in binary mixtures with the electron acceptors **2** and **3**. a) Lamellar smectic A-type mesophase for the CT complexes **1/2**. b) Rectangular columnar (Col_r) phase for the complex **1b/3**. The shaded ellipses symbolize the linear conformers of the polar triphenylaminotriazine cores (see Figure 3b).

face-centered position^[13] as shown in Figure 4b or that a more complex arrangement is displayed. In fact, we cannot rule out the presence of an oblique lattice symmetry.^[14]

The steric conformation of the polar core region of the triarylmelamines **1a, b** are, on average, the same for CT complexes with both **2** and **3**. Hence the Col_r phase of **1b/3** can be regarded as resulting from a destruction of the CT-induced lamellar phase (Figure 3a), which arises simply by a slight change in the chemical nature of the electron acceptor. Furthermore, one can imagine the rectangular columnar phase of the CT complex **1b/3** to be an intermediate between the hexagonal columnar mesophases formed by the triarylmelamines in their pure state and the lamellar mesomorphic structures induced by donor–acceptor interactions with **2**.

In summary, the triazines **1** are the first examples of electron donors that form columnar phases which give rise to the induction of smectic liquid crystalline structures through donor–acceptor interactions. The formation of the pure triarylmelamine structures as well as of the CT complexes arises from the distinct segregation of polar from nonpolar molecular regions along with certain specific anisometric conformations that become favored during the process of self-organization. The interplay between different driving forces for self-organization makes these materials attractive for further investigations regarding defined control of supramolecular structures through attractive interactions with complementary molecular species.

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- [1] D. Janietz, *J. Mater. Chem.* **1998**, *8*, 265.
- [2] It is, for example, well known that electron-rich, disklike aromatic compounds such as radial multialkynylbenzene derivatives and triphenylene ethers form charge-transfer complexes with flat, nitro-fluorenone-based electron acceptors. The CT interactions may cause the stabilization as well as the induction of columnar mesophases. The columnar phases formed by the binary mixtures are usually hexagonal (Col_h) or nematic columnar (N_{col}): H. Bengs, M. Ebert, O. Karthaus, B. Kohne, K. Praefcke, H. Ringsdorf, J. H. Wendorff, R. Wüstefeld, *Adv. Mater.* **1990**, *2*, 141; M. Ebert, G. Frick, C. Baehr, J. H. Wendorff, R. Wüstefeld, H. Ringsdorf, *Liq. Cryst.* **1992**, *11*, 293; K. Praefcke, J. D. Holbrey, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *24*, 19.
- [3] The association of 2,6-diacylaminopyridines with complementary uracil derivatives, for example, leads to the induction of columnar phases on either a hexagonal^[4,5] or a rectangular^[6] two-dimensional array. Here, however, the two components are not mesomorphic by themselves.
- [4] M. J. Brienne, J. Gabard, J.-M. Lehn, I. Stibor, *J. Chem. Soc. Chem. Commun.* **1989**, 1868.
- [5] C. Fouquey, J.-M. Lehn, A.-M. Levelut, *Adv. Mater.* **1990**, *2*, 254.
- [6] J.-M. Lehn, *Makromol. Chem. Macromol. Symp.* **1993**, *69*, 1.
- [7] D. Goldmann, PhD Thesis, Potsdam University, **1998**.
- [8] D. Goldmann, D. Janietz, R. Festag, C. Schmidt, J. H. Wendorff, *Liq. Cryst.* **1996**, *21*, 619.
- [9] D. Goldmann, R. Dietel, D. Janietz, C. Schmidt, J. H. Wendorff, *Liq. Cryst.* **1998**, *24*, 407.
- [10] D. Janietz, D. Goldmann, C. Schmidt, J. H. Wendorff, *Mol. Cryst. Liq. Cryst.* **1999**, *332*, 141.
- [11] The mixtures were prepared by combining solutions of the triazines **1** with either acceptor **2** or **3** in dichloromethane. Similar to triphenylene ethers, the dark brownish color that appears indicates complex formation through charge-transfer interactions. All thermal investigations were performed after evaporating the solvent, drying the residues in a vacuum, and annealing the samples in the isotropic state.
- [12] C. Tschierske, *J. Mater. Chem.* **1998**, *8*, 1485.
- [13] With respect to a rectangular columnar phase not with a face-centered but with an asymmetric position of the second molecule, see also G. Lattermann, S. Schmidt, R. Kleppinger, J. H. Wendorff, *Adv. Mater.* **1992**, *4*, 30.
- [14] Suggestion by one of the referees.

Combinatorial Surface Chemistry— Is it Possible?*

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Roger M. Leblanc*

Combinatorial chemistry and biological-based approaches have emerged as powerful aids for the discovery of novel pharmaceutical agents and biomaterials.^[1] Since its introduction in the early 1990s, combinatorial chemistry has revolutionized the medicinal chemistry area. The combinatorial

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

technique, applied to discover novel materials and catalysts, is making significant progress in another research area.^[2–4]

However, the basic essence of combinatorial chemistry has yet to be completely employed. Nature is the best combinatorial chemist, showing four deoxyribonucleotides and twenty amino acids under the whole biological world through the combination of these small molecular species. Indeed, one may think a long polypeptide chain is a combination of different amino acids connected through amide bonds. The long polypeptide chains then fold into a three-dimensional protein structure through noncovalent bonds between the amino acid residues. As a result, the active site of a protein can be envisaged merely as a combination of amino acids in a three-dimensional space.

This fundamental feature of proteins suggests a very interesting clue for the creation of their mimics. Amphiphilic lipid molecules are a class of molecules well studied by surface chemists. At the two-dimensional air–water interface, the hydrophilic polar moiety embeds into the water phase and the hydrophobic alkyl chain orients towards the air phase. When compressed at the interface, the amphiphilic molecules will move and assemble into organized supramolecular structures, known as Langmuir monolayers. If the polar moieties are functionalized with different peptides, such as a peptide library, the self assembly of these functional lipids may form proteinlike supramolecular structures, as illustrated in Figure 1.

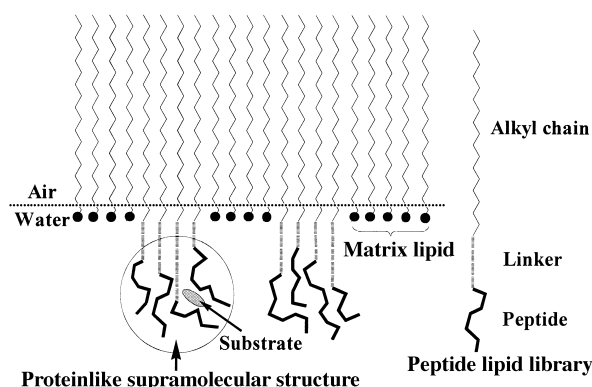
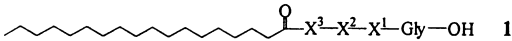


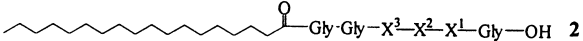
Figure 1. An illustration of the self assembly of peptide–lipid library components at the air–water interface to form proteinlike supramolecular structures.

Yu et al. recently reported that the self assembly of a peptide lipid led to the formation of a collagenlike structure at the air–water interface.^[5] Kunitake has shown that two peptide lipids could combine together at the air–water interface to form a unique binding site for a specific peptide guest.^[6] Furthermore, it was found that nonpeptide lipids could also combine together at the air–water interface to form receptors for different, small organic guest molecules.^[7] These previous works strongly support the feasibility of the proposed two-dimensional combinatorial approach.

We now for the first time report our study of Langmuir monolayers made of peptide lipid libraries. Library **LIB** and three sublibraries **SUB1**, **SUB2**, and **SUB3** (Table 1) were synthesized through the solid-phase peptide synthesis techni-

Table 1. The structures and the building blocks for the synthesis of the peptide lipid library and sublibraries.


1


2

Library	X ¹ , X ² , X ³	Components
LIB	Gly, Glu, Ser, His, Tyr	250
SUB1	Gly, Ser, Tyr	54
SUB2	Gly, Glu, Tyr	54
SUB3	Glu, Ser, His	54

que^[8] with a mixing–splitting method.^[9] Five amino acids—Gly, Glu, Ser, His, and Tyr—were used as building blocks. After the coupling of the last variable amino acid residue to the beads (Wang Resin), half of the resin was then taken out to couple with two more glycine units. The two extra Gly amino acids serve as a linker to extend the length of the remaining peptide lipids. The two portions of the resins were then combined and coupled with the succinimidyl ester of stearic acid (SA) to produce **1** and **2**, without and with the two extra Gly spacer units, respectively. Through the spacer extension, there are peptide lipids of different lengths, which, on combination, form a pseudo three-dimensional structure at the interface (Figure 1). As a result, the library **LIB** contains 250 components and each of the three sublibraries contains 54 components. The three sublibraries are part of the original library **LIB**.

The surface chemistry of the lipid libraries was studied. In contrast to traditional Langmuir monolayer studies, the present study used a lipid library instead of only one or a few lipids at the air–water interface. Surface pressure–area isotherm measurements show that, as a whole, these library and sublibrary samples formed monolayers at the air–water interface (Figure 2). A technical point was raised during the isotherm measurements of these lipid library and sublibrary samples. Since the library components are mixed together through the mixing–splitting synthesis, the library components are not addressed individually. An exact molecular weight of the library sample cannot be calculated. Therefore, when preparing the spreading solution, all the library and

sublibrary samples were presumed to have an mean molecular weight of 600 g mol^{−1}, corresponding to a molecule with a C₁₈ hydrocarbon alkyl chain and a peptide chain with three to five amino acid residues. The molecular areas in the isotherm may not reflect the actual average molecular area of the library components. Therefore, it is not appropriate to compare the molecular areas of monolayers from one library to another. However, this will not affect the molecular recognition and binding studies of the peptide lipid library, as discussed later, because only the molecular area changes upon binding of substrate are important.

We then have attempted to use these library and sublibrary monolayers for molecular recognition of maltose as well as other sugar molecules. It is known that the binding site of the maltose binding protein (MBP) is exceptionally rich in polar and aromatic amino acid residues.^[10] The polar, charged side chains hydrogen bond with the maltose hydroxyl group and the stacking of the aromatic residues provides the majority of the van der Waals contacts with maltose. Among the amino acid building blocks chosen for the lipid library and sublibrary synthesis, two are charged amino acids (His and Glu) and one is aromatic (Tyr). Since these amino acid residues exist in the different positions of the peptide–lipid library components, the spatial combination of these amino acid residues in the lipid library monolayers may lead to the formation of specific binding sites for maltose, similar to the binding site of MBP.

It was found that with 10 mM D-maltose as a subphase, the molecular areas of the library and sublibrary monolayers were all expanded. However, the molecular area expansions caused by the existence of maltose in the subphase are different from one library monolayer to the other (Figure 3). While the **LIB** monolayer only exhibits slight expansion on the maltose

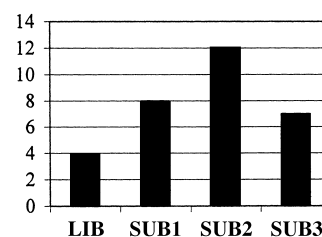


Figure 3. The increases of the molecular area (Å² molecule^{−1}) of the library and sublibrary monolayers on maltose subphase (10 mM in water, pH 5.8) compared to the pure water subphase. The area increases were calculated based on the molecular area increases at the surface pressure lifting point of the isotherms.

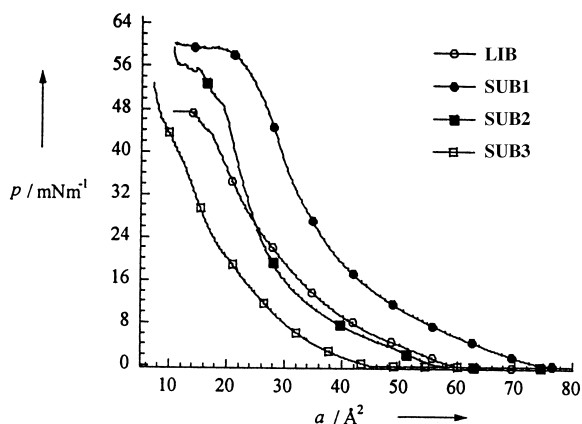


Figure 2. The surface pressure–area per molecule (p – a) isotherms of monolayers of the peptide lipid libraries **LIB**, **SUB1**, **SUB2**, and **SUB3** on a pure water subphase (pH 5.8, 20 °C).

subphase at the surface pressure lifting point (≈ 4 Å² molecule^{−1}) compared to pure water subphase, **SUB2** with Gly, Glu, and Tyr building blocks exhibits the largest expansion of molecular area (≈ 12 Å² molecule^{−1}).

In any combinatorial chemistry study, one key issue is to identify the active components from a library. Our study suggests that the peptide lipids in **SUB2** are more active towards maltose than the components from other sublibraries. This result can be explained by the following fact: For **SUB1** there is a lack of polar amino acid residues, while in **SUB3** there is a lack of aromatic amino acid residues. Only **SUB2** contains both polar charged (Glu) and aromatic (Tyr) amino

acid residues, the structural elements in the binding site of MBP.^[10] The spatial combination of these charged and aromatic amino acid residues in different positions of peptide lipids at the air–water interface lead to the formation of appropriate binding sites for maltose. The sublibrary **SUB2** monolayer can mimic the structural feature and function of MBP more efficiently than the other sublibrary monolayers. As for **LIB**, since it contains both active and inactive peptide lipids, the actual concentration of active peptide lipids in the monolayer is lower and the observed binding activity is therefore smaller, compared to the sublibraries.

It has been reported^[11] that the binding of maltose or its higher homologs to MBP induces major changes in both the fluorescence and UV absorption spectra of MBP, due to the van der Waals interactions between maltose and the aromatic residues of MBP in the binding site. Depending on the structures of the substrates, blue or red shifts of the absorption bands are observed. To verify the binding of maltose to the library monolayers, we have examined the UV/Vis absorption spectra of the **SUB2** monolayer on water and a 10 mM maltose subphase (Figure 4). Interestingly, like the MBP–maltose

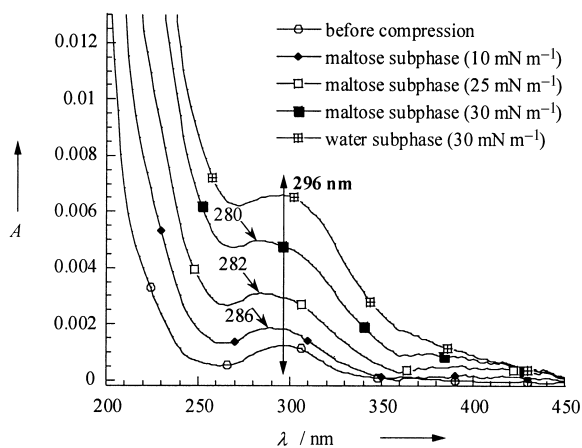


Figure 4. The UV/Vis absorption spectra (absorbance A , wavelength λ) of **SUB2** monolayer on 10 mM maltose subphase compared to the pure water subphase. Spectra of the monolayer on water at surface pressures lower than 30 mN m⁻¹ are not shown. The maximum UV absorption of the monolayer on the water subphase remains at 296 nm from the beginning of the compression until its collapse.

complex, the absorption band of the **SUB2** monolayer at around 296 nm (arising from Tyr residues) significantly blue shifted in the presence of maltose. Before compression, the maximum absorption of the **SUB2** monolayer appeared at 296 nm on both subphases and under continuous compression this absorption band remained at 296 nm on pure water subphase at all stages of compression. However, on a 10 mM maltose subphase, this absorption band shifted to 286, 282, and 280 nm at monolayer surface pressures of 10, 25, and 30 mN m⁻¹, respectively, a gradual and significant blue shift upon compression. Combined with the surface pressure–area isotherm measurements, these results confirm that maltose molecules bind to the **SUB2** monolayer with strong intermolecular interactions. The aromatic Tyr residue in the sublibrary should play a significant role for the binding activity. Although there is only one polar and one aromatic amino acid

in the library, the three-dimensional combination of the same amino acids in different positions can still lead to the formation of an efficient binding site.

As an important comparison, we have also examined the binding activity of the **SUB2** monolayer towards two other sugar molecules, D-glucose and sucrose. The surface pressure–area isotherms show the existence of glucose or sucrose in the aqueous subphase introduced almost no area expansion of the monolayer (Figure 5), in stark contrast to the maltose

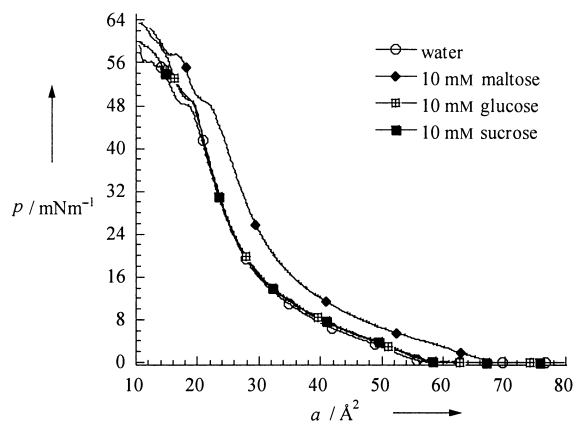


Figure 5. The surface pressure–area per molecule (p – a) isotherms of the **SUB2** monolayer on water (pH 5.8) and 10 mM sugar (maltose, glucose, and sucrose) subphases.

subphase. Furthermore, the UV/Vis absorption spectra of the **SUB2** monolayer with a 10 mM sucrose subphase show that the absorption band arising from Tyr residues appeared at 278 nm on this subphase and remained unchanged at this wavelength over the whole compression range. The dynamic property of the **SUB2** monolayer is not affected by the sucrose from the subphase. Both the surface pressure–area isotherms and UV/Vis absorption spectra indicate that the **SUB2** monolayer does not bind with sucrose as efficiently as with maltose. Even as a simple and small library, the **SUB2** monolayer already exhibits receptor specificity.

In summary, the present study has shown that a novel approach, that of combinatorial surface chemistry, is possible and can become a very facile and powerful technique in the design and creation of artificial proteins and enzymes. While peptide–lipid libraries can be conveniently synthesized using solid phase peptide synthesis techniques, a lipid library can be treated as a single lipid for monolayer studies by Langmuir techniques. With the presence of a large amount of molecular diversity, the chances of finding a good combination of peptide moieties to form a desired proteinlike supramolecular structures should increase.

The synthesis of peptide lipid libraries and experimental conditions for the surface chemistry studies are available in the Supporting Information. Surface pressure–area isotherms of peptide lipid library samples on maltose subphase and the UV/Vis absorption spectra of the **SUB2** monolayer on the sucrose subphase are also included.

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- [1] An extensive number of references are not listed here. For a recent general review, see: S. Borman, *Chem. Eng. News* **1998**, 76, 47.
- [2] A. H. Hoveyda, *Chem. Biol.* **1998**, 5, R187.
- [3] I. E. Maxwell, *Nature* **1998**, 394, 325.
- [4] R. F. Service, *Science* **1998**, 280, 1670; B. Jandeleit, D. J. Schaefer, T. S. Powers, H. W. Turner, W. H. Weinberg, *Angew. Chem.* **1999**, 111, 2648–2689; *Angew. Chem. Int. Ed.* **1999**, 38, 2494–2532.
- [5] Y. C. Yu, P. Berndt, M. Tirrell, G. B. Fields, *J. Am. Chem. Soc.* **1996**, 118, 12515.
- [6] T. Kunitake, *Pure Appl. Chem.* **1997**, 69, 1999.
- [7] K. Ariga, T. Kunitake, *Acc. Chem. Res.* **1998**, 31, 371.
- [8] G. B. Fields, R. L. Noble, *Int. J. Peptide Protein Res.* **1990**, 35, 161.
- [9] J. Nielsen, *Chem. Ind.* **1994**, 902.
- [10] S. P. Spurlino, G.-Y. Lu, F. A. Quijcho, *J. Biol. Chem.* **1991**, 266, 5202.
- [11] J. A. Hall, K. Gehring, H. Nikaido, *J. Biol. Chem.* **1997**, 272, 17605.

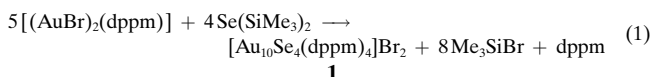
Selenium-Bridged Gold(I) Complex Cations [Au₁₀Se₄(dppm)₄]²⁺ and [Au₁₈Se₈(dppe)₆]²⁺**

Dieter Fenske,* Timo Langetepe, Manfred M. Kappes, Oliver Hampe, and Patrick Weis

The syntheses and possible applications of polynuclear complexes are presently being investigated by several research groups.^[1] For instance, transition metal complexes have been reported in which the metal centers are bridged by main group element atoms.^[2, 3] Sulfur-bridged cluster complexes of Groups 9 and 10 are among the known examples. Selenium- or tellurium-bridged compounds have been more rare up till now. However, chalcogen-bridged complexes of the coinage metals are predicted to feature physical properties of special interest. In contrast to the great number of known copper and silver compounds, only a few homologous gold species have been synthesized. This can certainly be attributed in part to the more restricted coordination chemistry of monovalent gold.^[4, 5] The complexes are often stabilized by protecting phosphane ligands, which suppress further reaction to binary chalcogenides. According to quantum-chemical investigations of PR₃-substituted Cu₂E cluster complexes (R = organic group; E = S, Se), the compounds that have been characterized up to now appear to be metastable in most cases.^[6] Nevertheless, the formation of various interesting molecules containing gold atoms has been described recently.^[7] Examples are [(Ph₃P)Au]₄S (Laguna et al.),^[7b] [(CSAu(PPh₃))₆]

(Schmidbaur et al.),^[7c] and [Au₁₂S₈]⁴⁻ (Strähle et al.).^[7c] However, only a few reports concern chalcogen-bridged gold complexes which are clad by bidentate phosphane ligands.^[8]

Herein [Au₁₀Se₄(dppm)₄]Br₂ (**1**) and [Au₁₈Se₈(dppe)₆]X₂ (**2**; X = Cl: **2a**, X = (PF₆): **2b**, X = BPh₄: **2c**; dppm = bis(diphenylphosphanyl)methane, dppe = bis(diphenylphosphanyl)ethane), two ionic compounds possessing such ligands and displaying remarkable stability, are introduced. Yellow crystals of **1** result from the reaction of [(AuBr)₂(dppm)] with Se(SiMe₃)₂ [Eq. (1)].



The molecular structure of **1** was determined by single-crystal X-ray structure analysis.^[9] In the cation of **1**, eight gold atoms (Au3–Au10) form an irregular, corrugated octagon which is centered by two further gold atoms (Au1, Au2; Figure 1). Thus, six Au₃ triangles and four Au₄ rectangles are

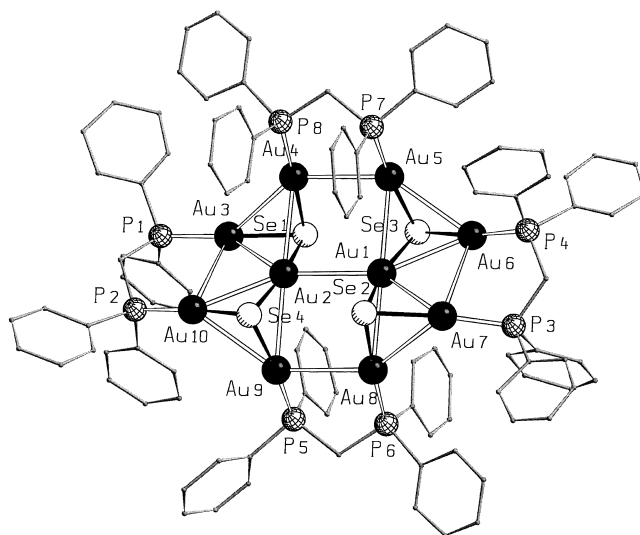


Figure 1. Structure of the cation of **1** in the crystal. Selected bond lengths [pm] and angles [°]: Au1–Au2 312.7(1), Au1–Au5 306.1(1), Au1–Se2 246.1(2), Au2–Au3 314.4(1), Au3–Au4 336.3(1), Au3–Au10, Au3–P1 227.8(6), Au3–Se1 242.9(2), Au4–Au5 309.6(1), Au4–Se1 242.3(2), Au4–P8 226.6(5), Se1–Se3 362.1(4), Au2–Se1–Au3 80.20(7), Au3–Au4–Au5 136.28(4), Au3–Au2–Au4 65.16(3), Au4–Au2–Au9 175.93(4), Se1–Au3–P1 168.5(2), Se1–Au4–P8 177.2(2), Se1–Au2–Se4 168.60(9).

generated. Four of the triangles (Au2, Au3, Au4; Au1, Au5, Au6; Au1, Au7, Au8; Au2, Au9, Au10) are μ_3 -bridged by selenium atoms in such a way that the two neighboring Au₃ faces bind one selenium atom above and one below the average plane of the gold atoms. Selenium atoms which are positioned on the same side of the Au₁₀ “plane” (Se1, Se3 and Se2, Se4) slightly approach each other through the Au₄ rectangle (Se1–Se3 362.1(4), Se2–Se 437.4(4) pm). The peripheral gold atoms (Au3–Au10) additionally bind to one phosphorus atom of the dppm ligands. Each of these eight metal centers is therefore nearly linearly coordinated by one phosphorus and one selenium atom. In contrast, the inner gold atoms (Au1, Au2) bind almost linearly to two selenium

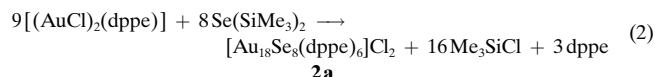
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neighbors. All Au–Se bond lengths (242.3(2)–246.1(2) pm) lie within the expected range, and the angles at the gold centers (164.6(1)–177.2(2)°) are somewhat smaller than the ideal angle of 180°. All Au–Se–Au angles in **1** are acute (76.3(1)–87.8(1)°). Binding interactions between gold atoms can be excluded because of the long intermetallic distances (303.1(2)–336.3(2) pm).

The reaction of [(AuCl)₂(dppe)] with Se(SiMe₃)₂ in dichloromethane gives orange crystals of **2a** in excellent yield [90%, Eq. (2)]. Ensuing reactions of **2a** with KPF₆ or NaBPh₄ lead to the formation of **2b** and **2c**, respectively, ionic compounds consisting of a complex cation and sterically isolated anion molecules.



Single crystals of **2a** were characterized by X-ray diffraction. The molecular structure of the cation in **2a** is given in Figure 2. With regard to the heavy-atom substructure, a

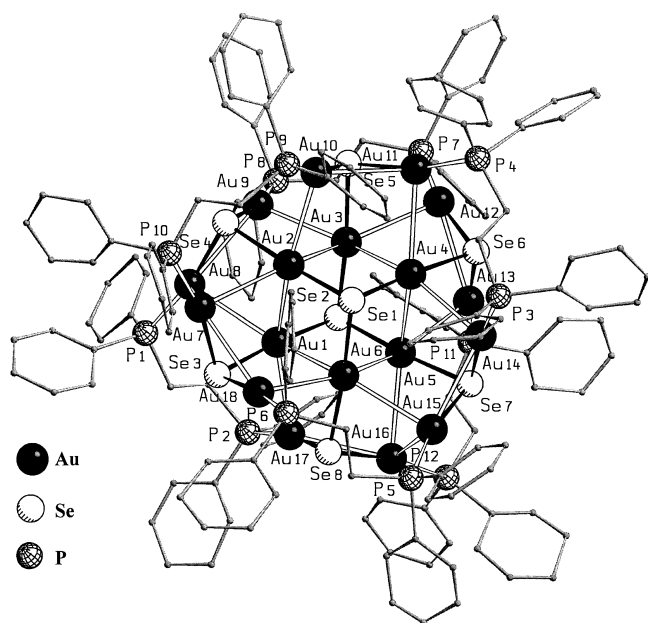


Figure 2. Structure of the cation of **2a** in the crystal. Selected bond lengths [pm] and angles [°]: Au1–Au2 294.3(2), Au1–Au8 312.4(2), Au1–Se2 246.3(4), Au1–Se3 247.8(4), Au7–Se3 245.4(4), Au7–P10 228.2(9); Au1–Se2–Au3 96.5(1), Au2–Au1–Au6 76.9(1), Au2–Au1–Se2 93.4(1), Au7–Se3–Au18 79.6(1), Se2–Au1–Se3 174.3(1), Se3–Au7–P10 166.9(2).

pseudo-threefold axis runs through selenium atoms Se1 and Se2. Two [Au₆Se₄(dppe)₃] fragments are interlocked in such a manner that a distorted Au₆Se₂ heterocubane arrangement (Au1–Au6, Se1, Se2) arises in the center of the polynuclear complex. This central Au₆Se₂ unit is surrounded by the twelve remaining gold atoms that are attached to phosphorus atoms from dppe ligands. Within the Au₆Se₂ heterocubane fragment, one finds Au–Au distances in the range between 294.0(2) and 300.2(2) pm; a range of 294.8(2)–315.3(2) pm is observed for the outer gold atoms bonded to the heterocubane. Atoms Au7–Au18 show intermetallic distances of 310.3(3)–

332.7(3) pm. Atoms Se1 and Se2 as well as the selenium atoms at the cation's periphery act as μ₃ bridges between gold atoms; the Au–Se bond lengths are 244.7(4)–246.3(4) pm for Se1 and Se2. The peripheral selenium atoms each display three short Au–Se bonds (243.0(3)–248.1(3) pm); there are also two weak interactions to neighboring gold atoms (332.4(4)–373.3(4) pm). One perceives differences in the Au–Se–Au angle between selenium atoms that belong to the inner Au₆Se₂ group those that are part of the outer Au–Se framework. For Se1 and Se2 these angles amount to 96.0(1)–97.3(1)°, whereas the selenium atoms of the cluster periphery show Au–Se–Au angles in the wider range of 78.6(1)–102.8(1)°. Similar observations have been reported for related compounds.^[7b, 7f] If intermetallic contacts are not taken into consideration, each gold center is almost linearly coordinated by either two selenium neighbors (Au1–Au6) or by one selenium and one phosphorus ligand (Au7–Au18). Compound **2** features Se–Au–Se angles of 173.8(1)–179.9(1)° at the inner gold atoms (Au1–Au6); the remaining twelve gold centers (Au7–Au18) form Se–Au–P angles in the range of 166.9(1)–173.0(2)°. The Au–Se bond lengths (243.0(3)–248.1(3) pm) are typical for such polynuclear complexes, and the Au–Au distances (294.0(2)–332.7(3) pm) correspond with intramolecular Au–Au distances in [μ-Au₂(dppf)]-[S[Au₂(dppf)]₂][OTf]₂ (290.5–327.2 pm; dppf = 1,1'-bis-(diphenylphosphanyl)ferrocene, OTf = trifluoromethanesulfonate (triflate)).^[8b] The structural determinations of **2a–c** proved to be very problematic, since localization of the anion molecules was always frustrated by cocrystallization of disordered CH₂Cl₂.

Nevertheless, the question concerning the charge of the cation and the nature of counterion could be answered unambiguously after electrospray/ionization mass spectrometry (ESI) experiments were carried out employing a high-resolution FT-ICR^[12] mass spectrometer.

Figure 3 shows the cation mass spectrum of **2b**, which is dominated by one single peak. The high-resolution signal is shown along with the simulated isotopic distribution in the inset. Excellent congruence is observed for a distribution centered around *m/z* 3283.89, corresponding to the dication

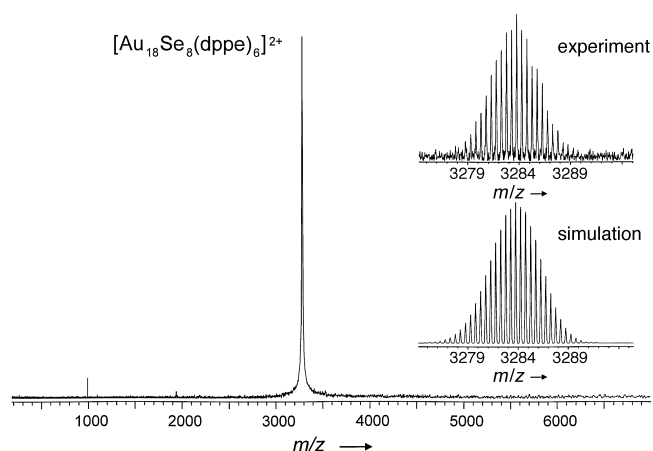


Figure 3. ESI mass spectrum of **2b** in CH₂Cl₂ in the range of *m/z* 200–7000. Inset: Comparison of the experimentally observed high resolution molecular peak with the simulation.

$[\text{Au}_{18}\text{Se}_8(\text{dppe})_6]^{2+}$. Thus the complex follows the classical perception of a gold(+I)/selenium(−II) compound. The actual charge distribution within the cluster and the possible existence of $d^{10}-d^{10}$ interactions, which were specified for binuclear gold complexes,^[10] are the subject of further spectroscopic measurements as well as essential ab initio investigations.

The only anionic species found in the mass spectrum of **2b** is PF_6^- . This clearly shows that a complete metathesis reaction took place followed by integration of the new anion molecules in the lattice.

It was thus possible to vaporize an inorganic ionic compound featuring a molecular mass of about 6568 g mol^{-1} almost without fragmentation by means of electrospray experiments.

A second mass spectrometric measurement was performed to investigate collision-induced fragmentation of the dication in **2b** by argon. The preferred dissociation path was found to be a scission of the complex framework into the fragments $[\text{Au}_5\text{Se}_2(\text{dppe})_2]^+$ and $[\text{Au}_{13}\text{Se}_6(\text{dppe})_3]^+$ with loss of one phosphane molecule. This directly suggests that the Au–Se and the Au–phosphane binding energies are of the same order of magnitude. More intensive studies of the dissociation behavior of the presented polynuclear complex is the subject of another publication.^[13]

The experimental observations indicate a high stability of the cationic molecular framework of **2**. Furthermore, it is conspicuous that **2** dissolves only in particular solvents, such as CH_2Cl_2 , DMF, or pyridine. The complex is stable to air and moisture and does not decompose below 80°C .

Reactions of $[(\text{AuCl})_2(\text{dppe})]$ with Me_3SiSeR ($\text{R} = \text{Et}$, $i\text{Pr}$, $t\text{Bu}$, $n\text{Bu}$) always yield **2a**, indicating that Se–C bond cleavage invariably occurs. The cation in **2** is so energetically advantaged, that it has not been possible yet to produce or isolate complexes containing SeR^- ligands by the described route.

Besides **2**, isostructural Au–Te species could be synthesized that are ligated by dppe, dppa, or dppb ligands (dppa = bis(diphenylphosphanyl)ethyne, dppb = bis(diphenylphosphanyl)butane).

In conclusion, reactions of gold halide–phosphane complexes with silylated selenium derivatives yield very stable polynuclear gold complexes. By means of single-crystal X-ray structure analyses, the following compounds were structurally determined: $[\text{Au}_{10}\text{Se}_4(\text{dppm})_4]\text{Br}_2$, $[\text{Au}_{18}\text{Se}_8(\text{dppe})_6]\text{Cl}_2$, $[\text{Au}_{18}\text{Se}_8(\text{dppe})_6][\text{BPh}_4]_2$, $[\text{Au}_{18}\text{Se}_8(\text{dppe})_6][\text{PF}_6]_2$. Electrospray mass spectrometry experiments of $[\text{Au}_{18}\text{Se}_8(\text{dppe})_6][\text{PF}_6]_2$ (**2b**) show an enormous stability of the complex. Collision-induced fragmentation by argon provokes splitting into three fragments.

Experimental Section

1: $[(\text{AuBr})_2(\text{dppm})]$ (1 mmol, 940 mg) was suspended in CH_2Cl_2 (10 mL) and then $\text{Se}(\text{SiMe}_3)_2$ (1 mmol, 0.25 mL) was added at room temperature. The slight yellow solution was stirred for 2 h and layered with *n*-heptane (10 mL). Pale yellow crystals of **1** were obtained in 67% yield.

2: $[(\text{AuCl})_2(\text{dppe})]$ (1 mmol, 860 mg) was suspended in CH_2Cl_2 (10 mL) and then $\text{Se}(\text{SiMe}_3)_2$ (0.88 mmol, 0.22 mL) was added at room temperature. Within 5 min the solution turned orange. After 4 h of stirring the solution was layered with *n*-heptane (10 mL). Orange needlelike crystals grew within 1 d, during which the solution discolored. Compound **2a** was

produced in 90% yield. Subsequently, the solution was removed by pipette and the crystals were redissolved in CH_2Cl_2 (10 mL). KPF_6 or NaBPh_4 (2 mmol) was then added, and the reaction mixture stirred for 2 h. Removal of the solid material and layering with *n*-heptane (10 mL) led to the formation of orange crystals of **2b** or **2c**, respectively, in 75% yield.

The Au, Se, P, and C elemental analyses of **1** and **2a–c** corresponded to the given formulas.

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- [1] *Clusters and Colloids. From Theory to Applications* (Ed.: G. Schmid), VCH, Weinheim, 1994.
- [2] See, for example, a) L. C. Roof, J. W. Kolis, *Chem. Rev.* **1993**, 93, 1037; b) I. Dance, K. Fisher, *Prog. Inorg. Chem.* **1994**, 41, 637; c) J. Arnold, *Prog. Inorg. Chem.* **1995**, 43, 353.
- [3] a) D. Fenske, J. Ohmer, J. Hachgenei, *Angew. Chem.* **1985**, 97, 933; *Angew. Chem. Int. Ed. Engl.* **1985**, 24, 993; b) D. Fenske, J. Ohmer, J. Hachgenei, K. Merzweiler, *Angew. Chem.* **1988**, 100, 1300; *Angew. Chem. Int. Ed. Engl.* **1988**, 27, 1277.
- [4] a) H. W. Roesky, T. Gries, P. G. Jones, K.-L. Weber, G. M. Sheldrick, *J. Chem. Soc. Dalton Trans.* **1984**, 1781; b) S.-P. Huang, M. G. Kanatzidis, *Inorg. Chem.* **1991**, 30, 1455; c) V. W. W. Yam, W. K. Lee, T. F. Lai, *J. Chem. Soc. Chem. Commun.* **1993**, 1571; d) P. J. Bonasia, G. P. Mitchell, F. J. Hollander, J. Arnold, *Inorg. Chem.* **1994**, 33, 1797; e) V. W. W. Yam, K. K. W. Lo, C. R. Wang, K. K. Cheung, *Inorg. Chem.* **1996**, 35, 5116; f) V. W. W. Yam, K. K. W. Lo, *Comments Inorg. Chem.* **1997**, 19, 209.
- [5] a) D. Fenske, H. Krautscheid, G. Baum, M. Semmelmann, *Angew. Chem.* **1993**, 105, 1364; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 1303; b) S. Dehnen, D. Fenske, A. C. Deveson, *J. Cluster Sci.* **1996**, 7, 351; c) S. Dehnen, D. Fenske, *Chem. Eur. J.* **1996**, 2, 1407; d) A. C. Deveson, S. Dehnen, D. Fenske, *J. Chem. Soc. Dalton Trans.* **1997**, 4491; e) J. F. Corrigan, D. Fenske, *Chem. Commun.* **1997**, 1837; f) D. Fenske, N. Zhu, T. Langetepe, *Angew. Chem.* **1998**, 110, 2783; *Angew. Chem. Int. Ed.* **1998**, 37, 2639.
- [6] a) S. Dehnen, A. Schäfer, D. Fenske, R. Ahlrichs, *Angew. Chem.* **1994**, 106, 786; *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 746; b) A. Schäfer, R. Ahlrichs, *J. Am. Chem. Soc.* **1994**, 116, 10686; c) S. Dehnen, A. Schäfer, R. Ahlrichs, D. Fenske, *Chem. Eur. J.* **1996**, 2, 429.
- [7] a) P. G. Jones, G. M. Sheldrick, E. Hädicke, *Acta Crystallogr. Sect. B* **1980**, 36, 2777; b) C. Lensch, P. G. Jones, G. M. Sheldrick, *Z. Naturforsch. B* **1982**, 37, 944; c) G. Marbach, J. Strähle, *Angew. Chem.* **1984**, 96, 695; *Angew. Chem. Int. Ed. Engl.* **1984**, 23, 715; d) P. G. Jones, C. Thöne, *Chem. Ber.* **1990**, 123, 1975; e) I. Schröter, J. Strähle, *Chem. Ber.* **1991**, 123, 2161; f) S. P. Huang, M. G. Kanatzidis, *Angew. Chem.* **1992**, 104, 799; *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 787; g) H. Schmidbaur, A. Kolb, E. Zeller, A. Schier, H. Beruda, *Z. Anorg. Allg. Chem.* **1993**, 619, 1575; h) F. Canales, M. C. Gimeno, P. G. Jones, A. Laguna, *Angew. Chem.* **1994**, 106, 811; *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 769; i) H. K. Yip, A. Schier, J. Riede, H. Schmidbaur, *J. Chem. Soc. Dalton Trans.* **1994**, 2333.
- [8] a) S. Hofreiter, M. Paul, H. Schmidbaur, *Chem. Ber.* **1995**, 128, 901; b) F. Canales, M. C. Gimeno, A. Laguna, P. G. Jones, *J. Am. Chem. Soc.* **1996**, 118, 4839; c) V. W. W. Yam, E. C. C. Cheng, K. K. Cheung, *Angew. Chem.* **1999**, 111, 193; *Angew. Chem. Int. Ed.* **1999**, 38, 197.
- [9] X-ray structure analyses: STOE-IPDS ($\text{MoK}\alpha$ radiation); data collection and refinement (SHELXS-86, SHELXL-93); empirical absorption correction (habit). **1**: monoclinic, space group $P2_1/c$ (no. 14), $Z = 4$, cell parameters (190 K): $a = 1086.0(2)$, $b = 4054.0(8)$, $c = 2916.0(6)$ pm, $\beta = 92.58(3)^\circ$, $V = 12825.5(5) \times 10^6$ pm³, $\mu(\text{MoK}\alpha) = 13.498\text{ mm}^{-1}$, $2\theta_{\text{max}} = 46.38^\circ$; of 19923 reflections, 12783 were independent, 12759 with $I > 2\sigma(I)$, 1233 parameters (Au, Se, P anisotropic, C isotropic); max. residual electron density $3.3\text{ e}\text{\AA}^{-3}$; $R_1 = 0.05$; $wR_2 = 0.15$. **2a**: monoclinic, space group $P2_1/n$ (Nr. 14), $Z = 4$, cell parameters (200 K): $a = 1779.1(4)$, $b = 3987.7(8)$, $c = 2934.8(6)$ pm, $\beta = 92.37(3)^\circ$, $V = 20803 \times 10^6$ pm³, $\mu(\text{MoK}\alpha) = 14.433\text{ mm}^{-1}$, $2\theta_{\text{max}} = 46.52^\circ$; of 28286 reflections, 28282 were independent, 22915 reflections with $I > 2\sigma(I)$, 1077 parameters (Au, Se, P anisotropic, C isotropic); max. residual electron density $8.5\text{ e}\text{\AA}^{-3}$; $R_1 = 0.10$; $wR_2 = 0.27$. The high R values were caused by highly disordered solvent molecules that could not be localized; localization of the anions was

not possible either. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140363 (**1**) and CCDC-140364 (**2a**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [10] W.-F. Fu, K.-C. Chan, V. M. Miskowski, C.-M. Che, *Angew. Chem.* **1999**, *111*, 2953; *Angew. Chem. Int. Ed.* **1999**, *38*, 2783.
- [11] a) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Science* **1989**, *246*, 46; b) *Electrospray Ionization Mass Spectrometry* (Ed.: R. B. Cole), Wiley, New York, **1997**, and references therein.
- [12] Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometry: A. G. Marshall, P. B. Grobbs, *Anal. Chem.* **1991**, *63*, 215A.
- [13] P. Weis, O. Hampe, M. M. Kappes, T. Langetepe, D. Fenske, unpublished results.

Channel-Forming Peptaibols Are Potent Elicitors of Plant Secondary Metabolism and Tendril Coiling**

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Attack by microorganisms and herbivorous insects may induce characteristic local and systemic responses in plants. Typical defense reactions include de novo biosynthesis of phytoalexins and, characteristic of insect damage, the emission of volatile compounds for long-distance interactions.^[1, 2] The molecular basis for recognition of either infection or herbivore interference at the plant's cell surface, however, is not well understood. The signal chain is often triggered by compounds from the attacking organism, generally referred to as elicitors. Subsequent intracellular signal transduction involves changes in the ion permeability of the plasma membrane,^[3] which initiates a complex network of intracellular events leading to an increase of biosynthetic activities. The involvement of ion channels in signaling was previously demonstrated with parsley cells and an oligopeptide elicitor,^[4] with tobacco cells and oligogalacturonides,^[5] and, more recently, with several proteinaceous elicitors involved in phytoalexin biosynthesis in plants.^[6]

In addition to macromolecular elicitors, certain fungi also produce low molecular weight peptide-type antibiotics with membrane-depolarising properties.^[7] Characteristic features of their chemical structures are the acylated N terminus, and the presence of α -aminoisobutyric acid (Aib) and C terminal α -amino alcohols (peptaibols). They occur mainly as large 18- to 20-membered peptaibols, such as alamethicin,^[7] ampu-
llosporin^[8] and chrysospermin,^[9] and as smaller 15- to 16-membered species, such as antiamoebin.^[10] Their antimicrobial activity is largely due to the formation of α -helical structures which produce voltage-dependent or voltage-independent ion channels within biological membranes.^[7, 11] Since the induction of phytoalexin biosynthesis in plants may be directly linked to ion fluxes and subsequent intracellular signaling,^[3, 4] this property of the peptaibols prompted us to study their potential effect on the secondary metabolism of plants.

Here we demonstrate for the first time that fungal peptaibols represent a novel and powerful class of elicitors that can induce multiple metabolic activities such as ethylene emission, biosynthesis of volatile substances, and tendril coiling.

As a representative model compound we chose alamethicin (ALA), a mixture of homologous peptaibols from the wide spread soil fungus *Trichoderma viride*, the major component of which contains eight Aib residues and two prolines.^[7, 11] The N terminus is acetylated, and the C-terminal residue is phenylalaninol (Table 1). ALA is capable of forming voltage-gated channels which exhibit very high conductance in lipid membranes.^[7, 11]

When ALA (5 μ M) was supplied to the shoots of young Lima bean plants (*Phaseolus lunatus*) through the transpiration stream,^[12] subsequent monitoring of the surrounding gas phase in a continuous flow system by photoacoustic spectroscopy^[13, 14] revealed the emission of ethylene as one of the plant's earliest responses to the peptaibol (Figure 1a). The emission of ethylene started approximately 3 h after the onset of the stimulus, reached a transient maximum after about 7.5 h and leveled off during the following 5 h; this response resembles the profile of ethylene emission after induction with the proteinaceous elicitor cellulysin^[14] or with jasmonic acid.^[15] Continued monitoring of the gas phase by absorption of emitted volatile compounds onto activated carbon in a closed system,^[12] followed by desorption and mass spectrometric analysis of the trapped compounds, demonstrated that the biosynthesis of certain terpenoids and aromatic components had also been increased by the treatment with ALA. The gas chromatogram (Figure 1b) shows that DMNT (5%) and methyl salicylate (MeSA) (4%) are the main compounds formed after TMTT (91%). Interestingly, the same profile of terpenoids can be induced by treatment of Lima bean leaves with 12-oxophytodienoic acid (12-OPDA),^[12] an early biosynthetic precursor of jasmonic acid (JA). JA itself, provokes a more complex pattern of volatile compounds when applied to Lima bean leaves.^[15] The induction of volatile compound biosynthesis by ALA was dose dependent with a threshold concentration in the range of approximately 0.5 μ M.

Owing to the presence of MeSA in the gas phase and the induction of biosynthesis of terpenoids, which represent a

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systemin.

Table 1. Peptaibols and peptides as elicitors of volatile compound production.

Peptaibols and peptides ^[12]	volatile compound bio-synthesis (in <i>P. lunatus</i>)
alamethicin F ^{[7] [a]} Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol	+
ampullosporin A ^[8] Ac-Trp-Ala-Aib-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Gln-Leu-Aib-Gln-Leuol	+
bergofungins ^[25] A: Ac-Val-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-iVal-Hyp-Aib-Pheol B: Ac-Val-Aib-Aib-Aib-Val-Gly-Leu-Val-Aib-Hyp-Gln-iVal-Hyp-Aib-Pheol C: Ac-Val-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-Aib-Hyp-Aib-Pheol	+
chrysospermin A ^{[9] [b]} Ac-Phe-Aib-Ser-Aib-Aib-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol	+
melittin ^[21] Gln-Gln-Arg-Lys-Arg-Lys-Ile-Try-Ser-Ile-Leu-Ala-Pro-Leu-Gly-Thr-Thr-Leu-Val-Lys-Leu-Val-Ala-Gly-Ile-Gly	–
valinomycin ^[22] Cyclo[-L-Val-D-Hylva-D-Val-L-Lac] ₃	–
bradykinin ^[22] Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Argol	–
systemin ^[24] Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp	–
substance P ^[23] Met-Leu-Gly-Phe-Phe-Gln-Gln-Pro-Lys-Pro-Arg	–

[a] Commercial alamethicin is a mixture of homologous peptides.^[7, 12] [b] A mixture of chrysospermins A–D was applied. Aib = 2-Amino-2-methylpropionic acid, Argol = arginanol, Hyp = hydroxyproline, Hylva = α -hydroxyisovaleric acid, Lac = lactic acid, Leuol = leucinol, Pheol = phenylalaninol, Trpol = tryptophanol. Amino acids have L-configuration unless specified otherwise.

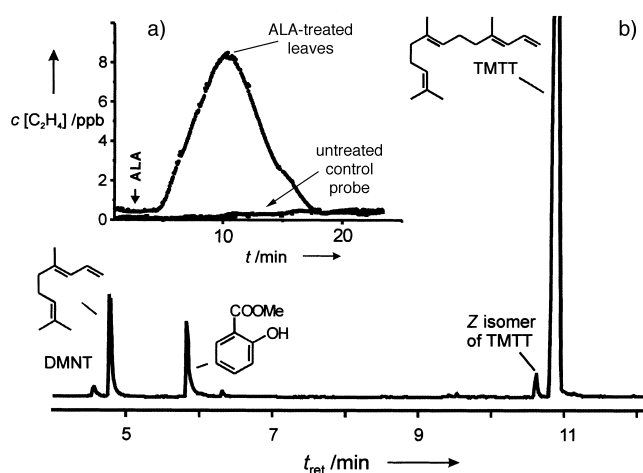


Figure 1. Profile of emitted volatile compounds from Lima bean leaves (*P. lunatus*) after treatment with alamethicin (ALA). Ethylene was determined in a continuous flow system by photoacoustic spectroscopy.^[13] a) Time course of ethylene emission. b) Gas chromatographic profile of other volatile compounds. The intensity of the signals is enhanced to show minor compounds. Quantitative composition of the blend: 4,11-dimethylnona-1,3,7-triene (DMNT), 5%; methyl salicylate (MeSA), 4%; 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), 91%. No volatile compounds were released from the untreated control plants.

typical JA-inducible class of volatile compounds, we next quantified the endogenous levels of SA and JA in ALA-treated leaves.^[16] As shown in Figure 2, both signaling pathways were activated. The typical early and transient production of JA (approximate 20-fold increase within 80 min), together with a steady and massive production of SA which began after 2 h and reached a plateau after 6 h (approximate 90-fold increase) was observed. Control plants, without ALA treatment, showed no volatile-compound production and did not exhibit raised levels of endogenous SA or JA. If the leaves

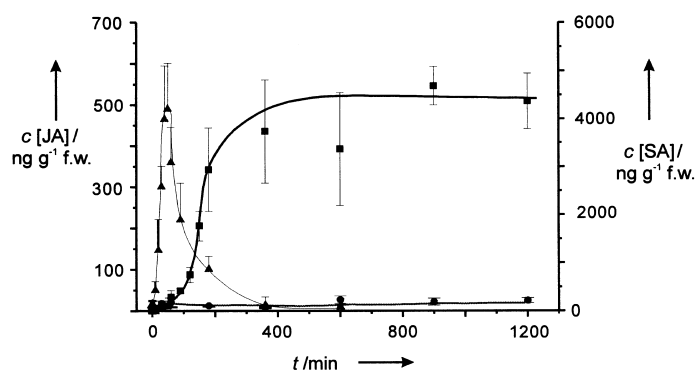


Figure 2. Quantity of endogenous jasmonate and salicylate produced over time in Lima bean leaves after treatment with ALA. ■ = Salicylate (SA), ▲ = jasmonate (JA), ● = SA or JA in untreated leaves (f.w. = fresh weight).

were treated with inhibitors of the octadecanoid pathway (such as phenidone^[17] or aristolochic acid^[18]) prior to induction with ALA, no volatile compounds were emitted. Since inhibitor-treated plants can start to produce such compounds again after exogenous application of JA, the involvement of the octadecanoid signaling pathway was unambiguously corroborated.

The biological activity of ALA is not restricted to Lima bean plants, but appears to be more general. The phylogenetic archetype fern (*Dryopteris filix-mas*) responds with a very pronounced emission of a complex pattern of sesquiterpenoids.^[15] The same class of compounds dominates in the spectrum of ALA-treated Mung bean (*Vigna radiata*), cotton (*Gossypium hirsutum*), or maize (*Zea mays*) plants.^[2, 15] The garden bean (*Phaseolus vulgaris*) showed a profile comparable to that of the Lima bean (similar to Figure 1). Soy bean (*Glycine max*) and garden pea (*Pisum sativum*) plants failed to produce volatile compounds.

The increase of JA production in the Lima bean plants prompted us to test ALA in tendrils-coiling experiments. Tendrils of *Bryonia dioica* respond to JA, MeJA, and 12-OPDA with a coiling reaction comparable to the free coiling reaction of mechanically stimulated tendrils.^[19] Tendrils of *Bryonia dioica*, *Pisum sativum*, and *Lathyrus* sp. were treated with solutions of ALA (5 μM) through the transpiration stream and, after 20 h, the degree of coiling was measured. Alternatively, tendrils were subjected to the coiling assay described in the literature.^[20] All tested plant species exhibited a rapid coiling response towards ALA. Surprisingly, inhibition of the octadecanoid cascade with phenidone did not hamper the coiling reaction, which indicates that signaling systems independent of the lipid-based pathway, such as ion fluxes, have to be considered as elements of mechanotransduction.^[20]

Besides ALA, many other peptaibols and peptides endowed with pore-forming or ion-transporting properties are known. To establish whether pore formation within a membrane or ion transport through a membrane is the underlying principle for the induction of volatile compound biosynthesis, other pore-forming peptaibols and some typical ion transporters were tested. The results are compiled in Table 1. The entire group of peptaibols induced the same pattern of volatile compounds in Lima bean leaves, suggesting a common mode of action. Although the basic peptide melittin is known as a pore-forming compound,^[21] it failed to induce volatile biosynthesis in Lima bean leaves. A typical ion transporter, K^+ selective valinomycin^[22] (9 μM), was also found to be inactive. Other biologically active small peptides which act through specific receptors, such as the undecapeptide "substance P",^[23] the nonapeptide bradykinin,^[22] and systemin, a signal peptide of tomato plants,^[24] did not induce volatile compound biosynthesis, supporting the pore-forming capability of peptaibols as the essential property for the elicitation process.

Channel-forming peptides, such as alamethicin, may be used as valuable tools to unravel the early events of plant defense under well defined conditions. First analyses of insect salivary secretions have already demonstrated the presence of pore-forming compounds,^[26] suggesting that membrane depolarisation also has to be considered as an important element of insect-induced plant defense.

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- [1] M. G. Hahn, *Annu. Rev. Plant Pathol.* **1996**, *34*, 387–412.
- [2] P. W. Paré, J. H. Tumlinson, *Plant Physiol.* **1999**, *121*, 325–331.
- [3] T. Jabs, M. Tschöpe, C. Colling, K. Hahlbrock, D. Scheel, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4800–4805.
- [4] J. L. Dangel, K. D. Hauße, S. Lipphardt, K. Hahlbrock, D. Scheel, *EMBO J.* **1987**, *6*, 2551–2556.
- [5] Y. Matthieu, A. Kurkdjian, H. Xia, J. Guern, A. Koller, M. D. Spiro, M. O'Neill, P. Albersheim, A. Darvill, *Plant J.* **1991**, *1*, 333–343.
- [6] B. Klüsener, E. W. Weiler, *FEBS Lett.* **1999**, *459*, 263–266.
- [7] D. S. Cafiso, *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 141–165.
- [8] M. Ritzau, S. Heinze, K.-J. Dornberger, A. Berg, W. Fleck, B. Schlegel, A. Härtl, U. Gräfe, *J. Antibiot.* **1997**, *50*, 722–728.
- [9] K.-J. Dornberger, W. Ihn, M. Ritzau, U. Gräfe, B. Schlegel, W. F. Fleck, *J. Antibiot.* **1995**, *48*, 977–989.
- [10] R. C. Pandey, J. C. Meng, C. Cook, R. L. Rinehardt, *J. Am. Chem. Soc.* **1977**, *99*, 8469–8483.

- [11] M. S. P. Sansom, *Quart. Rev. Biophys.* **1993**, *26*, 365–321.
- [12] Experimental details concerning plant cultivation, application of test compounds, volatile compound collection, and mass spectrometric analysis are described in: T. Koch, T. Krumm, V. Jung, J. Engelberth, W. Boland, *Plant Physiol.* **1999**, *121*, 153–162. Alamethicin, melittin, and valinomycin were purchased from Sigma–Aldrich. Bradykinin and substance P were from Calbiochem. All test compounds were applied at a concentration of 10 $\mu\text{g mL}^{-1}$.
- [13] B. Beßler, S. Schmitgen, F. Kühnemann, R. Gäbler, W. Urban, *Planta* **1998**, *205*, 140–144.
- [14] J. Piel, R. Atzorn, R. Gäbler, F. Kühnemann, W. Boland, *FEBS Lett.* **1997**, *416*, 143–148.
- [15] W. Boland, J. Hopke, J. Donath, F. Nüske, F. Bublitz, *Angew. Chem.* **1995**, *107*, 1715–1717; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1600–1602.
- [16] J. Engelberth, T. Koch, N. Bachmann, J. Rechtenbach, W. Boland, *Plant Physiol.* **1999**, in press.
- [17] C. Cucurou, J. P. Battioni, D. C. Thang, N. H. Nam, D. Mansuy, *Biochemistry* **1991**, *30*, 8964–8970.
- [18] M. D. Rosenthal, B. S. Vishwanath, R. C. Franson, *Biochim. Biophys. Acta* **1989**, *1001*, 1–8.
- [19] E. W. Weiler, T. Albrecht, B. Groth, Z.-Q. Xia, M. Luxem, H. Liss, L. Andert, P. Spengler, *Phytochemistry* **1993**, *32*, 591–600.
- [20] B. Klüsener, G. Boheim, H. Liss, J. Engelberth, E. W. Weiler, *EMBO J.* **1995**, *14*, 2708–2714.
- [21] A. W. Bernheimer, B. Rudy, *Biochim. Biophys. Acta* **1986**, *864*, 123–141.
- [22] D. W. Urry, *Top. Curr. Chem.* **1985**, *128*, 175–218.
- [23] M. M. Klavdieva, *Front. Neuroendocrinol.* **1996**, *17*, 155–179.
- [24] A. Schaller, C. A. Ryan, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11 802–11 806.
- [25] A. Berg, B. Schlegel, W. Ihn, U. Demuth, U. Gräfe, *J. Antibiotics* **1999**, *52*, 666–669.
- [26] D. Spittler, V. Jung, W. Boland, unpublished results.

Sol–Gel Polycondensation of Tetraethoxysilane in a Cholesterol-Based Organogel System Results in Chiral Spiral Silica

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Exploitation of new organic gelators that can gelate various organic solvents has become an active area of research.^[1–11] These organogels are of particular interest because they are different from polymer gels. Fibrous aggregates of low molecular weight compounds formed by noncovalent interactions are responsible for such gelation phenomena. Hence, the xerogels exhibit various superstructures, reflecting the monomeric structure of each gelator. This is why the study of organogels is considered to be a new field of supramolecular chemistry.^[11]

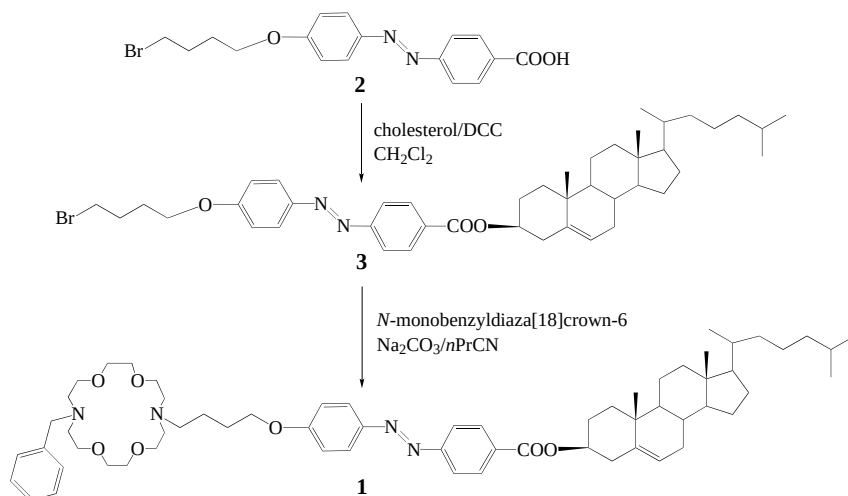
Recently, it was found that certain cholesterol derivatives can gelate even tetraethoxysilane (TEOS), which results in

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silica by sol–gel polycondensation.^[12, 13] Sol–gel polycondensation of gelated TEOS solutions affords novel silica with a hollow fiber structure, because the organogel fibers act as a template to create an inner tube in the TEOS polycondensation process.^[12, 13] One may therefore consider that the unique superstructures of organogel fibers can be transcribed in the silica structure: Superstructures of organic aggregates temporarily formed by noncovalent interactions can be permanently fixed in inorganic silica. It was later proven that the presence of the positive charge in the organogel fibers is indispensable for transcribing the organogel superstructure into the silica structure.^[13] This prerequisite is rationalized in terms of an electrostatic interaction between oligomeric “anionic” silica and “cationic” organogel fibers during the sol–gel polycondensation process.

Organogel fibers formed from cholesterol-based gelators frequently result in a helical structure.^[3, 7] This fact tempted us to transcribe the helical organogel fiber structure into the inorganic silica. We have noticed, however, that the presence of a cationic group tends to reduce the gelation ability and that these systems rarely result in the formation of a helical structure.^[12] An idea to overcome this dilemma came to mind: The positive charge can be readily incorporated by a metal–crown interaction. We therefore designed compound **1** (see Scheme 1), which has a cholesterol skeleton as a chiral aggregate-forming site and an azacrown moiety as a metal binding site.^[14] We found that **1** not only gels various organic solvents, but in the presence of metal cations also acts as a template for sol–gel polycondensation of TEOS to produce the novel “chiral spiral silica”. To the best of our knowledge, this is a very rare example of “chirality” being induced in the inorganic silica by the organogel template method.^[15]

Compound **1** (m.p. 113.3–115.2 °C) was synthesized according to Scheme 1 and identified by IR and ¹H NMR spectroscopy, mass spectrometry, and elemental analysis. The gelation ability was estimated for 17 different organic solvents containing **1** ($5.00 \times 10^{-2} \text{ mol L}^{-1}$).^[16] As summarized in Table 1, **1** can gelate 8 out of the 17 solvents, and therefore acts as a versatile gelator of organic solvents. The gelation ability for alcohols in general is excellent.



Scheme 1. Synthesis of **1**. DCC = dicyclohexyl carbodiimide.

Table 1. The gelation ability^[a] of **1** in organic solvents.

Solvent	Result ^[b]	Solvent	Result ^[b]
methanol	I	acetone	I
ethanol	I	acetonitrile	I
1-propanol	G	cyclohexane	PG
1-butanol	G	methylcyclohexane	PG/R
<i>tert</i> -butyl alcohol	G	acetic anhydride	G
isobutanol	G	acetic acid	S
1-octanol	G	dichloromethane	S
dimethyl sulfoxide	G		
1,2-diaminoethane	PG		
1,3-diaminopropane	G		

[a] The concentration of the gelator was 5.0 wt %. [b] G = stable gel formed at room temperature; I = insoluble; PG = partially gelatinized; R = recrystallization; S = solution.

To characterize the chirality of the organogel, the circular dichroism (CD) spectrum of the organogel formed by **1** in the presence and absence of AgNO₃ or CsClO₄ was measured (Figure 1). Since the λ values for $\theta = 0$ (θ = ellipticity) in the

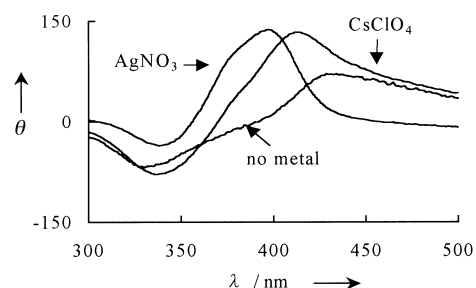


Figure 1. CD spectra of **1** ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) in 1-butanol in the absence and presence of CsClO₄ ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) or AgNO₃ ($5.00 \times 10^{-2} \text{ mol L}^{-1}$; concentration in the gel phase). The concentration of **1** required to gelate the 1-butanol solution was so high that the CD spectra could not be measured with conventional optical cells. They were obtained by sandwiching the gel between two glass plates to make a thin layer. Hence, the ellipticity θ is given in arbitrary units.

CD spectra (365 and 358 nm in the presence of CsClO₄ ($3.15 \times 10^{-3} \text{ mol L}^{-1}$) and AgNO₃ ($3.15 \times 10^{-3} \text{ mol L}^{-1}$), respectively) are very close to the λ_{max} of the absorption spectrum (360 nm), one can regard these bands as positive exciton-coupling bands: Compound **1** forms aggregates and the dipoles in the chromophoric azobenzene moieties are orientated in a clockwise direction (i.e., with an *R* configuration). Although the gel formed in the absence of a metal salt was CD-active, the spectrum did not show a clear exciton-coupling band.

The scanning electron microscopy (SEM) pictures of the xerogels obtained by a freeze-and-pump method from gel-phase solutions of **1** in *tert*-butyl alcohol below the sol–gel phase-transition temperature ($[\mathbf{1}] = 5.00 \times 10^{-2} \text{ mol L}^{-1}$) are shown in Figure 2.^[17] In the absence of a metal salt, the major structures are film-like lamella, some of which show a pseudocylindrical structure (Figure 2A).

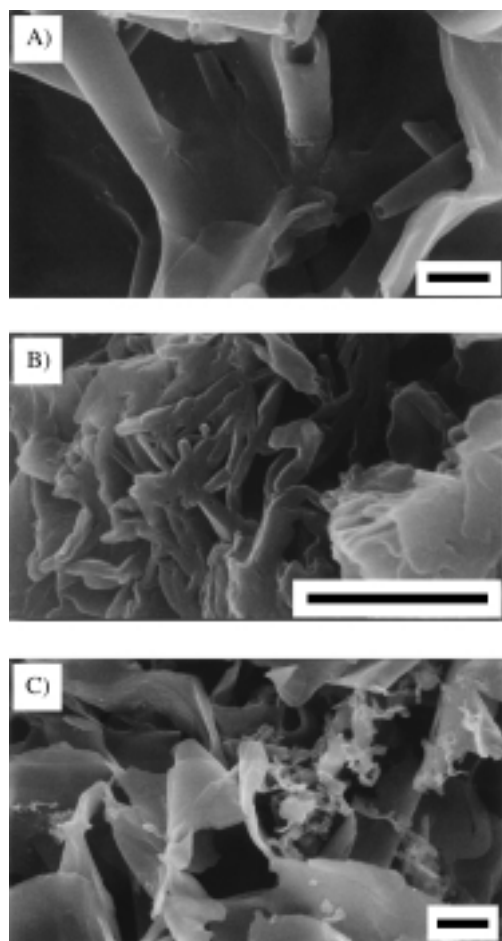


Figure 2. SEM pictures of the xerogels obtained from the gel-phase solution of **1** ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) in *tert*-butyl alcohol A) in the absence of and B) in the presence of AgNO_3 ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) or C) CsClO_4 ($5.00 \times 10^{-2} \text{ mol L}^{-1}$). The gel was first cooled in liquid nitrogen and then the solvent was removed under vacuum at 15°C . In each case the bar corresponds to $1.5 \mu\text{m}$.

In the presence of AgNO_3 or CsClO_4 , on the other hand, both the fibrous structure and the lamellar structure can be recognized (Figure 2B and C, respectively). These results, together with the CD spectra, suggest that the exciton-coupling band originates from the chirality present in the fibrous structure.

Sol–gel polycondensation of TEOS was carried out in the gel phase of **1** in the absence and the presence of AgNO_3 or CsClO_4 . A typical mixture consists of 1-butanol (98 mg), TEOS (16 mg), water (5.7 mg), and benzylamine (5.6 mg) as a catalyst. This mixture was left at room temperature for one day. The SEM pictures taken at this stage are shown in Figure 3. The silica obtained in the absence of metal salt showed the nonimpressive granular structure, which is also obtained from conventional TEOS polycondensation in solution (not shown). This result indicates that 1) the presence of the positive charge is indispensable for transcribing the organogel structure into the silica structure^[12, 14] and 2) as shown by the CD spectrum in Figure 1, the aggregates formed in the absence of a metal salts are not so highly orientated that they affect the silica structure.

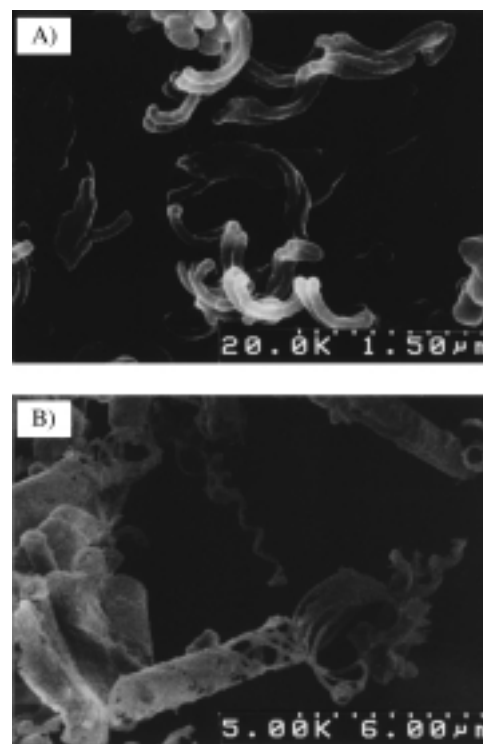


Figure 3. SEM pictures of the spiral silica obtained from **1** ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) in the presence of A) AgNO_3 ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) and B) CsClO_4 ($5.00 \times 10^{-2} \text{ mol L}^{-1}$) before calcination. A similar structure was also observed after calcination, indicating that the organogel fibers are surrounded by the silica.

In contrast, the silica obtained in the presence of AgNO_3 shows a novel spiral structure (Figure 3A). As far as can be recognized, all the spirals possess a right-handed helical motif. Since the exciton-coupling band of the organogel also shows *R* helicity in the presence of AgNO_3 , we consider that microscopic helicity to be reflected by a macroscopic spiral helicity. Figure 3B shows the SEM picture of the silica obtained in the presence of CsClO_4 —again the well-grown spiral structure is seen, which features a right-handed helical motif. In this picture, one can also recognize a filmlike structure, which is presumably constructed by transcription of the lamellar structure of the organogel (which is also seen in Figure 2C). The silica obtained in the presence of NaClO_4 or KClO_4 showed a hollow fiber structure as observed previously,^[12, 14] but the spiral structure was not recognized (not shown).

To further corroborate that the organogel fibers really acted as a template for the growth of spiral silica, transmission electron microscopy (TEM) pictures were taken after removal of **1** by calcination.^[12, 14] We found that the spirals have a tubular structure with an inner diameter of 25–50 nm (Figure 4). These results support the view that anionic oligomeric siloxanes are adsorbed onto metal-containing cationic organogel fibers, and the silica spirals thus grown reflect the chiral factor present in the organogel fibers.

In conclusion, sol–gel polycondensation of TEOS in the gel phase supported by an azacrown-appended cholesterol gelator results in a unique spiral silica with a right-handed helical motif. This is a very rare example of chirality being created in

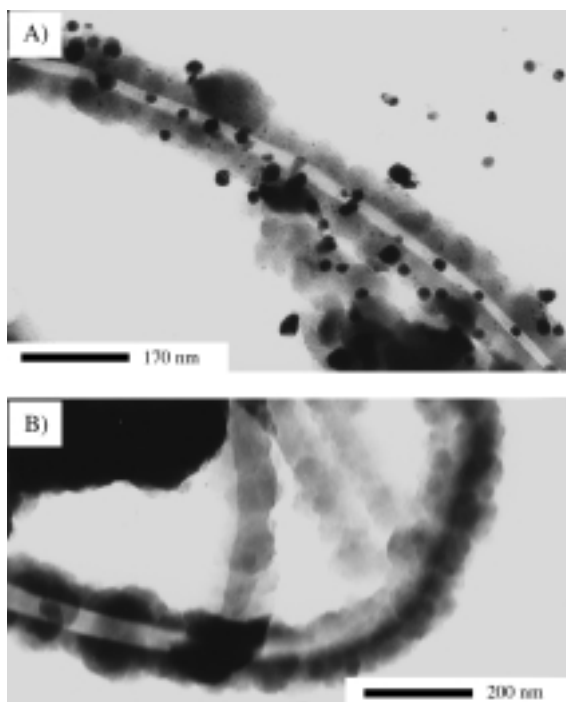


Figure 4. TEM pictures of the spiral silica obtained from **1** in the presence of A) AgNO_3 (the dots are silver particles) and B) CsClO_4 after calcination. The final calcination was carried out at 500°C for 4 h under aerobic conditions. The silica obtained from the experiment in the presence of CsClO_4 was confirmed with a electron probe microanalyzer (Si: 45.45, O: 44.34, Cs: 9.32 wt %).

the inorganic material. The results indicate the versatility of the template method for the creation of various silica structures.^[15]

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- [1] a) E. J. de Vries, R. M. Kellogg, *J. Chem. Soc. Chem. Commun.* **1993**, 238; b) M. de Loos, J. van Esch, I. Stokroos, R. M. Kellogg, B. L. Feringa, *J. Am. Chem. Soc.* **1997**, *119*, 12675.

- [2] M. Aoki, K. Nakasima, H. Kawabata, S. Tsutsui, S. Shinkai, *J. Chem. Soc. Perkin Trans. 2* **1993**, 347.
- [3] a) K. Hanabusa, K. Okui, K. Karaki, T. Koyoma, H. Shirai, *J. Chem. Soc. Chem. Commun.* **1992**, 1371; b) K. Hanabusa, A. Kawakami, M. Kimura, H. Shirai, *Chem. Lett.* **1997**, 191, and references therein; c) K. Hanabusa, M. Yamada, M. Kimura, H. Shirai, *Angew. Chem.* **1996**, *108*, 2086; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1949.
- [4] J.-E. S. Sohna, F. Fages, *Chem. Commun.* **1997**, 327.
- [5] E. Ostun, P. Kamaras, R. G. Weiss, *Angew. Chem.* **1996**, *108*, 1423; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1324, and references therein.
- [6] P. Terech, I. Furman, R. G. Weiss, *J. Phys. Chem.* **1995**, *99*, 9558, and references therein.
- [7] K. Murata, M. Aoki, T. Suzuki, T. Harada, H. Kawabata, T. Komori, F. Ohseto, K. Ueda, S. Shinkai, *J. Am. Chem. Soc.* **1994**, *116*, 6664, and references therein.
- [8] T. D. James, K. Murata, T. Harada, K. Ueda, S. Shinkai, *Chem. Lett.* **1994**, 273.
- [9] S. W. Jeong, K. Murata, S. Shinkai, *Supramol. Sci.* **1996**, *3*, 83.
- [10] T. Brotin, R. Utermohlen, F. Fages, H. Bouas-Laurent, J.-P. Desvergne, *J. Chem. Soc. Chem. Commun.* **1991**, 416.
- [11] Reviews: a) P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, 3133; b) S. Shinkai, K. Murata, *J. Mater. Chem.* **1998**, *8*, 485.
- [12] a) Y. Ono, K. Nakashima, M. Sano, Y. Kanekiyo, K. Inoue, J. Hojo, S. Shinkai, *Chem. Commun.* **1998**, 1477; b) Y. Ono, Y. Kanekiyo, K. Inoue, J. Hojo, S. Shinkai, *Chem. Lett.* **1999**, 23.
- [13] a) P. Yang, D. Zhao, D. I. Margolese, B. F. Chmelka, G. D. Stucky, *Nature* **1998**, *396*, 152; b) J. Y. Ying, C. P. Mehnert, M. S. Wong, *Angew. Chem.* **1999**, *111*, 58; *Angew. Chem. Int. Ed.* **1999**, *38*, 56; c) E. Lindner, T. Schneller, F. Auer, H. A. Mayer, *Angew. Chem.* **1999**, *111*, 2288; *Angew. Chem. Int. Ed.* **1999**, *38*, 2154; d) J. S. Beck, J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T.-W. Chu, D. H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, J. L. Schlenker, *J. Am. Chem. Soc.* **1992**, *114*, 10834.
- [14] J. H. Jung, Y. Ono, S. Shinkai, *J. Chem. Soc. Perkin Trans. 2* **1999**, 1289.
- [15] Although several inorganic materials with a helical structure have been reported, none were prepared by the chiral template method presented here. a) K. Robbie, M. J. Brett, A. Lakhtakia, *J. Vac. Sci. Technol. A* **1995**, *13*, 2991; b) Y. Akiyama, F. Mizukami, Y. Kiyozumi, K. Maeda, H. Izutsu, K. Sakaguchi, *Angew. Chem. Int. Ed.* **1999**, *111*, 1510; *Angew. Chem. Int. Ed.* **1999**, *38*, 1420; c) H. Yang, N. Coombs, G. A. Ozin, *Nature* **1997**, *386*, 692.
- [16] For experimental details of the gelation test and the xerogel preparation, see ref. [7].
- [17] Because a freeze-and-pump method was used to prepare xerogels suitable for SEM observation, the xerogel was obtained from a solution in *tert*-butyl alcohol (m.p. 25.7°C) instead of 1-butanol (m.p. -90°C).

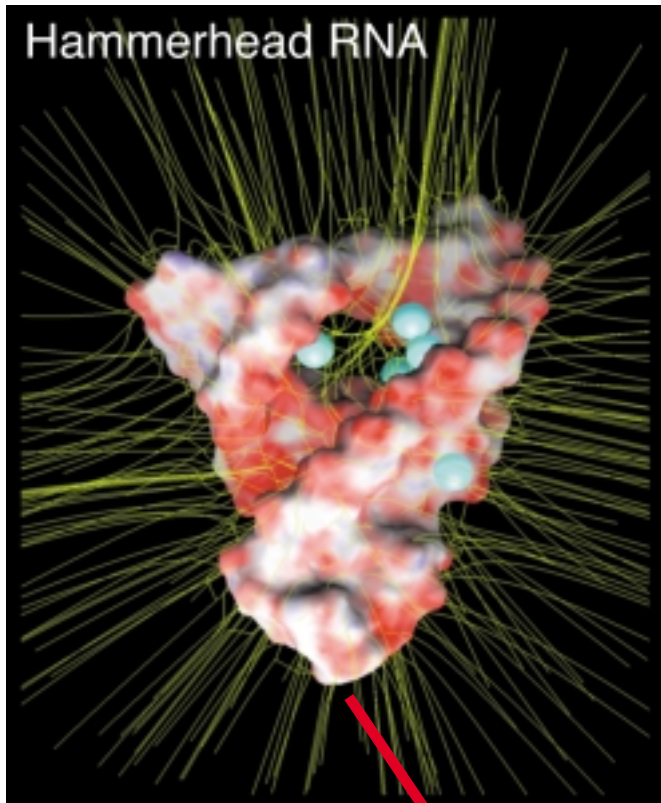
Cover Picture

Michael Winkler and Wolfram Sander

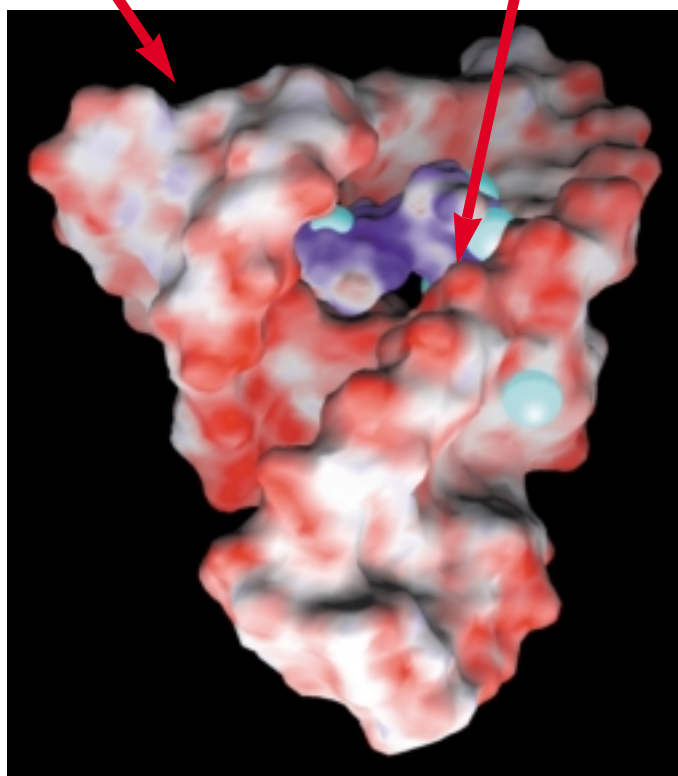
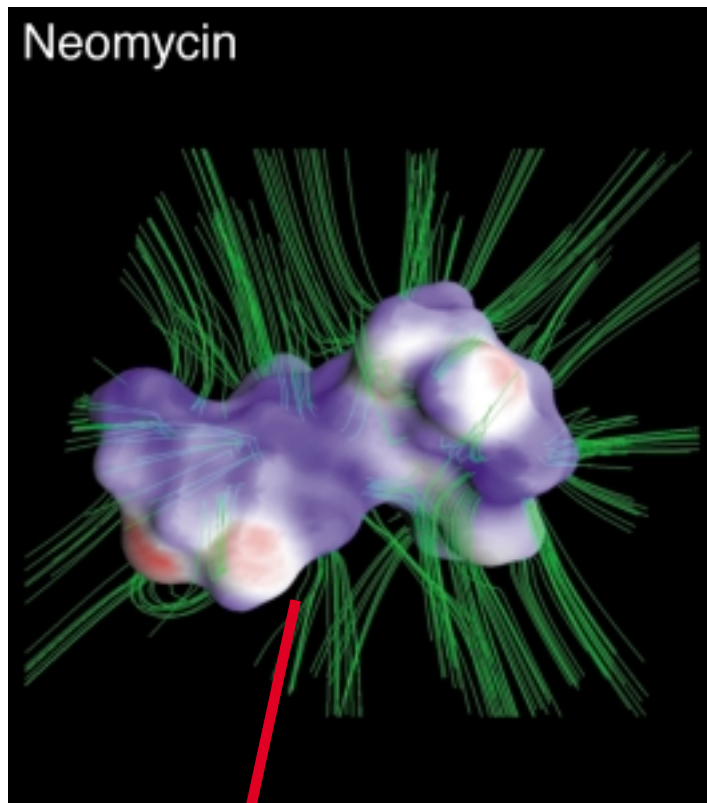
The cover picture shows schematically the formation of the phenyl cation by photoionization of halobenzenes and its further reaction with nitrogen to give the benzene diazonium ion. The phenyl cation, as well as its reaction products, could be isolated in argon matrices and characterized by IR spectroscopy. The upper part of the picture shows the argon resonance lamp used for the photoionization of the halobenzenes. The structures of the molecules shown were obtained by DFT calculations. Further details of the matrix isolation of the phenyl cation are described by M. Winkler and W. Sander on page 2014.



Hammerhead RNA



Neomycin



Electrostatic
Complementarity

Strategies for the Design of Drugs Targeting RNA and RNA – Protein Complexes

Thomas Hermann*

In many steps of gene replication and expression, RNA molecules participate as key players, which renders them attractive targets for therapeutic intervention. While the function of nucleic acids as carriers of genetic material is based on their sequence, a number of important RNAs are involved in processes that depend on the defined three-dimensional structures of these molecules. As for proteins, numerous complex folds of RNA exist. The development of drugs that bind specifically to RNA folds opens exciting new ways to expand greatly the existing repertoire of protein-targeted

therapeutics. Most functions of RNAs involve interactions with proteins that contain RNA-binding domains. Effector molecules targeted at RNA may either alter the functional three-dimensional structure of the nucleic acid, so the interaction with proteins is thereby inhibited or enhanced, or, as interface inhibitors, they may directly prevent the formation of competent RNA – protein complexes. While the same tools used for the design of protein-targeted drugs may be considered for studying effectors binding to nucleic acids, the differences between proteins and RNAs in the forces which

dominate their three-dimensional folding call for novel drug design strategies. In the present review, I will outline how our rapidly expanding knowledge of RNA three-dimensional structure and function facilitates rational approaches to develop RNA-binding compounds. Putative RNA targets for therapeutic intervention will be discussed along with recent advances in understanding RNA – small molecule and RNA – protein interactions.

Keywords: aminoglycosides • antibiotics • drug research • RNA recognition • RNA structures

1. Introduction

The large number of steps in gene replication and expression which involve RNA molecules in pivotal roles might reflect the descendance of the present-day organisms from an ancestral “RNA world” in which both storage and replication of the genetic information were accomplished solely by RNA.^[1] Whether such a nucleic acid based prelude to extant life existed or not, the wealth of different contemporary RNA folds justifies another notion of a “world of RNA molecules”. Despite the key roles it plays in biological processes, RNA has been considered a drug target only recently.^[2] In addition to its functional importance, RNA offers further advantages of an attractive drug target, namely its accessibility in ribonucleoprotein complexes, the absence of cellular RNA repair mechanisms, and the diversity of three-dimensional folds^[3] to allow the selective binding of effector molecules. Technical progress in RNA synthesis and structure

determination is fueling a rapidly expanding knowledge about RNA three-dimensional structure and its roles for biological functions and is paving the way for rational design of therapeutic compounds which bind specifically to RNA folds.

Here, I will attempt a survey of current efforts to exploit RNA as a drug target. In Section 2, I will give an overview of the many roles RNAs play in biological reactions. Emphasis will be on identifying RNA molecules as potential targets in pathological processes. Basic principles of RNA recognition by peptides and proteins will be discussed in Section 3. Finally, in Section 4 I will describe how our current knowledge on RNA recognition by small molecules may foster the development of techniques for the structure-based design of drugs targeted at RNAs.

2. Roles of RNAs in Biological Processes

Gene replication and expression are probably the most ancient cellular processes involving many different RNAs (Figure 1). While following the flow of genetic information down from DNA through messenger RNA (mRNA) to proteins, I will briefly describe the role of RNA molecules

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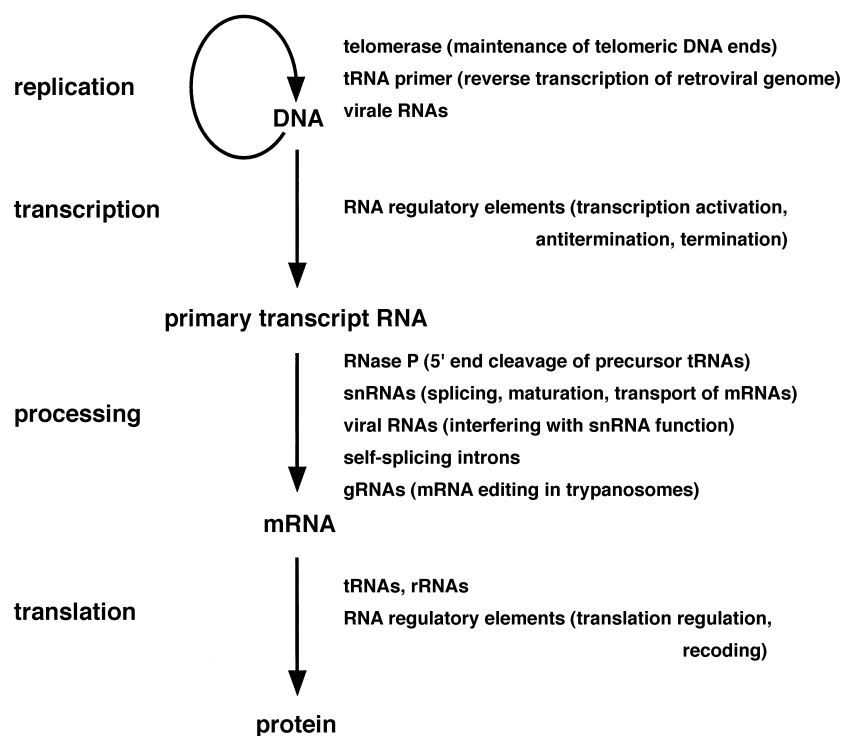


Figure 1. Schematic overview of the participation of RNAs in some of the most important processes of gene replication and expression. No distinction is made between normal and pathological processes. For further details, see text. gRNA = guide RNA, rRNA = ribosomal RNA, snRNA = small nuclear RNA, tRNA = transfer RNA.

in steps which are either obligatory for viability or specific to pathological conditions. The rapidly expanding and extremely exciting field of epigenetic mechanisms of gene regulation^[4], which involves RNA molecules as central triggers of gene silencing and chromosomal modification, has been omitted for space limits. This overview is not meant to be an exhaustive or rigorous summary of the roles RNA molecules play in cells but merely a selected collection of highlights.

2.1. Replication

At their termini, eucaryotic chromosomes contain stretches of tandemly repeated species-specific DNA sequences, termed telomeres, which are required for continued main-

tenance of chromosome length during replication.^[5] Telomeric DNA is synthesized by the ribonucleoprotein enzyme telomerase which uses its RNA component as an internal template for terminal addition of deoxynucleotides.^[6] In germ cells and the early-stage embryo of vertebrates, telomerase is active but it is shut off in somatic cells. In the majority of human cancers, telomerase activity is upregulated, implying that malignancy may be linked to telomerase-dependent immortalization. Inhibition of telomerase has, thus, been suggested as a strategy for anticancer drug therapy.^[7] The RNA component of human telomerase comprising approximately 450 nucleotides (nt) has been targeted with RNA-cleaving ribozymes^[8] and antisense nucleic acids.^[9] Either modified nucleic acids^[10] or highly selective small molecule inhibitors binding to the telomerase RNA–protein complex will be needed to overcome the limitations of ribozyme and antisense approaches in vivo and exploit the ribonucleoprotein as a target for chemotherapy.^[11] Recent finding of telomerase activity in kinetoplastid parasitic protozoa such as *Trypanosoma* and *Leishmania* has led to the suggestion that drugs which interfere with telomerase might

be considered as a new therapeutic strategy for the treatment of parasitic diseases.^[12]

In retrovirus infection, the pathogen-encoded enzyme reverse transcriptase (RT) converts the viral single-stranded RNA genome into double-stranded DNA for insertion into the eucaryal host chromosome.^[13] Initiation of reverse transcription is primed by a cellular transfer RNA (tRNA) which hybridizes to the primer binding site (PBS) of the viral RNA.^[14] In several retroviruses, such as the type 1 human immunodeficiency virus (HIV-1), additional interactions between the primer tRNA and the viral genomic RNA enhance the specificity of the initiation of reverse transcription.^[15] Based on data from mutational and probing experiments, a model of the initiation complex, formed between primer tRNA₃^{Lys} and HIV-1 RNA bound to the RT, has been constructed which suggests an intricate three-



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dimensional fold^[16] which serves as a putative target for RNA-binding drugs. Structural details of the contacts between nucleic acid substrates and HIV-1 RT in the transcription complex have also been elucidated by crystal structure analyses.^[17]

Another intermolecular RNA–RNA interaction playing an important role in the HIV-1 retroviral cycle is the dimer formation between two copies of the viral genome.^[18] Dimerization is initiated by an RNA loop–loop “kissing” interaction^[19] which, *in vivo*, is required for both efficient replication and encapsulation of the virus.^[20] Models for the three-dimensional structure of the hairpin dimer initiation complex have been obtained by solution techniques.^[21] Both a solution and a crystal structure of the mature complex are available.^[22]

A kissing interaction between two RNAs is also responsible for a mechanism of plasmid copy number control widespread in eubacteria. Plasmids are not essential for the survival of bacteria, but they encode a wide variety of genetic determinants of medical importance, including antibiotic resistance and specific virulence traits.^[23] In antisense control of plasmid replication, two plasmid-encoded complementary RNAs form an initial contact through a loop–loop kissing complex^[24] which could be targeted by small molecules. In some eubacterial plasmids, including *Escherichia coli* ColE1, sense–antisense RNA pairing is dependent on an RNA-binding protein, Rop in ColE1, which facilitates the annealing process.^[25]

The 1700 nt RNA genome of hepatitis delta virus (HDV), a small pathogenic RNA satellite virus of the hepatitis B virus, is replicated in a rolling-circle mechanism which gives rise to multimeric genome copies. Catalytic RNA motifs within both the genomic and antigenomic RNAs, the HDV ribozymes, process the multimers to generate linear monomers of the viral genome.^[26] HDV ribozymes are inhibited by aminoglycoside antibiotics^[27] which bind specifically to the RNA fold. While the three-dimensional structure of an HDV ribozyme has been determined by crystal structure analysis,^[28] the exact binding position of aminoglycosides is, as yet, unknown. Aminoglycosides also inhibit the self-cleavage of the hammerhead ribozymes,^[29, 30] small catalytic RNAs involved in the replication of plant pathogenic viroids.^[31] RNA self-cleavage in both HDV and hammerhead ribozymes proceeds by metal ion dependent mechanisms.^[32] Displacement of catalytic metal ions by electrostatically complementary cationic compounds has been suggested as the general principle of aminoglycoside action on these ribozymes.^[33, 34]

2.2. Transcription

During transcription, the first step in gene expression, RNA polymerase synthesizes an RNA copy of the DNA template. Transcriptional initiation, RNA chain elongation, and termination are tightly controlled both in eubacteria^[35] and eucaryotes.^[36] RNA structures mediate several of the regulatory mechanisms,^[37] such as transcription termination by the eubacterial mRNA-binding Rho factor,^[38] the tRNA-dependent transcription antitermination in eubacteria^[39] and, impor-

tantly, the *trans* activation of viral genome transcription in HIV and related retroviruses.^[40]

The *trans*-activating region (TAR) near the 5′ terminus of HIV mRNA forms a hairpin structure that binds the viral Tat protein. The contact between the Tat protein and the cellular cyclin-dependent kinases, which phosphorylate the C terminal domain of RNA-polymerase II, enhances both the initiation and the processivity of viral mRNA synthesis.^[41] Since Tat–TAR interaction is absolutely essential for viral replication, in the search for anti-HIV drugs, considerable effort has concentrated on identifying and developing compounds which interfere with the formation and function of this protein–RNA complex.^[42–50] Small molecule inhibitors of the Tat–TAR interaction have been obtained by rational design,^[45] combinatorial synthesis,^[43] high-throughput screening (HTS) assays,^[44, 46] and a combination of these approaches.^[47] Structure-based design of ligands specific for TAR^[45, 50] will be greatly facilitated by the availability of three-dimensional structures of the free RNA,^[51] the RNA complexed with argininamide,^[52] and peptides derived from the Tat protein.^[53]

Interestingly, it has been found that in the red clover necrotic mosaic virus (RCNMV), transcriptional *trans* activation is mediated not by a protein but by an RNA specifically binding to a TAR-like element.^[54] While it is as yet unknown whether similar systems exist in other organisms, the RCNMV RNA-mediated *trans* activation provides another example in which an RNA accomplishes a function which, in many cases, depends on a protein.

Recently it has been reported that the HIV TAR structure is also required for another crucial process in the viral replication cycle^[55] namely the RNA encapsulation into viral particles.^[56] The protein partner interacting with TAR for the packaging activity has not yet been identified. Encapsulation requires additional protein–RNA interactions, namely those between the viral nucleocapsid protein (NC) and the ψ signal sequence, which consists of three viral mRNA hairpins.^[57] Structure-based design of inhibitors of the NC–RNA interaction could be facilitated by the availability of the three-dimensional solution structure of HIV-1 NC bound to the SL3 hairpin of ψ RNA which has been determined by NMR spectroscopy.^[58]

2.3. Processing, Transport, and Localization

Primary RNA transcripts undergo a variety of different processing and localization events, depending on the type of RNA (mRNA, rRNA, tRNA, etc.) and the organism. In all contemporary cells, functional tRNAs are tailored by the ribonucleoprotein enzyme RNase P cleaving the 5′ terminus from longer precursor sequences.^[59] RNase P is composed of one (*Eubacteria*) or several (*Eucarya* and *Archaea*) protein subunits and one RNA component of 300–400 nt which, in all cases, contains the catalytic center of the endonuclease activity. The RNA component of eubacterial RNase P can catalyze the cleavage reaction in the absence of the protein subunit. The RNAs of eucaryotic and archaeal RNase P, however, require the protein constituents for activity. RNase P from eubacteria and eucaryotes, thus, may be

different enough to allow the development of compounds which specifically interfere with the eubacterial enzyme. Aminoglycosides and the nucleoside derivative puromycin have been reported to inhibit RNase P of *Escherichia coli*^[60] and the ciliate protozoan *Tetrahymena thermophila*,^[61] by interaction with the RNA component. Analogues of vitamin A (retinoids) act as inhibitors of RNase P of *Dictyostelium discoideum*, however, the binding site of the compounds is not known.^[62] Refined three-dimensional models for the RNA component of eubacterial RNase P are available^[63] which were derived from comparative phylogenetic analyses and biochemical data.

mRNA for export to the cytoplasm is generated by a processing machinery involving small nuclear ribonucleoproteins (snRNPs) in the nucleoplasm, whereas ribosomal rRNAs (rRNAs) are matured and assembled by a different set of snRNPs, the snoRNPs (small nucleolar ribonucleoproteins), in the nucleolus.^[64] Vertebrate cells contain around 200 different snRNPs consisting of one or several protein subunits and an RNA component (snRNA) of 60–300 nt. The most prominent function of snRNPs is splicing, a process which involves many snRNAs assembled into large “spliceosomes” which remove introns from primary transcripts to yield mature mRNA.^[65] While information about the extent to which snRNAs contribute to catalytic steps in processing is currently scarce, they participate in many specific protein–RNA and RNA–RNA interactions. The splicing apparatus of parasites has been suggested as a target for drugs against fungal infections^[66] and trypanosomes.^[67] Information on molecular details of protein–RNA interactions in snRNPs is becoming accessible as several three-dimensional structures of snRNP proteins and protein–RNA complexes have been recently solved by crystallography and NMR spectroscopy.^[68]

A number of viruses encode the small RNAs that associate with protein components of host cell snRNPs and, so, form viral snRNPs^[64] which might play roles in the infection cycle by manipulating the host RNA processing machinery. Such snRNP-forming RNAs have been found, among others, in infection of human B lymphocytes with Epstein–Barr virus (EBV),^[69] in infections of tumor and lymphoma cells with Kaposi sarcoma-associated herpes virus (KSHV),^[70] and as components of the viral replication complexes of simian hemorrhagic fever virus (SHFV).^[71]

Splicing and transport of HIV mRNA is controlled by an interaction between the viral Rev protein and the Rev response element (RRE),^[72] an internal loop RNA structure located in the *env* gene of HIV. The exact role of Rev binding to RRE is not fully clear, but Rev–RRE interaction is required for nuclear export of unspliced and partially spliced viral mRNA encoding viral structural proteins. Similar splicing control systems are found in other retroviruses including the human T-cell leukemia virus (HTLV).^[73] While the primary structures of the HTLV regulatory protein, Rex, and its cognate RNA element, RxRE, are distinct from HIV Rev/RRE, the mechanism of action is similar for both systems.^[74] The finding that aminoglycoside antibiotics such as neomycin B can selectively bind to RRE RNA and prevent the Rev–RRE interaction in vivo^[75, 76] has initiated an intense search for RRE-binding compounds by rational design and

combinatorial synthesis.^[77–80] Development of compounds targeted at the Rev–RRE system will benefit from three-dimensional structure data now available for complexes between the RNA-binding domain of Rev and both RRE^[81] and aptamer RNAs.^[82]

A peculiar case of mRNA processing is the RNA-catalyzed excision of introns independent from the cellular splicing machinery. Among this family of catalytic RNAs are self-splicing group I introns, the first ribozymes found.^[83] Group I introns are potential therapeutic targets since they occur in relevant genes of pathogenic microorganisms but not in mammalian cells.^[84–86] Group I introns form large RNA folds with a conserved core containing the splice site and the binding pocket for the guanosine cofactor.^[87] The three-dimensional structure of group I introns has been modeled using phylogenetic data.^[88] Crystal structures of core domains have been obtained recently.^[89] Aminoglycosides,^[84, 90] peptide antibiotics of the tuberactinomycin family,^[91] and L-arginine derivatives^[85] have been reported to inhibit group I intron self-splicing. HTS assays have been developed in order to find other inhibitors.^[86, 92]

A rare posttranscriptional modification process is uridine (U) insertion/deletion editing of mitochondrial mRNA in trypanosomes such as *Trypanosoma* and *Leishmania*.^[93] The site specificity of U insertions and deletions is determined by short guide RNA (gRNA) molecules which hybridize in proximity to the editing region and provide structural elements recognized by the proteins of the editing machinery.^[94] Since similar processes are absent in humans, mitochondrial mRNA editing provides an attractive target for RNA-directed drugs in therapy of trypanosome-caused diseases.

Besides covalent processing and modification, localization and stability control of mRNA mediated by specific interactions with proteins, are widespread mechanisms for directing the translation of messages in distinct regions of eucaryotic cells.^[95, 96] It has only recently become evident that a large number of mRNAs are tightly attached to the cytoskeleton by RNA-binding microtubule- and actin-associated proteins.^[96] mRNA localization has two complementary functions, either to cause a high local concentration of the encoded protein or to prevent high levels of the protein from being ubiquitous in all cells. A common theme in RNA localization is interaction between a *cis*-acting element, usually located within the 3′ untranslated region (3′-UTR) of the mRNA, and a *trans*-acting protein factor. The number of discovered protein–RNA interactions controlling mRNA localization and stability in developmental regulation and pathological processes is rapidly increasing, pointing to a wide variety of mechanisms and only few examples are discussed here.

Defects in the recognition of mRNA regulatory elements by proteins have been thought to be involved in the human autoimmune disease paraneoplastic opsoclonus-myoclonus ataxia (POMA) and the fragile X mental retardation syndrome (FMR), the most common form of hereditary mental retardation. In POMA, a disorder associated with breast cancer and motor dysfunction, specific binding of the neuronal protein Nova-1 to RNA targets, including the mRNAs coding for Nova-1 itself and the inhibitory glycine receptor

alpha2 (GlyRa2),^[97] is disrupted by antibodies.^[98] The three-dimensional structure of the RNA-binding K homology (KH) domains of Nova protein has been determined by X-ray crystallography^[99] revealing similarities to the RNA-binding regions of FMR1,^[100] an mRNA-recognizing protein involved in FMR.^[101]

cis-Acting sequence motifs, dubbed “zipcode RNA”, located in the 3'-UTR of mRNAs, are responsible for the binding of localization and stability control factors.^[102] In β -actin mRNA, a conserved 54 nt motif in the 3'-UTR is recognized by a zipcode binding protein (ZBP-1) which localizes the mRNA to the cell periphery of fibroblasts.^[103] ZBP-1 shares sequence similarity with four other known proteins: 1) the general mRNA localization factor Vera in *Xenopus*, 2) the coding region determinant-binding protein (CRD-BP) in mice,^[104] 3) the KH domain protein (KOC) which is overexpressed in human cancer cells,^[105] and 4) the p62 consensus sequence RNA-binding domain protein (CS-RBD) in human hepato-cellular carcinoma (HCC) cells.^[106] Vera, a highly conserved component of the mRNA localization machinery, binds specifically to an RNA motif in the 3'-UTR of Vg1 mRNA (vegetal mRNA 1).^[107] CRD-BP, a KH domain RNA-binding protein, is not involved in localization but stabilization of mRNA. It increases the RNA half-life by binding to a motif within the coding region of the oncogene c-myc mRNA, thereby preventing endonucleolytic degradation.^[105]

Similar mechanisms of mRNA stabilization have been reported for urokinase receptor (uPAR) mRNA which is the target of a stability-decreasing regulatory protein,^[108] for vascular endothelial growth factor (VEGF) mRNA which, at its 3'-UTR, binds heterogeneous nuclear ribonucleoprotein L (hnRNP L),^[109] and for the tumor-specific c-fos and MYCN mRNAs which have 3'-UTR recognition sites for regulatory RNA-binding proteins which are possible malignancy-promoting factors.^[110]

Expression of the human GLUT1 transporter, responsible for shuttling of glucose over the blood–brain barrier, is regulated by a 10 nt RNA element in the 3'-UTR which enhances the stability of GLUT1 mRNA.^[111] While the protein recognizing the GLUT1 stabilization motif is as yet unknown, brain-derived peptides have been shown to exert stabilizing activity, probably by interaction with the mRNA.^[112] Interestingly, the RNA-controlled regulation of mRNA stability is involved in the overproduction of cytokines and amyloid precursor protein (APP) in cancer and Alzheimer's disease, respectively.^[113] Overproduction of APP can be caused by accumulation of APP mRNA which is stabilized by proteins binding to the 3'-UTR. A 29 nt AU-rich RNA motif in the 3'-UTR of APP mRNA has been demonstrated to be a specific recognition site for several cytoplasmic RNA-binding proteins,^[113] including heterogeneous nuclear ribonucleoprotein C (hnRNP C) which, upon binding, increases APP mRNA stability.^[114, 115] Other factors, yet unknown, might recognize the same 29 nt motif and accelerate APP mRNA degradation in normal cells, since mutant mRNAs lacking the 29 nt element have been shown, in an *in vitro* translation system in the absence of stabilizing proteins, to be more stable than wild-type sequences.^[115]

2.4. Translation

The ribosome, which synthesizes the proteins encoded on mRNA, has been identified as the target of some of the oldest antibiotics, the aminoglycosides, which were later found to specifically recognize the eubacterial 16S ribosomal RNA component (rRNA).^[116] Aminoglycosides bind to the A site of the decoding region of rRNA^[117] which leads to miscoding during translation.^[118] The aminoglycoside–rRNA interaction is remarkable as the drugs are able to specifically target the eubacterial A site fold which differs from the eucaryotic A site by essentially a single base exchange (at position 1408, a guanine is found in all eucaryotes while it is always an adenine in eubacteria^[119]). The high recognition specificity of aminoglycosides is further highlighted by the finding that a genetically determined point mutation in human mitochondrial rRNA leads to increased binding affinity for aminoglycosides which causes hypersensitivity to these drugs.^[120] In order to elucidate the molecular origin of binding specificity, considerable effort has been concentrated on studying the aminoglycoside–rRNA interaction by biochemical^[121] and biophysical methods.^[122–127] The three-dimensional solution structures of a 27 nt model RNA containing the eubacterial A site complexed with the aminoglycosides paromomycin and gentamicin have been determined by NMR spectroscopy.^[122, 125] A promising technique based on the analysis of fragmentation patterns obtained in mass spectroscopy has been applied for studying the interaction sites of aminoglycosides on ribosomal A site RNA constructs.^[127] Synthetic analogues^[128] and mimetics^[129–132] of aminoglycosides were tested for their ability to target the ribosomal decoding region and a high-throughput screen has been developed to identify molecules with high affinity for the 16S rRNA A site.^[133]

A number of antibiotics known to inhibit the peptidyl transferase reaction in the ribosome by different mechanisms have been investigated for their binding regions on 23S rRNA.^[134] A remarkable inhibition mechanism has been uncovered for thiostrepton, a large thiazole-containing cyclic peptide, which binds to the conserved 58 nt GTPase center of eubacterial 23S rRNA^[135] and, thereby, stabilizes a tertiary interaction within this RNA^[136] and interferes with a conformational change within the ribosomal L11 protein.^[137] Recent crystallographic analyses of complexes between L11 binding site RNAs and the L11 protein^[138] provide a molecular basis for detailed investigations of thiostrepton action on the ribosome.

Interestingly, thiostrepton also interacts specifically with 28S rRNAs encoded on extrachromosomal DNA in the plastide-like organelles of *Plasmodium falciparum*.^[139, 140] Similar to its action on 23S rRNA, thiostrepton stabilizes a tertiary interaction in the plastid rRNA,^[141] which ultimately leads to growth inhibition of the parasite.^[140]

The licosamides, pyrrolidine–saccharide antibiotics, may exhibit a striking case of inhibition by substrate mimicry at the peptidyl transferase center of rRNA. It has been suggested that the licosamide clindamycin has a three-dimensional structure resembling the first adenine of tRNA linked to an L-proline–methionine dipeptide.^[142]

The pyrrolidine antibiotic anisomycin provides a beautiful example of a small molecule triggering a complex process upon specific binding to RNA. In eucaryotic ribosomes, interaction of the antibiotic with the 28S rRNA initiates the stress-activated protein kinase JNK1, probably by inducing a conformational change in the rRNA.^[143] Mechanistic studies on compounds which target rRNA and, thus, interfere with ribosome function will, in future, be greatly facilitated by current efforts to elucidate high-resolution structures of the ribosome.^[144, 145]

Structural elements within mRNA play an important role in a number of recoding mechanisms, in which decoding alternatives of the message are exploited during translation.^[146] A prominent example is programmed ribosomal frameshifting to read through a stop codon in retroviral Gag–Pol polyprotein synthesis; this occurs in response to three conserved RNA elements consisting of a frameshift site, a spacer region, and a downstream enhancer motif.^[147] The downstream enhancer provides a stable RNA structure that causes the ribosome to pause during elongation so that kinetically unfavorable alternative decoding events can occur. In the RNA of some retroviruses, the enhancer sequence forms a pseudoknot^[148] while others, such as HIV, contain a stable hairpin motif.^[149] As replication of the retrovirus depends on production of a balanced ratio of the Gag protein and the Gag–Pol polyprotein, both artificial stabilization and destabilization of the frameshift enhancer motif might provide strategies for RNA-targeted therapy in retrovirus infection. The three-dimensional structures of frameshifting RNA pseudoknots are known from NMR spectroscopy and crystallography.^[150] Interestingly, RNA pseudoknots have been demonstrated to form specific binding sites for small molecules such as the peptide antibiotic viomycin^[151] and biotin.^[152]

A peculiar frameshifting signal that does not depend on a downstream enhancer has been found in the mRNA coding for a thymidine kinase in a herpes virus mutant.^[153] The frameshift site itself consists of a G-rich sequence which probably forms a unique three-dimensional structure recognized either by the ribosome or an additional factor.

Alternative decoding of a stop codon occurs in cotranslational selenocysteine incorporation during synthesis of selenoproteins.^[154] In eucaryotes, the recoding event is triggered by the selenocysteine insertion sequence (SECIS) which forms a conserved structural motif in the 3'-UTR of selenoprotein mRNAs.^[155] The SECIS element provides the binding site for SECIS-binding proteins (SBP)^[155] which act as elongation factors to confer alternative decoding. Based on sequence analyses, it has been suggested that SECIS elements may occur in the genome of pathogenic viruses.^[156]

Expression of several proteins involved in iron transport and uptake is regulated on the translational level by the interaction between an iron-responsive element (IRE) in the 3'-UTR of the mRNAs and iron regulatory proteins (IRP).^[157] The IREs are a family of conserved, approximately 30 nt RNA hairpin motifs with defined three-dimensional structures^[158] which are recognition sites for different IRPs, thereby providing finely tuned mRNA-specific responses to external iron signals.^[159] Mutations in an IRP, which perhaps interfere with RNA binding, have been suggested to be the

cause for the abnormal distribution of iron in the brain in Alzheimer's disease.^[160]

Autoregulation of translation by binding of the protein to its own mRNA has been reported for human thymidylate synthase (TS),^[161] which is an important target in cancer chemotherapy.^[162] TS also binds specifically to other cellular mRNAs coding for the nuclear oncogene c-myc,^[163] interferon-induced proteins,^[164] and the tumor suppressor p53.^[165] Two distinct domains of TS mRNA, one in the 5'-UTR and one within the coding message, are involved in TS protein recognition.^[166] For the binding site in the 5'-UTR, a hairpin structure has been predicted which, when isolated in a smaller RNA construct, was also shown to specifically bind aminoglycoside antibiotics.^[167] Based on these findings, a strategy for anticancer therapy has been suggested which requires small molecules targeted at the 5'-UTR TS binding site in the mRNA to inhibit translation of TS message.^[167]

3. Recognition of RNA by Peptides and Proteins

Most functions of RNAs require interaction with RNA-binding proteins. Protein–RNA complexes are instructive for understanding RNA recognition by small molecules as, firstly, proteins may use similar strategies for binding to RNA and, secondly, protein-binding sites are potential targets for drug action. A prominent example for a structural element recognized in common by a protein and a small molecule is a non-Watson–Crick G–G base pair within the HIV RRE RNA which is critical for binding of the viral Rev protein as well as for neomycin B binding.^[175, 168]

RNA recognition strategies of peptides and proteins will only be briefly surveyed here, as several excellent recent reviews on this topic are available.^[169–173] An exponential increase of structural data on a wealth of novel protein–RNA interactions is expected soon from further refinement of currently available electron density maps of the ribosome, with a resolution of around 8 Å for the complete ribosome,^[144] and around 5 Å for the separate small and large subunits.^[145]

3.1. General Principles of Protein–RNA Interactions

While the structural variety of DNA-targeted proteins reflects strategies for binding double-stranded nucleic acid helices, which is almost invariably the form of cellular DNA, RNA-binding proteins have evolved to recognize intricate three-dimensional RNA folds, comparable in their complexity to protein structures. The major role of RNAs participating as functional players in cellular processes, rather than being simply an intermediate storage of genetic information, is attested by the occurrence of most RNAs as at least partially folded single-stranded molecules. Double-stranded RNA in regular A-form helices is only marginally suited for specific intermolecular interactions. The discriminatory base edges are buried in a narrow deep (“major”) groove^[174] and the readily accessible shallow (“minor”) groove contains less information for recognition. Yet, large parts of the RNA folds participate in double-stranded base-paired regions. Distur-

tions of the regular A-form by non-Watson–Crick base pairs, interspersed looped-out residues, and platforms of consecutive nucleotides lead, however, to an accessible widened deep groove.^[171, 172, 175] Proteins not only recognize but may also enhance such distortions of the RNA structure. Insertion of an α helix (Figure 2A) or a flexible protein loop into a

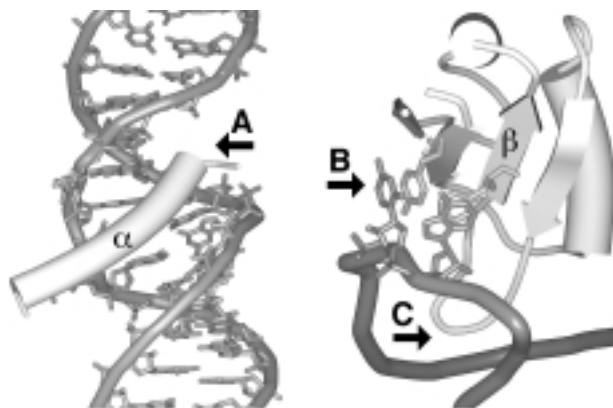


Figure 2. Strategies for RNA recognition by proteins, illustrated by schematic views of a Rev peptide–RRE complex (left)^[81] and a U1A protein–RNA complex (right).^[216] RNA is shown in dark grey, protein in light grey. In the right-hand panel, only the RNA backbone is shown as a tube. A: An α helix can be inserted into the RNA deep groove which has been widened by looped-out residues, noncanonical base pairs, or base triples. Side chains of residues in the α helix can form contacts with the base-pair edges and with the sugar phosphate backbone in the RNA. B: Aromatic residues on the surface of β -strands are accessible for stacking with unpaired bases in RNA loops. C: Flexible protein loops can penetrate into RNA folds and form H-bonds and ionic interactions, preferably with the RNA backbone. The figure was prepared from coordinates deposited in the protein data bank (PDB), Brookhaven (PDB codes: 1ETF, left panel; 1URN, right panel).

widened deep groove is a common theme in several protein–RNA complexes.^[172, 173] Exposed β -strands in proteins provide interaction surfaces for unpaired RNA regions which can bind by stacking of splayed-out bases on aromatic amino acid side chains (Figure 2B).^[170, 171] Flexible protein loops participate predominantly in interactions with the RNA backbone (Figure 2C), often leading to reduced loop mobility.^[171, 173]

A remarkable difference is observed in RNA complexes of small peptides versus RNAs bound to larger proteins.^[172, 176, 177] In peptide–RNA complexes, adaptive conformational transitions are frequently observed for the peptide upon binding to a largely unaltered RNA scaffold. On the contrary, larger proteins predominantly bind RNA as rigidly folded domains which provide exposed surfaces, cavities, and clefts for the RNA substrate which adapts by conformational changes.

3.2. Molecular Details of Protein–RNA Interactions

Protein–RNA interactions mostly involve the protein side chains (approximately 90 % of the interactions in all complexes of known structure^[178]), which act predominantly as H-bond donors. In the RNA, negatively charged phosphate groups of the backbone are the most important H-bond

acceptors. In protein–RNA complexes of known structure, about 20 % of the intermolecular interactions involve the RNA 2'-OH group, equally often as a hydrogen donor and as an acceptor.^[178] RNA-binding surfaces of peptides and proteins are rich in the basic amino acids arginine and lysine which account for about 60 % of intermolecular H-bonds in the RNA complexes.^[178] Obviously, acidic residues are seldom found at the interface of proteins and RNA. The important role of arginine in protein–RNA interactions is due to both its potency as a versatile basic H-bond donor and its flat guanidinium side chain which allows for additional favorable stacking interactions with RNA bases.

Intermolecular stacking interactions in protein–RNA complexes can be extensive, involving residues of both components in an alternating fashion, termed interdigitation.^[172] Hydrophobic parts of RNA bases, accessible in triples, platforms, and noncanonical base pairs can act as surfaces for aligning nonpolar side chains of proteins.^[172] Important contact sites for H-bonds are provided by the Watson–Crick edges of RNA bases^[172, 175], namely the 6 and 7 positions of purines and the 4 position of pyrimidines which, in helical regions, are all located in the deep groove.

Only one example of recognition by a peptide in the RNA shallow groove is known in which a ten-residue peptide specifically binds to an RNA helix when the central base pair is G–U but not when it is replaced by G–C.^[179] This system highlights a remarkable specificity potential in protein–RNA interactions which allows for discrimination of a shallow-groove asymmetry as subtle as a single amino group of a G–U pair.

Non-Watson–Crick base pairs can generally provide excellent recognition sites for proteins in RNA folds.^[175] A prominent example is sheared G–A pairs for which a characteristic “in-plane breathing” motion has been observed in molecular dynamics (MD) simulations of RNA;^[180] this facilitates potential interactions of functional groups on the Watson–Crick edges of the bases with protein side chains.

Finally, there are ions and water which are intrinsic parts of the RNA three-dimensional structure^[181] and play important roles in protein–RNA interactions. In three-dimensional structures of protein–nucleic acid complexes, well-oriented water molecules are often found at the binding interfaces where they extend shape complementarity by filling cavities.^[178] As polyanions, RNAs are inevitably surrounded by an important number of counterions competing with proteins for binding sites.^[182] Proteins can bind to RNAs at low ionic strength by an entropically favored displacement of cations from electronegative pockets on the RNA. At higher salt concentrations, however, this process is reversed due to salt-induced destabilization of polar intermolecular H-bonds and the competition between hydration equilibria of charged groups and salt ions.^[183]

4. RNA Recognition by Small Molecules

In future, therapeutic strategies relying on complex biomolecular effectors such as antisense and ribozyme RNA may be facilitated by emerging techniques for the delivery of large,

often highly charged molecules and by safe systems for gene therapy and engineered viral vectors. For the time being, however, lead design, independently of whether proteins or nucleic acids are the targets, will continue to concentrate on small synthetic molecules and derivatives of natural low molecular weight compounds. In the following discussion, the focus will mainly be on the recognition of naturally occurring RNA targets by small molecules. Aptamers—nucleic acid molecules which are obtained by *in vitro* selection (SELEX) of random sequence libraries and bind a substrate molecule with both high affinity and specificity—will be discussed only in few selected cases. Recently, *in vitro* selected aptamer RNAs have been shown to also recognize their cognate ligands *in vivo*.^[184] Thus, knowledge of principles of adaptive molecular recognition in nucleic acid aptamer complexes^[177] is likely to influence rational drug design strategies for natural RNA targets. Concise overviews on the selection of aptamers,^[185] their three-dimensional structures,^[177, 186, 187] and their uses as diagnostic and therapeutic agents^[188] and in conventional drug design^[189] are given by recent reviews.

4.1. Philosophy of Targeting RNA Folds

In the early days of investigating small molecule–RNA interactions, many concepts of molecular recognition had been adapted from knowledge on DNA complexes. Thus, intercalators and groove binders were among the first compounds systematically analyzed for their RNA-binding capacity.^[190] The application of recognition strategies derived from DNA targets on RNA is severely limited by the very different three-dimensional structures of functional RNA molecules^[3] which are not restricted to regular double helices. In the light of RNA structure–function relations, regular undistorted A-form helices of double-stranded RNA are unlikely to be efficient targets for effector molecules. Consequently, in the search for compounds targeted at RNA molecules, the notion of sequence specificity is not a useful concept and is perhaps limited to the partially understood connection between the sequence of an RNA and its three-dimensional structure.

The goal of achieving specific RNA recognition is conceptually closer to targeting proteins than DNA, but with different weights on the distinct energetic contributions to molecular interactions. Such contributions come from electrostatic forces, H-bond formation, and different nonbonded interactions, including van der Waals, stacking, and hydrophobic forces, which are maximized by molecular shape complementarity.

In the following, I will discuss how these forces direct the binding of small molecules to RNA folds and how drug-design strategies might benefit from our current understanding of RNA–small molecule interactions.

4.2. Electrostatic Forces and Metal Ion Binding

Electrostatic forces play a major role in interactions with RNA because of the polyanionic nature of RNA. The majority of RNA-binding molecules (Figure 3) both natural, such as the aminoglycoside^[191] and tuberactinomycin^[91] antibiotics, and designed, including synthetic aminoglycoside derivatives and mimetics,^[30, 49, 79, 128–132, 192] diphenylfurans,^[77] cyclophanes,^[47, 193] macrocycles,^[80] polyamine–acridine conjugates,^[45] arginine derivatives,^[48, 49] and peptoid/peptid oligom-

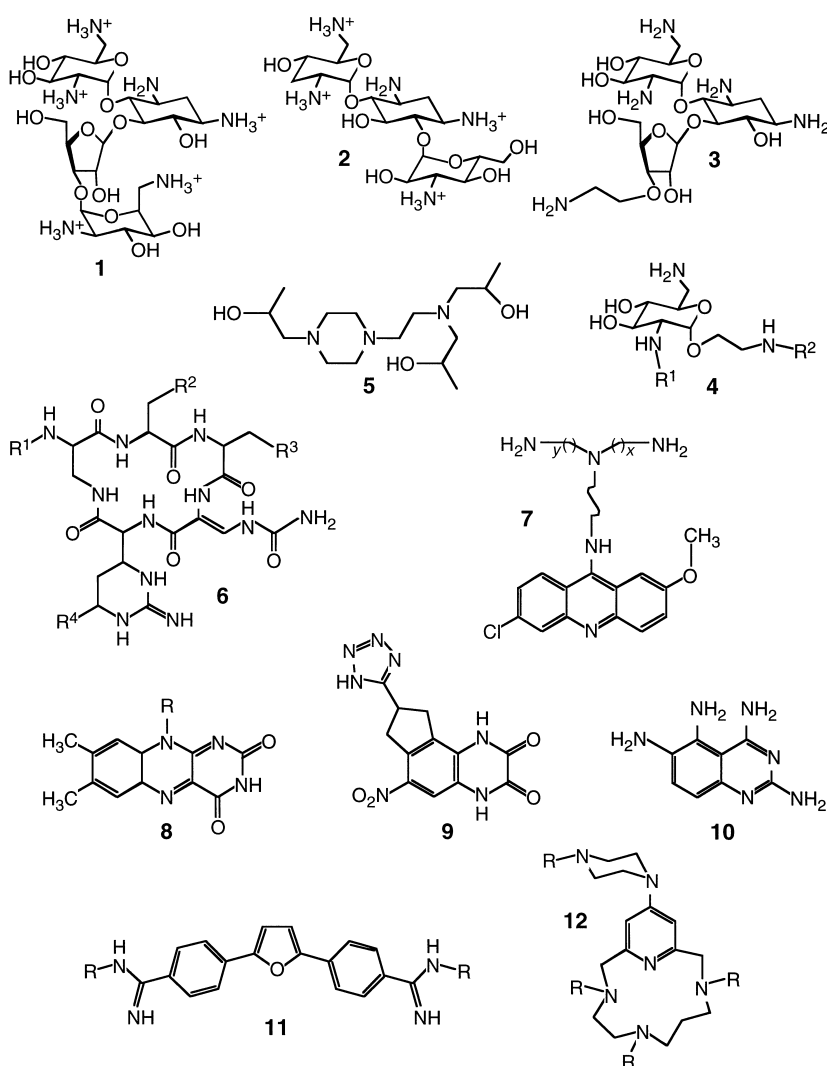


Figure 3. A collection of RNA-binding compounds which specifically recognize different RNA folds (see text for further details): natural aminoglycoside antibiotics, neomycin B (**1**) and tobramycin (**2**);^[191] simplified semisynthetic aminoglycoside mimetics, **3** and **4**;^[128, 130] an aminoglycoside surrogate **5**;^[131] natural tuberactinomycin antibiotics **6** (R^1 and/or R^3 are usually cationic substituents);^[91] In-PRiNts **7**;^[45] photocleaving flavin derivatives **8**;^[204] a quinoxaline-2,3-dione derivative **9**;^[46] a 2,4-diaminoquinoxaline derivative **10**;^[46] substituted diphenyl furans **11**;^[77] and piperazinyl polyazacyclophanes **12**.^[47] Most of the compounds carry positive charges at physiological pH, however, protonation is indicated here only for the natural aminoglycosides, for which exact pK_a values are available.^[217]

ers,^[43] are cationic compounds carrying several positively charged groups. Electrostatic interactions between the negatively charged RNA backbone and cationic groups enhance the binding affinity in RNA–drug complexes. But what about their role in mediating binding specificity? Lacking spatial directionality, electrostatic forces are considered to contribute to nonspecific binding. They can govern, however, specific recognition in the binding of conformationally constrained polycationic molecules to RNA folds as revealed by investigations on the interaction of aminoglycosides with different RNA targets.^[30, 34, 128, 129, 192] In a quantitative approach using surface plasmon resonance, it has been demonstrated that increasing the ionic competition by adding salt to RNA–aminoglycoside complexes decreased both the specific and nonspecific drug binding to about the same extent.^[128] In other words, the electrostatic component of the binding energy contributed, as expected, to the unspecific binding but also, to a remarkable extent, to the specific binding. Two mechanisms confer the specificity to electrostatic interactions in RNA–drug recognition.

Firstly, electrostatic interactions with RNA are often part of polar H-bonds between anionic phosphate oxygens as acceptors and positively charged H-donor groups in the bound molecule (Figure 4A). While electrostatic forces between the

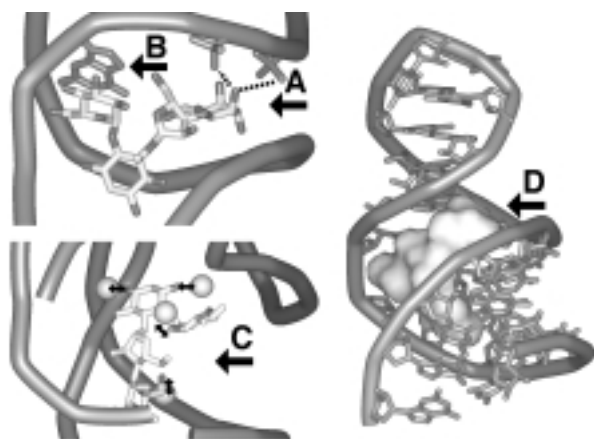


Figure 4. Some principles of RNA recognition by small molecules, illustrated by schematic views of a 16S A site RNA–paromomycin complex (top left and right)^[122] and a hammerhead RNA–neomycin complex (bottom left).^[33] RNA is shown in dark grey, in the left panels as the backbone tube only. The drug is shown in light grey, in the left panels as a stick representation, on the right as a space-filling surface representation. A: H-bonds can form between polar donor and acceptor groups in the drug and the RNA. Frequently, negatively charged phosphate groups of the RNA backbone participate as acceptors in H-bonds with cationic substituents in the small molecule (here, an ammonium group). B: Flat, but not necessarily planar hydrophobic molecule fragments, such as the sugar ring shown here, provide stacking surfaces for RNA bases. C: By exploiting structural electrostatic complementarity, small molecules can provide frameworks to orient cationic groups properly in space, so as to complement the electronegative metal ion binding pockets of the RNA fold. In the figure, a solution conformer of neomycin has been docked to the crystal structure of the hammerhead RNA in such a way that the positively charged ammonium groups of the drug point (arrows) towards the crystallographic Mg^{II} binding sites (spheres). D: Shape complementarity between the binding surfaces of the small molecule and the RNA maximizes intermolecular van der Waals interactions and contributes to binding specificity. The top left and right panels were prepared from coordinates deposited in the protein data bank (PDB), Brookhaven (file 1A3M), the bottom left panel is adapted from Ref. [33].

charged heavy atoms contribute to the binding energy, the oriented nature of the H-bond provides directional specificity in the interaction. Examples for this type of polar H-bond are the contacts between RNA phosphate oxygens and ammonium groups of aminoglycosides observed in three-dimensional solution structures of RNA–aminoglycoside complexes.^[122, 125, 194, 195]

Secondly, the three-dimensional folding of an RNA chain into a scaffold of spatially oriented anionic groups creates electronegative pockets where negative charge is focused.^[196] The spatial distribution of negatively charged pockets in an RNA fold provides a three-dimensional pattern that can be specifically targeted by compounds exhibiting structural electrostatic complementarity.^[33, 34, 50] This principle is exploited by aminoglycosides binding to RNA targets, which has been extensively investigated for the hammerhead ribozyme. This catalytic RNA is a paradigm system for the study of RNA–drug interactions because, in addition to the binding affinity, the specificity of RNA recognition can be analyzed by monitoring inhibition of the ribozyme. Following on the experimental observation that neomycin B (**1** in Figure 3) inhibits the self-cleavage of the hammerhead ribozyme by competing with the binding of catalytic metal ions to the RNA,^[29] it has been shown that solution conformers of aminoglycosides provide conformationally constrained scaffolds carrying cationic ammonium groups in the proper spatial orientation so as to simultaneously displace several Mg^{II} ions from their binding sites in electronegative pockets of the hammerhead fold (Figure 4C).^[33] MD simulations of hammerhead RNA–aminoglycoside complexes suggest that ammonium groups of the aminoglycosides can mimic the interaction of Mg^{II} ions with the RNA down to an atomic level (Figure 5A, B).

Experimental studies on hammerhead RNA–drug complexes containing designed aminoglycoside derivatives in which the number, the basicity, or the flexibility of the attachment of ammonium groups has been systematically altered^[30, 128, 192] support the model of structural electrostatic complementarity.^[34] When either the number or the charge density of ammonium groups in aminoglycosides is increased, the inhibitory activity of the compounds is enhanced.^[30, 34, 192] The binding specificity of aminoglycosides is diminished when the conformational constraint on the aminoglycoside scaffold is partially relieved by replacing one of the ring substructures by a more flexible aliphatic chain carrying one or more amine groups (Compounds **3** and **4** in Figure 3).^[128]

The concept of structural electrostatic complementarity has been successfully used to explain the structural basis of aminoglycoside inhibition of self-splicing group I introns.^[197] The Mg^{II} ions needed for catalysis could be displaced from the active site of a group I intron by docking solution conformations of neomycin B to the RNA.

While the direct participation of metal ions in RNA function may be limited to ribozymes, the role of cations in stabilizing the three-dimensional structure of probably all RNAs offers a general strategy for targeting metal ion binding pockets with positively charged groups, in order to specifically anchor small molecules to RNA folds. A hierarchical procedure has been developed to, first, calculate electronegative

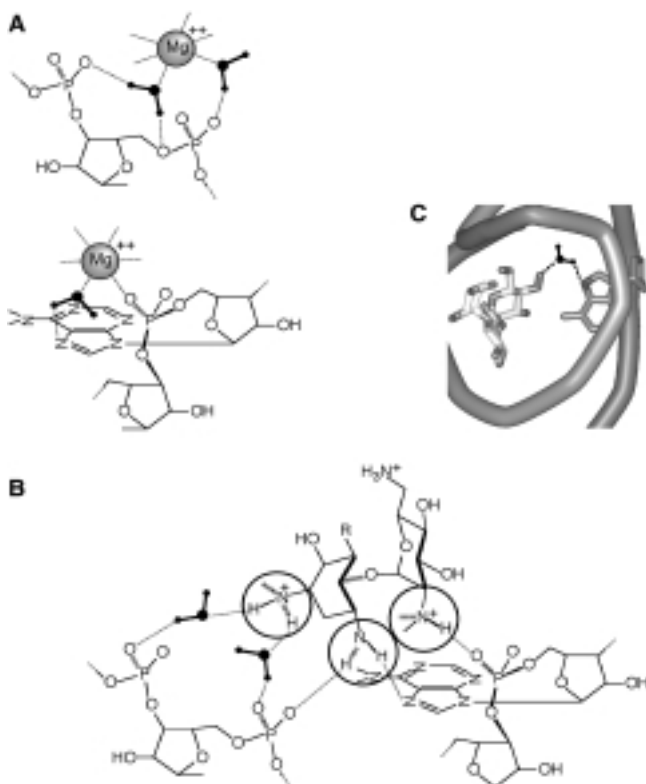


Figure 5. The Mg^{2+} ions bound to the hammerhead ribozyme in the crystal structure.^[218] (A) can be displaced by ammonium groups of aminoglycosides engaging in interactions with the RNA which resemble the contacts of the metal ions down to an atomic level (B).^[33] Water molecules (represented by black ball-and-stick models) mediate the binding by conferring a certain plasticity in the contacts. C: A proposed water-mediated contact between a hydroxyl group of the drug (stick representation) and the N7 atom of an adenine of the RNA (tube representation) in the solution structure of a tobramycin–aptamer RNA complex.^[194] Figures A and B were adapted from Ref. [33], Figure C was prepared from coordinates kindly provided by D. J. Patel.

pockets in RNA folds^[196] and, second, predict, on the basis of structural electrostatic complementarity, docking sites for cationic compounds.^[50, 198] The prediction method was used to construct complexes of aminoglycosides bound to HIV-1 TAR which explain, on a molecular basis, the experimentally established allosteric mechanism^[199] by which neomycin induces dissociation of Tat–TAR complexes.^[50]

4.3. H-Bonds and Water-Mediated Contacts

For the H-bond forming capacity of single atoms in RNA–small molecule complexes, the observations from protein–RNA complexes (see Section 3.2) apply accordingly. It has already been pointed out that polar hydrogen bonds, predominantly between phosphate groups of the RNA backbone as acceptors and hydrogen atoms of the small molecule, can contribute significantly to RNA–drug binding. A large portion of the available data on H-bonding in RNA–drug complexes stems from studies on RNA–aminoglycoside complexes,^[200] among them NMR studies on aminoglycoside

complexes of aptamer RNAs^[194, 195, 201] and oligonucleotides derived from the eubacterial A site rRNA.^[122, 124, 125]

In experimentally determined three-dimensional structures of RNA–aminoglycoside complexes, both the ammonium and hydroxyl groups of the drugs are H-bond donors which interact predominantly with phosphate oxygen atoms of the RNA backbone (Figure 4A), and with the N7 and O4 atoms of purines and uridine, respectively.^[122, 124, 125, 194, 195, 201] Similar observations have been made in model complexes obtained by docking of aminoglycosides to RNA targets.^[33, 202]

The stereochemical variety of substitution sites for the ammonium and hydroxyl groups in aminoglycosides, along with their constrained conformational flexibility, provides a versatile tool for orienting H-bond donors in space so as to provide a tight H-bonding network for recognizing RNA folds. The versatility of aminoglycosides in RNA recognition is attested by the wide range of targets which bind distinct compounds of these family of drugs with high affinity. Thus, it has been suggested that the principal sugar building blocks of aminoglycosides could be used as synthons for the design of RNA-targeted drugs.^[33] Both conventional synthetic chemistry^[128, 129, 131] and combinatorial synthesis^[79, 130] have been used to simplify aminoglycoside building blocks and to obtain synthetically readily accessible carbohydrate mimetics (Compounds **3** and **4** in Figure 3).^[132, 203]

H-bond formation involving the 2-amino group of guanine is likely to be responsible for the shallow-groove recognition of G–U wobble pairs by a small peptide.^[179] Likewise, a specific H-bond between the 2-amino group of guanine and a carbonyl group of the isalloxazine moiety has been proposed in a model for the shallow-groove recognition of G–U pairs by photocleaving flavin derivatives (**8** in Figure 3).^[204, 205]

Water may participate in H-bond formation in RNA–small molecule complexes, as has been suggested for a tobramycin–RNA aptamer complex in which a water-mediated H-bond between a hydroxyl group of the drug and an adenine N7 atom could occur (Figure 5C).^[194] Such water-mediated H-bond contacts between the RNA and the small molecule, again preferably to phosphate oxygen atoms and N7 atoms of purines, have frequently been observed in MD simulations of hammerhead RNA–aminoglycoside complexes (Figure 5B).^[33]

4.4. Stacking and Intercalation

The bases in RNA folds provide surfaces for stacking interactions driven by hydrophobic effects, that is, the exclusion of polar solvent from the proximity of the flat unipolar surfaces, van der Waals interactions and, eventually, interactions between aromatic π -electron systems.^[206] The full range of these interactions contributes in classical intercalation of planar aromatic moieties between nucleic acid base pairs which is the binding mode of a variety of DNA-targeted antibiotics and antitumor agents.^[207] Different classes of DNA intercalators have been systematically studied for their affinity for RNA^[208] and most of the DNA binders were found to intercalate as well in A-form RNA duplexes.^[190] For both classical intercalators such as ethidium,^[209] and com-

pounds intercalating in a partial “bookmarking” fashion into single-stranded base stacks, such as copper phenanthroline,^[210] the interactions with RNA have been analyzed in modeled complexes of HIV TAR and tRNA, respectively.

Due to the physical nature of the interactions involved, classical intercalation is relatively nonspecific. In an attempt at rational design of inhibitors targeting the HIV Tat–TAR complex, an aromatic acridine moiety for stacking and a flexible polycationic anchor for electrostatic contacts with the RNA backbone have been connected through an aliphatic linker to give rise to the inhibitor of protein–ribonucleotide sequences (In-PRiNts;^[45] **7** in Figure 3). While this approach yielded a number of promising Tat–TAR inhibitors, large activity differences, not easily linked to compound structure, were found.^[45] This suggests that additional restrictions, such as constrained conformational flexibility, might increase inhibitor specificity. For the In-PRiNts type of RNA binders, a stacking interaction different from classical intercalation has been proposed which involves additional H-bonding between the substituted acridine moiety and the RNA base pair edges.^[45] Nonclassical threading intercalation has also been discussed as the binding mode of synthetic diphenylfuran cations (**11** in Figure 3) which selectively inhibit HIV Rev binding to RRE RNA.^[77]

Recently, specific inhibition of the HIV Tat–TAR interaction has been reported for derivatives of quinoxaline-2,3-dione and 2,4-diaminoquinoxaline (**9** and **10** in Figure 3) which specifically recognize an internal bulge and a loop in TAR RNA, respectively.^[46] Although detailed structural models for the TAR–drug complexes are not available for these two compounds, it is likely that stacking interactions contribute to their RNA-binding affinity as both molecules contain flat aromatic moieties. The presence of several potential H-bond donating and accepting groups in the quinoxalinediones and diaminoquinoxalines suggests that these compounds, similar to the In-PRiNts, might form additional H-bonds within the stacking plane. Extended interactions, as observed in the In-PRiNts, diphenylfurans, quinoxalinediones, and diaminoquinoxalines, increase the specificity of intercalation, attested also by the frequent use of combinations of stacking and H-bonding as the recognition principle in RNA aptamers.^[177]

Sufficiently planar, unpolar molecule fragments can engage in a more general fashion in stacking interactions driven by van der Waals forces and hydrophobic effects. Spectacular examples are seen in aminoglycosides which stack the less polar side of six-membered sugar rings on purine bases (Figure 4B) in the three-dimensional structures of paromomycin and gentamicin bound to an A site oligonucleotide^[122, 125] and tobramycin bound to aptamer RNA.^[194] An inverse and maybe more fragile situation with a flat aromatic molecule stacking on the ribose moiety of the RNA backbone is found in the model of a complex between a photocleaving flavin derivative (**8** in Figure 3) bound to the shallow groove of an RNA helix with a central G–U wobble pair.^[205] Stacking of the isoalloxazine core of the flavin on a ribose orients the small molecule to direct a carbonyl group into H-bonding with the free 2-amino group of guanine in the shallow-groove asymmetry created by the G–U pair.

4.5. Shape Complementarity and Conformational Adaption

The complementarity of molecular shapes can provide a major driving force in the formation of biomolecular complexes providing high binding affinity and specific ligand discrimination. This is most impressively demonstrated by the three-dimensional structures of RNA aptamer complexes.^[177, 186, 187] Characteristically, aptamers encapsulate their substrate within a tightly fitting cavity. For example, in a tobramycin–RNA aptamer complex, 75 % of the accessible surface area of the aminoglycoside is buried inside the RNA fold.^[194] Ligand binding to aptamer RNAs is typically associated with major conformational changes as the nucleic acids adapt to their substrate.^[177, 187]

For natural RNA targets, which, in contrast to aptamers, did not evolve in order to tightly bind to a specific small molecule, shape complementarity with effector compounds might be more difficult to achieve. It has been pointed out that in complexes of aminoglycosides bound to natural targets, considerably less accessible surface of the drugs is buried relative to aptamers.^[50] In the complexes between eubacterial ribosomal A site oligonucleotides and aminoglycosides, however, a reasonably good complementarity between the binding surfaces of the RNA and the drugs are observed (Figure 4D).^[122, 125]

Shape complementarity can account for remarkable site discrimination, as was reported for a photocleaving phenanthroline rhodium complex that selectively recognizes and cleaves G–U wobble base pairs within double-helical regions of folded RNAs, such as tRNAs.^[211]

Conformational changes in RNA upon binding of other molecules can be small, as for the A site oligonucleotide–aminoglycoside system,^[123] or considerably larger, as for the HIV TAR RNA interacting with argininamide.^[52] The development of small molecules that, upon binding to an RNA fold, prevent a conformational change necessary for the biological function of the target, is another promising strategy in the search for therapeutic compounds. The thiazole-containing peptide antibiotic thiostrepton recognizes a complex structure in the conserved GTPase center of eubacterial 23S rRNA and stabilizes a tertiary interaction which might be required to be flexible for a functional interaction with the ribosomal L11 protein.^[136–138] Rational design of effector molecules interfering with RNA conformational flexibility requires a detailed picture of the three-dimensional structure and function of the RNA target. Recently, extensive molecular modeling and simulation studies have led to the suggestion that the allosteric mechanism by which neomycin B induces dissociation of HIV Tat–TAR complexes^[199] is based on a conformational arrest in which the aminoglycoside locks TAR in a conformer with low affinity for Tat.^[50]

Finally, shape complementarity in RNA–ligand interactions is mediated and enhanced by structural water molecules at the complex interface, as they are commonly found in protein–drug complexes.^[212] By reorienting, water molecules can provide a certain plasticity in H-bond formation (see Section 4.3) and fill interfacial cavities. In order to include information about the positions of tightly-bound water

molecules in rational drug design, preferred hydration sites have to be determined by crystal structure analyses,^[213] special NMR spectroscopic techniques,^[214] and MD simulations.^[215]

5. Summary and Outlook

We are beginning to uncover the many pathological processes involving RNA molecules as key players. It is therefore becoming evident that RNA is a potential prime target for therapeutic intervention. Small molecules may interfere with RNA functions by a number of mechanisms. By binding to an RNA fold, small molecules can alter the conformation or the flexibility of the RNA, and, thereby, inhibit the interaction with proteins or even enhance protein–RNA association. Thus, RNA-binding effectors can trigger responses that depend on either the formation or the disruption of protein–RNA complexes. Outstanding examples are the aminoglycosides and thiostrepton discussed above, which interfere with the function of protein–RNA complexes by preventing their formation (aminoglycosides) or by increasing the rigidity of the RNA component (thiostrepton). Another class of RNA-targeted molecules may act as interface inhibitors and directly prevent the binding of proteins.

The sequencing of the whole human genome will cause an unprecedented leap in the number of potential drug targets, namely RNA transcripts and their various processed active forms. The exorbitant number of available RNA targets requires efficient novel approaches of high-throughput screening (HTS) for small molecules interfering with RNA functions. The development of compounds identified by HTS into highly specific effectors targeted at distinct RNA folds is hardly imaginable without a detailed structural understanding of RNA recognition. This structural knowledge comes from analyses employing X-ray crystallography and NMR spectroscopy on RNA alone, as well as in complexes with proteins and small molecules. With our growing understanding of the RNA three-dimensional structure and function, firm grounds will be provided for the development of specific RNA-directed effector molecules.

Structure-based approaches will be at the heart of RNA-targeted drug design and the evolution of lead compounds identified by HTS into therapeutics. This is particularly true if our efforts in targeting RNA go beyond the group of RNAs which are unique to eubacteria and certain organelles, and if we set out to develop drugs which exploit the minimal structural differences between RNA targets common to humans and pathogens.

Our view on drug design may still be biased by the protein world and some of the difficulties encountered in the search for RNA-binding compounds are due to limitations in applying methods developed for studying protein targets. Just as new experimental tools have been developed for studying RNA in the laboratory, theoretical investigations of RNA and its interactions call for novel approaches. Initial steps have been made in the synthesis of RNA-targeted compounds and tools are emerging for their rational design.

The physical laws describing intermolecular interactions are the same for proteins and RNAs. The example of electrostatic forces shows, however, that the focus on energetic contributions is shifted and requires a higher level of sophistication in theoretical treatment. The emerging improvements in algorithms and techniques for studying RNA complexes by crystallography and NMR spectroscopy, along with novel molecular design approaches, will be profitable for structure-based drug design, irrespective of whether the target is an RNA or a protein.

While I have tried to cover the literature until September 1999, the selection of references was subjective, and I apologize for any contribution omitted from the review. I thank Dinshaw J. Patel for support and helpful discussions, and Eric Westhof, Eugene Skripkin, and Seema Qamar for useful comments. I am especially grateful to my wife, Tatjana Singer, for critical reading of the manuscript and numerous valuable suggestions. Research in the laboratory of D.J.P is supported by the NIH (GM-54777).

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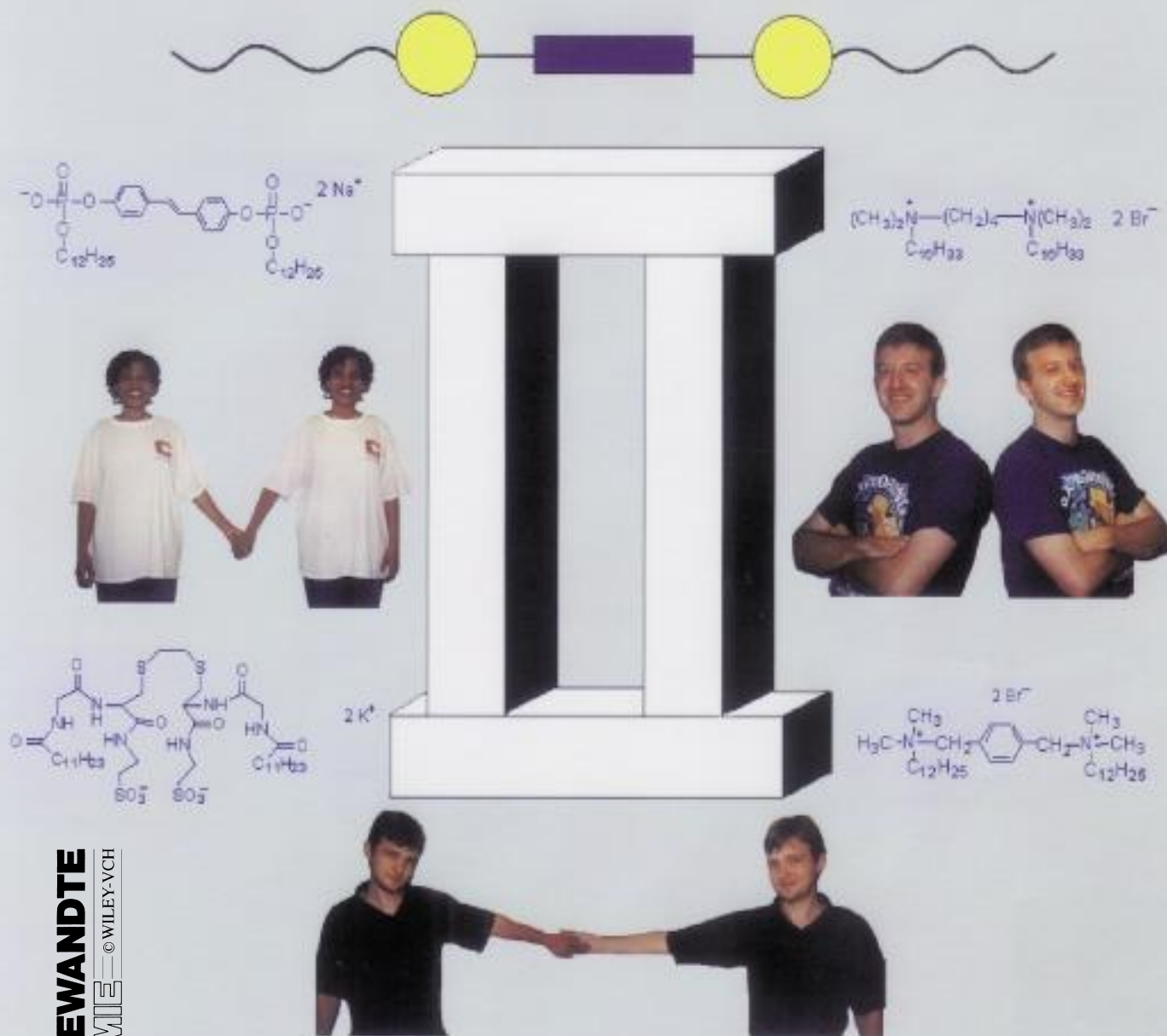
- [1] *The RNA World*, 2. Ed. (Eds.: R. F. Gesteland, T. R. Cech, J. F. Atkins), Cold Spring Harbor Laboratory Press, New York, **1999**.
- [2] a) N. D. Pearson, C. D. Prescott, *Chem. Biol.* **1997**, *4*, 409–414; b) T. Hermann, E. Westhof, *Curr. Opin. Biotechnol.* **1998**, *9*, 66–73; c) K. Michael, Y. Tor, *Chem. Eur. J.* **1998**, *4*, 2091–2098; d) M. Afshar, C. D. Prescott, G. Varani, *Curr. Opin. Biotechnol.* **1999**, *10*, 59–63; e) Y. Tor, *Angew. Chem.* **1999**, *111*, 1681–1685; *Angew. Chem. Int. Ed.* **1999**, *38*, 1579–1582.
- [3] a) A. R. Ferré-D'Amaré, J. A. Doudna, *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 57–73; b) P. B. Moore, *Annu. Rev. Biochem.* **1999**, *67*, 287–300; c) R. T. Batey, R. P. Rambo, J. A. Doudna, *Angew. Chem.* **1999**, *111*, 2472–2491; *Angew. Chem. Int. Ed.* **1999**, *38*, 2326–2343; d) T. Hermann, D. J. Patel, *J. Mol. Biol.* **1999**, *294*, 829–849.
- [4] a) A. Fire, *Trends Genet.* **1999**, *15*, 358–363; b) D. C. Baulcombe, *Curr. Biol.* **1999**, *9*, R599–R601; c) R. A. Jorgensen, R. A. Atkinson, R. L. S. Forster, W. J. Lucas, *Science* **1998**, *279*, 1486–1487; d) N. Brockdorff, *Curr. Opin. Genet. Devel.* **1998**, *8*, 328–333.
- [5] V. A. Zakian, *Science* **1995**, *270*, 1601–1607.
- [6] E. H. Blackburn in *Lit. [1]*, pp. 609–635.
- [7] a) N. Axelrod, *Nat. Med.* **1996**, *2*, 158–159; b) E. Raymond, D. Sun, S. F. Chen, B. Windle, D. D. Von Hoff, *Curr. Opin. Biotechnol.* **1996**, *7*, 583–591; c) H. W. Sharma, J. Y. Maltese, X. Zhu, H. E. Kaiser, R. Narayanan, *Anticancer Res.* **1996**, *16*, 511–515; d) A. J. Lustig, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3339–3341.
- [8] Y. Kanazawa, K. Ohkawa, K. Ueda, E. Mita, T. Takehara, Y. Sasaki, A. Kasahara, N. Hayashi, *Biochem. Biophys. Res. Commun.* **1996**, *225*, 570–576.
- [9] A. I. Glukhov, O. V. Zimnik, S. A. Gordeev, S. E. Severin, *Biochem. Biophys. Res. Commun.* **1998**, *248*, 368–371.
- [10] a) J. C. Norton, M. A. Piatyszek, W. E. Wright, J. W. Shay, D. R. Corey, *Nat. Biotechnol.* **1996**, *14*, 615–619; b) A. E. Pitts, D. R. Corey, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 11549–11554.
- [11] S. E. Hamilton, D. R. Corey, *Chem. Biol.* **1996**, *3*, 863–867.
- [12] M. I. N. Cano, J. M. Dungan, N. Agabian, E. H. Blackburn, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3616–3621.
- [13] A. M. Skalka, S. P. Goff, *Reverse Transcriptase*, Cold Spring Harbor Laboratory Press, New York, **1993**.
- [14] R. Marquet, C. Isel, C. Ehresmann, B. Ehresmann, *Biochimie* **1995**, *77*, 113–124.
- [15] C. Isel, C. Ehresmann, G. Keith, B. Ehresmann, R. Marquet, *J. Mol. Biol.* **1995**, *247*, 236–250.

- [16] C. Isel, E. Westhof, C. Massire, S. F. J. Le Grice, B. Ehresmann, C. Ehresmann, R. Marquet, *EMBO J.* **1999**, *18*, 1038–1048.
- [17] a) J. Ding, K. Das, Y. Hsiou, S. G. Sarafianos, A. D. Clark, A. Jacobo-Molina, C. Tantillo, S. H. Hughes, E. Arnold, *J. Mol. Biol.* **1998**, *284*, 1095–1111; b) H. Huang, R. Chopra, G. L. Verdine, S. C. Harrison, *Science* **1998**, *282*, 1669–1675.
- [18] J. C. Paillart, R. Marquet, E. Skripkin, C. Ehresmann, B. Ehresmann, *Biochimie* **1996**, *78*, 639–653.
- [19] E. Skripkin, J. C. Paillart, R. Marquet, B. Ehresmann, C. Ehresmann, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4945–4949.
- [20] a) J. C. Paillart, L. Berthou, M. Ottmann, J. L. Darlix, R. Marquet, B. Ehresmann, C. Ehresmann, *J. Virol.* **1996**, *70*, 8348–8354; b) G. P. Harrison, G. Miele, E. Hunter, A. M. L. Lever, *J. Virol.* **1998**, *72*, 5886–5896.
- [21] a) A. Mujeeb, J. L. Clever, T. M. Billeci, T. L. James, T. G. Parslow, *Nat. Struct. Biol.* **1998**, *5*, 432–436; b) F. Jossinet, J.-C. Paillart, E. Westhof, T. Hermann, E. Skripkin, J. S. Lodmell, C. Ehresmann, B. Ehresmann, R. Marquet, *RNA* **1999**, *5*, 1222–1234.
- [22] a) A. Mujeeb, T. G. Parslow, A. Zarrinpar, C. Das, T. L. James, *FEBS Lett.* **1999**, *458*, 387–392; b) E. Ennifar, M. Yusupov, P. Walter, R. Marquet, B. Ehresmann, C. Ehresmann, P. Dumas, *Structure* **1999**, *7*, 1439–1449.
- [23] L. A. Actis, M. E. Tolmasky, J. H. Crosa, *Front. Biosci.* **1999**, *4*, D43–D62.
- [24] E. Gerhart, H. Wagner, S. Brantl, *Trends Biochem. Sci.* **1998**, *23*, 451–454.
- [25] P. F. Predki, L. M. Nayak, M. B. Gottlieb, L. Regan, *Cell* **1995**, *80*, 41–50.
- [26] M. D. Been, G. S. Wickham, *Eur. J. Biochem.* **1997**, *247*, 741–753.
- [27] a) J. Rogers, A. H. Chang, U. von Ahsen, R. Schroeder, J. Davies, *J. Mol. Biol.* **1996**, *259*, 916–925; b) J.-S. Chia, H.-L. Wu, H.-W. Wang, D.-S. Chen, P.-J. Chen, *J. Biomed. Sci.* **1997**, *4*, 208–216.
- [28] A. R. Ferré-D'Amaré, K. Zhou, J. A. Doudna, *Nature* **1998**, *395*, 567–574.
- [29] a) T. K. Stage, K. J. Hertel, O. C. Uhlenbeck, *RNA* **1995**, *1*, 95–101; b) B. Clouet-d'Orval, T. K. Stage, O. C. Uhlenbeck, *Biochemistry* **1995**, *34*, 11186–11190.
- [30] H. Wang, Y. Tor, *J. Am. Chem. Soc.* **1997**, *119*, 8734–8735.
- [31] R. H. Symons, *Nucleic Acids Res.* **1997**, *25*, 2683–2689.
- [32] R. G. Kuimelis, L. W. McLaughlin, *Chem. Rev.* **1998**, *98*, 1027–1104.
- [33] T. Hermann, E. Westhof, *J. Mol. Biol.* **1998**, *276*, 903–912.
- [34] Y. Tor, T. Hermann, E. Westhof, *Chem. Biol.* **1998**, *5*, R277–R283.
- [35] S. M. Uptain, C. M. Kane, M. J. Chamberlin, *Annu. Rev. Biochem.* **1997**, *66*, 117–172.
- [36] J. Greenblatt, *Curr. Opin. Cell Biol.* **1997**, *9*, 310–319.
- [37] P. H. von Hippel, W. A. Rees, K. S. Wilson, *Nucleic Acids Symp. Ser.* **1995**, *33*, 1–4.
- [38] T. Platt, *Mol. Microbiol.* **1994**, *11*, 983–990.
- [39] T. M. Henkin, *Mol. Microbiol.* **1994**, *13*, 381–387.
- [40] a) M. J. Gait, J. Karn, *Trends Biochem. Sci.* **1993**, *18*, 255–259; b) A. D. Frankel, J. A. T. Young, *Annu. Rev. Biochem.* **1998**, *67*, 1–25.
- [41] K. Yankulov, D. Bentley, *Curr. Biol.* **1998**, *8*, R447–R449.
- [42] a) M. J. Gait, J. Karn, *Trends Biotechnol.* **1995**, *13*, 430–438; b) H.-Y. Mei, A. A. Galan, N. S. Halim, D. P. Mack, D. W. Moreland, K. B. Sanders, H. N. Truong, A. W. Czarnik, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2755–2760; c) J. Wang, S. Y. Huang, I. Choudhury, M. J. Leibowitz, S. Stein, *Anal. Biochem.* **1995**, *232*, 238–242; d) C. Bailly, P. Colson, C. Houssier, F. Hamy, *Nucleic Acids Res.* **1996**, *24*, 1460–1464; e) L. Dassonneville, F. Hamy, P. Colson, C. Houssier, C. Bailly, *Nucleic Acids Res.* **1997**, *25*, 4487–4492; f) A. C. Lim, J. K. Barton, *Bioorg. Med. Chem.* **1997**, *5*, 1131–1136; g) K. Michaelis, M. Kalesse, *Angew. Chem.* **1999**, *111*, 2382–2385; *Angew. Chem. Int. Ed.* **1999**, *38*, 2243–2245.
- [43] F. Hamy, E. R. Felder, G. Heizmann, J. Lazdins, F. Aboul-Ela, G. Varani, J. Karn, T. Klimkait, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3548–3553.
- [44] H.-Y. Mei, D. P. Mack, A. A. Galan, N. S. Halim, A. Heldsinger, J. A. Loo, D. W. Moreland, K. A. Sannes-Lowery, L. Sharmeen, H. N. Truong, A. W. Czarnik, *Bioorg. Med. Chem.* **1997**, *5*, 1173–1184.
- [45] F. Hamy, V. Brondani, A. Florsheimer, W. Stark, M. J. Blommers, T. Klimkait, *Biochemistry* **1998**, *37*, 5086–5095.
- [46] H.-Y. Mei, M. Cui, A. Heldsinger, S. M. Lemrow, J. A. Loo, K. A. Sannes-Lowery, L. Sharmeen, A. W. Czarnik, *Biochemistry* **1998**, *37*, 14204–14212.
- [47] H. An, B. D. Haly, P. D. Cook, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2345–2350.
- [48] A. Farese-Di Giorgio, S. Pairot, N. Patino, R. Condom, C. Di Giorgio, A. Aumelas, A. M. Aubertin, R. Guedj, *Nucleosides Nucleotides* **1999**, *18*, 263–275.
- [49] A. Litovchick, A. G. Evdokimov, A. Lapidot, *FEBS Lett.* **1999**, *445*, 73–79.
- [50] T. Hermann, E. Westhof, *J. Med. Chem.* **1999**, *42*, 1250–1261.
- [51] a) F. Aboul-ela, J. Karn, G. Varani, *Nucleic Acids Res.* **1996**, *24*, 3974–3981; b) J. A. Ippolito, T. A. Steitz, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9819–9824.
- [52] a) J. D. Puglisi, R. Tan, B. J. Calnan, A. D. Frankel, J. R. Williamson, *Science* **1992**, *257*, 76–80; b) J. D. Puglisi, L. Chen, A. D. Frankel, J. R. Williamson, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3680–3684; c) A. S. Brodsky, J. R. Williamson, *J. Mol. Biol.* **1997**, *267*, 624–639.
- [53] a) J. D. Puglisi, L. Chen, S. Blanchard, A. D. Frankel, *Science* **1995**, *270*, 1200–1203; b) X. Ye, R. A. Kumar, D. J. Patel, *Chem. Biol.* **1995**, *2*, 827–840.
- [54] T. L. Sit, A. A. Vaewhongs, S. A. Lommel, *Science* **1998**, *281*, 829–832.
- [55] A. T. Das, B. Klaver, B. Berkhout, *J. Virol.* **1998**, *72*, 9217–9223.
- [56] J. L. Clever, D. A. Eckstein, T. G. Parslow, *J. Virol.* **1999**, *73*, 101–109.
- [57] J. L. Clever, T. G. Parslow, *J. Virol.* **1997**, *71*, 3407–3414.
- [58] R. N. de Guzman, Z. R. Wu, C. C. Stalling, L. Pappalardo, P. N. Borer, M. F. Summers, *Science* **1998**, *279*, 384–388.
- [59] S. Altman, L. Kirsebom in Lit. [1], pp. 351–380.
- [60] a) N. E. Mikkelsen, M. Brännvall, A. Virtanen, L. A. Kirsebom, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6155–6160; b) A. Vioque, *FEBS Lett.* **1989**, *246*, 137–139.
- [61] H. L. True, D. W. Celandier, *J. Biol. Chem.* **1996**, *271*, 16559–16566.
- [62] E. Papadimou, S. Georgiou, D. Tsambaos, D. Drinas, *J. Biol. Chem.* **1998**, *273*, 24375–24378.
- [63] a) C. Massire, L. Jaeger, E. Westhof, *J. Mol. Biol.* **1998**, *279*, 773–793; b) J. L. Chen, J. M. Nolan, M. E. Harris, N. R. Pace, *EMBO J.* **1998**, *17*, 1515–1525.
- [64] Y.-T. Yu, E. C. Scharl, C. M. Smith, J. A. Steitz in Lit. [1], pp. 487–524.
- [65] C. B. Burge, T. Tuschl, P. A. Sharp in Lit. [1], pp. 525–560.
- [66] L. Silver, K. Bostian, *Eur. J. Clin. Microbiol. Infect. Dis.* **1990**, *9*, 455–461.
- [67] C. C. Wang, *Annu. Rev. Pharmacol. Toxicol.* **1995**, *35*, 93–127.
- [68] a) G. Varani, K. Nagai, *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 407–445; b) C. Kambach, S. Walke, K. Nagai, *Curr. Opin. Struct. Biol.* **1999**, *9*, 222–230.
- [69] D. P. Toczyski, A. G. Matera, D. C. Ward, J. A. Steitz, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3463–3467.
- [70] W. Zhong, D. Ganem, *J. Virol.* **1997**, *71*, 1207–1212.
- [71] Y. K. Hwang, M. A. Brinton, *J. Virol.* **1998**, *72*, 4341–4351.
- [72] V. W. Pollard, M. H. Malim, *Annu. Rev. Microbiol.* **1998**, *52*, 491–532.
- [73] H. P. Bogerd, G. L. Huckaby, Y. F. Ahmed, S. M. Hanly, W. C. Greene, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 5704–5708.
- [74] P. Askjaer, J. Kjems, *J. Biol. Chem.* **1998**, *273*, 11463–11471.
- [75] M. L. Zapp, S. Stern, M. R. Green, *Cell* **1993**, *74*, 969–978.
- [76] G. Werstuck, M. L. Zapp, M. R. Green, *Chem. Biol.* **1996**, *3*, 129–137.
- [77] L. Ratmeyer, M. L. Zapp, M. R. Green, R. Vinayak, A. Kumar, D. W. Boykin, W. D. Wilson, *Biochemistry* **1996**, *35*, 13689–13696.
- [78] a) W. D. Wilson, L. Ratmeyer, M. Zhao, D. Ding, A. W. McConaughie, A. Kumar, D. W. Boykin, *J. Mol. Recognit.* **1996**, *9*, 187–196; b) M. L. Zapp, D. W. Young, A. Kumar, R. Singh, D. W. Boykin, W. D. Wilson, M. R. Green, *Bioorg. Med. Chem.* **1997**, *5*, 1149–1155.
- [79] W. K. C. Park, M. Auer, H. Jaksche, C.-H. Wong, *J. Am. Chem. Soc.* **1996**, *118*, 10150–10155.
- [80] K. Li, M. Fernandez-Saiz, C. T. Rigl, A. Kumar, K. G. Ragunathan, A. W. McConaughie, D. W. Boykin, H. J. Schneider, W. D. Wilson, *Bioorg. Med. Chem.* **1997**, *5*, 1157–1172.

- [81] J. L. Battiste, H. Mao, N. S. Rao, R. Tan, D. R. Muhandiram, L. E. Kay, A. D. Frankel, J. R. Williamson, *Science* **1996**, 273, 1547–1551.
- [82] a) X. Ye, A. Gorin, A. D. Ellington, D. J. Patel, *Nat. Struct. Biol.* **1996**, 3, 1026–1033; b) X. Ye, A. Gorin, R. Frederick, W. Hu, A. Majumdar, W. Xu, G. McLendon, A. Ellington, D. J. Patel, *Chem. Biol.* **1999**, 6, 657–669.
- [83] T. R. Cech, B. L. Golden in Lit. [1], pp. 321–349.
- [84] U. von Ahsen, J. Davies, R. Schroeder, *Nature* **1991**, 353, 368–370.
- [85] Y. Liu, M. J. Leibowitz, *Nucleic Acids Res.* **1995**, 23, 1284–1291.
- [86] H. Y. Mei, M. Cui, S. T. Sutton, H. N. Truong, F.-Z. Chung, A. W. Czarnik, *Nucleic Acids Res.* **1996**, 24, 5051–5053.
- [87] V. Lehnert, L. Jaeger, F. Michel, E. Westhof, *Chem. Biol.* **1996**, 3, 993–1009.
- [88] F. Michel, E. Westhof, *J. Mol. Biol.* **1990**, 216, 585–610.
- [89] a) J. H. Cate, A. R. Gooding, E. Podell, K. Zhou, B. L. Golden, C. E. Kundrot, T. R. Cech, J. A. Doudna, *Science* **1996**, 273, 1678–1685; b) B. L. Golden, A. R. Gooding, E. R. Podell, T. R. Cech, *Science* **1998**, 282, 259–264.
- [90] U. von Ahsen, J. Davies, R. Schroeder, *J. Mol. Biol.* **1992**, 226, 935–941.
- [91] H. Wank, J. Rogers, J. Davies, R. Schroeder, *J. Mol. Biol.* **1994**, 236, 1001–1010.
- [92] H.-Y. Mei, M. Cui, S. M. Lemrow, A. W. Czarnik, *Bioorg. Med. Chem.* **1997**, 5, 1185–1195.
- [93] L. Simpson in Lit. [1], pp. 585–608.
- [94] a) T. Hermann, B. Schmid, H. Heumann, U. Göringer, *Nucleic Acids Res.* **1997**, 25, 2311–2318; b) J. Köller, U. F. Müller, B. Schmid, A. Missel, V. Kruft, K. Stuart, H. U. Göringer, *J. Biol. Chem.* **1997**, 272, 3749–3752.
- [95] a) L. D. Etkin, H. D. Lipshitz, *FASEB J.* **1999**, 13, 419–420; b) P. Lasko, *FASEB J.* **1999**, 13, 421–433; c) K. L. Mowry, C. A. Cote, *FASEB J.* **1999**, 13, 435–445; d) G. J. Bassell, Y. Oleynikov, R. H. Singer, *FASEB J.* **1999**, 13, 447–454.
- [96] R. P. Jansen, *FASEB J.* **1999**, 13, 455–466.
- [97] R. J. Buckanovich, R. B. Darnell, *Mol. Cell Biol.* **1997**, 17, 3194–3201.
- [98] R. J. Buckanovich, Y. Y. Yang, R. B. Darnell, *J. Neurosci.* **1996**, 16, 1114–1122.
- [99] H. A. Lewis, H. Chen, C. Edo, R. J. Buckanovich, Y. Y. Yang, K. Musunuru, R. Zhong, R. B. Darnell, S. K. Burley, *Structure* **1999**, 7, 191–203.
- [100] G. Musco, G. Stier, C. Joseph, M. A. Castiglione Morelli, M. Nilges, T. J. Gibson, A. Pastore, *Cell* **1996**, 85, 237–245.
- [101] V. Brown, K. Small, L. Lakkis, Y. Feng, C. Gunter, K. D. Wilkinson, S. T. Warren, *J. Biol. Chem.* **1998**, 273, 15521–15527.
- [102] E. H. Kislaukis, R. H. Singer, *Curr. Opin. Cell Biol.* **1992**, 4, 975–978.
- [103] A. F. Ross, Y. Oleynikov, E. H. Kislaukis, K. L. Taneja, R. H. Singer, *Mol. Cell Biol.* **1997**, 17, 2158–2165.
- [104] G. A. Doyle, N. A. Betz, P. F. Leeds, A. J. Fleisig, R. D. Prokipcak, J. Ross, *Nucleic Acids Res.* **1998**, 26, 5036–5044.
- [105] F. Mueller-Pillasch, U. Lacher, C. Wallrapp, A. Micha, F. Zimmerhackl, H. Hameister, G. Varga, H. Friess, M. Buchler, H. G. Beger, M. R. Vila, G. Adler, T. M. Gress, *Oncogene* **1997**, 14, 2729–2733.
- [106] J. Y. Zhang, E. K. Chan, X. X. Peng, E. M. Tan, *J. Exp. Med.* **1999**, 189, 1101–1110.
- [107] J. O. Deshler, M. I. Highett, T. Abramson, B. J. Schnapp, *Curr. Biol.* **1998**, 8, 489–496.
- [108] S. Shetty, S. Idell, *Am. J. Physiol.* **1998**, 274, L871–L882.
- [109] S. C. Shih, K. P. Claffey, *J. Biol. Chem.* **1999**, 274, 1359–1365.
- [110] a) D. Chagnonovich, S. L. Cohn, *Eur. J. Cancer* **1997**, 33, 2064–2067; b) R. A. Ross, D. L. Lazarova, G. T. Manley, P. S. Smitt, B. A. Spengler, J. B. Posner, J. L. Biedler, *Eur. J. Cancer* **1997**, 33, 2071–2074.
- [111] R. J. Boado, W. M. Pardridge, *Brain Res. Mol. Brain Res.* **1998**, 59, 109–113.
- [112] R. J. Boado, *Neurosci. Lett.* **1998**, 255, 147–150.
- [113] a) S. H. Zaidi, R. Denman, J. S. Malter, *J. Biol. Chem.* **1994**, 269, 24000–24006; b) S. H. Zaidi, J. S. Malter, *J. Biol. Chem.* **1994**, 269, 24007–24013.
- [114] S. H. Zaidi, J. S. Malter, *J. Biol. Chem.* **1995**, 270, 17292–17298.
- [115] L. E. Rajagopalan, C. J. Westmark, J. A. Jarzembowski, J. S. Malter, *Nucleic Acids Res.* **1998**, 26, 3418–3423.
- [116] D. Moazed, H. F. Noller, *Nature* **1987**, 327, 389–394.
- [117] P. Purohit, S. Stern, *Nature* **1994**, 370, 659–662.
- [118] H. F. Chambers, M. A. Sande in *The Pharmacological Basis of Therapeutics*, 9. Ed. (Eds.: J. G. Hardman, L. E. Limbird, P. B. Molinoff, R. W. Ruddon, A. Goodman Gilman), McGraw-Hill, New York, **1996**, pp. 1103–1121.
- [119] Y. Van de Peer, E. Robbrecht, S. de Hoog, A. Caers, P. De Rijk, R. De Wachter, *Nucleic Acids Res.* **1999**, 27, 179–183.
- [120] a) T. Hutchin, I. Haworth, K. Higashi, N. Fischel-Ghodsian, M. Stoneking, N. Saha, C. Arnos, G. Cortopassi, *Nucleic Acids Res.* **1993**, 21, 4174–4179; b) K. Hamasaki, R. R. Rando, *Biochemistry* **1997**, 36, 12323–12328.
- [121] a) J. Woodcock, D. Moazed, M. Cannon, J. Davies, H. F. Noller, *EMBO J.* **1991**, 10, 3099–3103; b) M. I. Recht, D. Fourmy, S. C. Blanchard, K. D. Dahlquist, J. D. Puglisi, *J. Mol. Biol.* **1996**, 262, 421–436; c) M. I. Recht, S. Douthwaite, K. D. Dahlquist, J. D. Puglisi, *J. Mol. Biol.* **1999**, 286, 33–43; d) M. Famulok, A. Hüttenhofer, *Biochemistry* **1996**, 35, 4265–4270; e) Y. Wang, K. Hamasaki, R. R. Rando, *Biochemistry* **1997**, 36, 768–779; f) S. C. Blanchard, D. Fourmy, R. G. Eaton, J. D. Puglisi, *Biochemistry* **1998**, 37, 7716–7724.
- [122] D. Fourmy, M. I. Recht, S. C. Blanchard, J. D. Puglisi, *Science* **1996**, 274, 1367–1371.
- [123] D. Fourmy, S. Yoshizawa, J. D. Puglisi, *J. Mol. Biol.* **1998**, 277, 333–345.
- [124] D. Fourmy, M. I. Recht, J. D. Puglisi, *J. Mol. Biol.* **1998**, 277, 347–362.
- [125] S. Yoshizawa, D. Fourmy, J. D. Puglisi, *EMBO J.* **1998**, 17, 6437–6448.
- [126] C.-H. Wong, M. Hendrix, E. S. Priestley, W. A. Greenberg, *Chem. Biol.* **1998**, 5, 397–406.
- [127] a) R. H. Griffey, M. J. Greig, H. An, H. Sasnor, S. Manalili, *J. Am. Chem. Soc.* **1999**, 121, 474–475; b) R. H. Griffey, S. A. Hofstadler, K. A. Sannes-Lowery, D. J. Ecker, S. T. Crooke, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 10129–10133.
- [128] a) P. B. Alper, M. Hendrix, P. Sears, C.-H. Wong, *J. Am. Chem. Soc.* **1998**, 120, 1965–1978; b) W. A. Greenberg, E. S. Priestley, P. S. Sears, P. B. Alper, C. Rosenbohm, M. Hendrix, S.-C. Hung, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, 121, 6527–6541.
- [129] M. Hendrix, P. B. Alper, E. S. Priestley, C.-H. Wong, *Angew. Chem.* **1997**, 109, 119–122; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 95–98.
- [130] C.-H. Wong, M. Hendrix, D. D. Manning, C. Rosenbohm, W. A. Greenberg, *J. Am. Chem. Soc.* **1998**, 120, 8319–8327.
- [131] J. B. H. Tok, R. R. Rando, *J. Am. Chem. Soc.* **1998**, 120, 8279–8280.
- [132] P. Sears, C.-H. Wong, *Angew. Chem.* **1999**, 111, 2446–2471; *Angew. Chem. Int. Ed.* **1999**, 38, 2300–2324.
- [133] K. Hamasaki, R. R. Rando, *Anal. Biochem.* **1998**, 261, 183–190.
- [134] a) J. Egebjerg, S. Douthwaite, R. A. Garrett, *EMBO J.* **1989**, 8, 607–611; b) I. G. Leviev, C. Rodriguez-Fonseca, H. Phan, R. A. Garrett, G. Heilek, H. F. Noller, A. S. Mankin, *EMBO J.* **1994**, 13, 1682–1686; c) G. T. Tan, A. DeBlasio, A. S. Mankin, *J. Mol. Biol.* **1996**, 261, 222–230; d) C. Spickler, M.-N. Brunelle, L. Brakier-Gingras, *J. Mol. Biol.* **1997**, 273, 586–599; e) B. T. Porse, R. A. Garrett, *J. Mol. Biol.* **1999**, 286, 375–387; f) N. B. Matassova, M. V. Rodnina, R. Endermann, H.-P. Kroll, U. Pleiss, H. Wild, W. Wintermeyer, *RNA* **1999**, 5, 939–956.
- [135] P. C. Ryan, D. E. Draper, *J. Mol. Biol.* **1991**, 221, 1257–1268.
- [136] D. E. Draper, Y. Xing, L. G. Laing, *J. Mol. Biol.* **1995**, 249, 231–238.
- [137] a) Y. Xing, D. E. Draper, *Biochemistry* **1996**, 35, 1581–1588; b) B. T. Porse, I. Leviev, A. S. Mankin, R. A. Garrett, *J. Mol. Biol.* **1998**, 276, 391–404.
- [138] a) G. L. Conn, D. E. Draper, E. A. Lattman, A. G. Gittis, *Science* **1999**, 284, 1171–1174; b) B. T. Wimberly, R. Guymon, J. P. McCutcheon, S. W. White, V. Ramakrishnan, *Cell* **1999**, 97, 491–502.
- [139] B. Clough, M. Strath, P. Preiser, P. Denny, I. R. Wilson, *FEBS Lett.* **1997**, 406, 123–125.
- [140] G. A. McConkey, M. J. Rogers, T. F. McCutchan, *J. Biol. Chem.* **1997**, 272, 2046–2049.
- [141] M. J. Rogers, Y. V. Bukhman, T. F. McCutchan, D. E. Draper, *RNA* **1997**, 3, 815–820.
- [142] A. L. Fitzhugh, *Bioorg. Med. Chem. Lett.* **1998**, 8, 87–92.
- [143] M. S. Iordanov, D. Pribnow, J. L. Magun, T.-H. Dinh, J. A. Pearson, S. L.-Y. Chen, B. E. Magun, *Mol. Cell Biol.* **1997**, 17, 3373–3381.

- [144] a) J. H. Cate, M. M. Yusupov, G. Z. Yusupova, T. N. Earnest, H. F. Noller, *Science* **1999**, 285, 2095–2104; b) G. M. Culver, J. H. Cate, G. Z. Yusupova, M. M. Yusupov, H. F. Noller, *Science* **1999**, 285, 2133–2135.
- [145] a) W. M. Clemons, Jr., J. L. C. May, B. T. Wimberly, J. P. McCutcheon, M. S. Capel, V. Ramakrishnan, *Nature* **1999**, 400, 833–840; b) N. Ban, P. Nissen, J. Hansen, M. Capel, P. B. Moore, T. A. Steitz, *Nature* **1999**, 400, 841–847.
- [146] J. F. Atkins, A. Böck, S. Matsufuji, R. F. Gesteland in Lit. [1], pp. 637–673.
- [147] a) D. Hatfield, S. Oroszlan, *Trends Biochem. Sci.* **1990**, 15, 186–190; b) P. J. Farabaugh, *Annu. Rev. Genet.* **1996**, 30, 507–528.
- [148] a) M. Chamorro, N. Parkin, N. E. Varmus, *Proc. Natl. Acad. Sci. USA* **1992**, 89, 713–717; b) N. M. Wills, R. F. Gesteland, J. F. Atkins, *EMBO J.* **1994**, 13, 4137–4144.
- [149] H. Kang, *Biochim. Biophys. Acta* **1998**, 1397, 73–78.
- [150] a) C. W. Pleij, *Genet. Eng.* **1995**, 17, 67–80; b) X. Chen, H. Kang, L. X. Shen, M. Chamorro, H. E. Varmus, I. Tinoco, *J. Mol. Biol.* **1996**, 260, 479–483; c) L. Su, L. Chen, M. Egli, J. M. Berger, A. Rich, *Nat. Struct. Biol.* **1999**, 6, 285–292.
- [151] M. G. Wallis, B. Streicher, H. Wank, U. von Ahsen, E. Clodi, S. T. Wallace, M. Famulok, R. Schroeder, *Chem. Biol.* **1997**, 4, 357–366.
- [152] C. Wilson, J. Nix, J. Szostak, *Biochemistry* **1998**, 37, 14410–14419.
- [153] B. C. Horsburgh, H. Kollmus, H. Hauser, D. M. Coen, *Cell* **1996**, 86, 949–959.
- [154] S. C. Low, M. J. Berry, *Trends Biochem. Sci.* **1996**, 21, 203–208.
- [155] R. Walczak, E. Westhof, P. Carbon, A. Krol, *RNA* **1996**, 2, 367–379.
- [156] a) C. S. Ramanathan, E. W. Taylor, *Biol. Trace Elem. Res.* **1997**, 56, 93–106; b) L. Grate, *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **1998**, 17, 398–403.
- [157] E. C. Theil, *Met. Ions Biol. Syst.* **1998**, 35, 403–434.
- [158] K. J. Addess, J. P. Basilion, R. D. Klausner, T. A. Rouault, A. Pardi, *J. Mol. Biol.* **1997**, 274, 72–83.
- [159] Y. Ke, J. Wu, E. A. Leibold, W. E. Walden, E. C. Theil, *J. Biol. Chem.* **1998**, 273, 23637–23640.
- [160] M. A. Smith, K. Wehr, P. L. R. Harris, S. L. Siedlak, J. R. Connor, G. Perry, *Brain Res.* **1998**, 788, 232–236.
- [161] E. Chu, C. J. Allegra, *Bioessays* **1996**, 18, 191–198.
- [162] E. Chu, C. J. Allegra, *Adv. Enzyme Regul.* **1996**, 36, 143–163.
- [163] E. Chu, T. Takechi, K. L. Jones, D. M. Voeller, S. M. Copur, G. F. Maley, F. Maley, S. Segal, C. J. Allegra, *Mol. Cell. Biol.* **1995**, 15, 179–185.
- [164] E. Chu, T. Cogliati, S. M. Copur, A. Borre, D. M. Voeller, C. J. Allegra, S. Segal, *Nucleic Acids Res.* **1996**, 24, 3222–3228.
- [165] E. Chu, S. M. Copur, J. Ju, T. M. Chen, S. Khleif, D. M. Voeller, M. Mizunuma, M. Patel, G. F. Maley, F. Maley, C. J. Allegra, *J. Mol. Cell. Biol.* **1999**, 19, 1582–1594.
- [166] E. Chu, D. Voeller, D. M. Koeller, J. C. Drake, C. H. Takimoto, F. Maley, C. J. Allegra, *Proc. Natl. Acad. Sci. USA* **1993**, 90, 517–521.
- [167] J. B.-H. Tok, J. Cho, R. R. Rando, *Biochemistry* **1999**, 38, 199–206.
- [168] J. Cho, R. R. Rando, *Biochemistry* **1999**, 38, 8548–8554.
- [169] K. Nagai, I. W. Mattaj, *RNA-Protein Interactions*, Oxford University Press, Oxford, **1994**.
- [170] K. Nagai, *Curr. Opin. Struct. Biol.* **1996**, 6, 53–61.
- [171] S. Cusack, *Curr. Opin. Struct. Biol.* **1999**, 9, 66–73.
- [172] D. J. Patel, *Curr. Opin. Struct. Biol.* **1999**, 9, 74–87.
- [173] D. E. Draper, L. P. Reynaldo, *Nucleic Acids Res.* **1999**, 27, 381–388.
- [174] K. M. Weeks, D. M. Crothers, *Science* **1993**, 261, 1574–1577.
- [175] T. Hermann, E. Westhof, *Chem. Biol.* **1999**, R335–343.
- [176] A. D. Frankel, C. A. Smith, *Cell* **1998**, 92, 149–151.
- [177] T. Hermann, D. J. Patel, *Science* **2000**, 287, 820–825.
- [178] K. Nadassy, S. J. Wodak, J. Janin, *Biochemistry* **1999**, 38, 1999–2017.
- [179] M. Frugier, P. Schimmel, *Proc. Natl. Acad. Sci. USA* **1997**, 94, 11291–11294.
- [180] T. Hermann, P. Auffinger, E. Westhof, *Eur. Biophys. J.* **1998**, 27, 153–165.
- [181] a) D. E. Draper, V. K. Misra, *Nat. Struct. Biol.* **1998**, 5, 927–930; b) A. L. Feig, O. C. Uhlenbeck in Lit. [1], pp. 287–319; c) E. Westhof in *Water and Biological Macromolecules* (Ed.: E. Westhof), CRC Press, Boca Raton, FL, **1993**, pp. 226–243.
- [182] C. F. Anderson, M. T. Record, *Annu. Rev. Phys. Chem.* **1995**, 46, 657–700.
- [183] T. Hermann, E. Westhof, *Nat. Struct. Biol.* **1999**, 6, 540–544.
- [184] a) G. Werstuck, M. R. Green, *Science* **1998**, 282, 296–298; b) M. Blind, W. Kolanus, M. Famulok, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 3606–3610; c) H. Shi, B. E. Hoffman, J. T. Lis, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 10033–10038.
- [185] a) J. R. Lorsch, J. W. Szostak, *Acc. Chem. Res.* **1996**, 29, 103–110; b) B. E. Eaton, *Curr. Opin. Chem. Biol.* **1997**, 1, 10–16; c) Y. Li, R. R. Breaker, *Curr. Opin. Struct. Biol.* **1999**, 9, 315–323; d) M. Famulok, *Curr. Opin. Struct. Biol.* **1999**, 9, 324–329.
- [186] a) J. Feigon, T. Dieckmann, F. W. Smith, *Chem. Biol.* **1996**, 3, 611–617; b) D. J. Patel, *Curr. Opin. Chem. Biol.* **1997**, 1, 32–46.
- [187] D. J. Patel, A. K. Suri, F. Jiang, L. Jiang, P. Fan, R. A. Kumar, S. Nonin, *J. Mol. Biol.* **1997**, 272, 645–664.
- [188] a) S. E. Osborne, I. Matsumura, A. D. Ellington, *Curr. Opin. Chem. Biol.* **1997**, 1, 5–9; b) M. Famulok, G. Mayer, *Curr. Top. Microbiol. Immunol.* **1999**, 243, 123–136.
- [189] A. D. Ellington, *Drug Dev. Res.* **1994**, 33, 102–115.
- [190] W. D. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski, D. Boykin, *Biochemistry* **1993**, 32, 4098–4104.
- [191] R. Schroeder, U. von Ahsen, *Nucleic Acids Mol. Biol.* **1996**, 10, 53–74.
- [192] H. Wang, Y. Tor, *Angew. Chem.* **1998**, 110, 117–120; *Angew. Chem. Int. Ed.* **1998**, 37, 109–111.
- [193] M. Fernandez-Saiz, H.-J. Schneider, J. Sartorius, W. D. Wilson, *J. Am. Chem. Soc.* **1996**, 118, 4739–4745.
- [194] L. Jiang, D. J. Patel, *Nat. Struct. Biol.* **1998**, 5, 769–774.
- [195] L. Jiang, D. J. Patel, *Structure* **1999**, 7, 817–827.
- [196] T. Hermann, E. Westhof, *Structure* **1998**, 6, 1303–1314.
- [197] I. Hoch, C. Berens, E. Westhof, R. Schroeder, *J. Mol. Biol.* **1998**, 282, 557–569.
- [198] T. Hermann, E. Westhof in *RNA-Binding Antibiotics* (Eds.: M. G. Wallis, R. Schroeder), Landes Bioscience, Austin, TX, **2000**, pp. 148–158.
- [199] S. Wang, P. W. Huber, M. Cui, A. W. Czarnik, H.-Y. Mei, *Biochemistry* **1998**, 37, 5549–5557.
- [200] T. Hermann, E. Westhof, *Biopolymers* **1999**, 48, 155–165.
- [201] L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, *Chem. Biol.* **1997**, 4, 35–50.
- [202] F. Leclerc, R. Cedergren, *J. Med. Chem.* **1998**, 41, 175–182.
- [203] P. Sears, C.-H. Wong, *Chem. Commun.* **1998**, 1161–1169.
- [204] P. Burgstaller, M. Famulok, *J. Am. Chem. Soc.* **1997**, 119, 1137–1138.
- [205] P. Burgstaller, T. Hermann, C. Huber, E. Westhof, M. Famulok, *Nucleic Acids Res.* **1997**, 25, 4018–4027.
- [206] W. Saenger, *Principles of Nucleic Acid Structure*, Springer, New York, **1984**.
- [207] B. C. Baguley, *Anticancer Drug Des.* **1991**, 6, 1–35.
- [208] C. S. Chow, F. M. Bogdan, *Chem. Rev.* **1997**, 97, 1489–1513.
- [209] S. Yao, W. D. Wilson, *J. Biomol. Struct. Dynam.* **1992**, 10, 367–387.
- [210] T. Hermann, H. Heumann, *RNA* **1995**, 1, 1009–1017.
- [211] C. S. Chow, J. K. Barton, *Biochemistry* **1992**, 31, 5423–5429.
- [212] C. S. Poornima, P. M. Dean, *J. Comput. Aided Mol. Des.* **1995**, 9, 521–531.
- [213] a) S. R. Holbrook, S. H. Kim, *Biopolymers* **1997**, 44, 3–21; b) M. Egli, *Antisense Nucleic Acid Drug. Dev.* **1998**, 8, 123–128.
- [214] a) G. Otting, *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, 31, 259–285; b) A. T. Phan, J. L. Leroy, M. Gueron, *J. Mol. Biol.* **1999**, 286, 505–519.
- [215] a) P. Auffinger, E. Westhof, *Curr. Opin. Struct. Biol.* **1998**, 8, 227–236; b) M. Feig, B. M. Pettitt, *Structure* **1998**, 6, 1351–1354.
- [216] C. Oubridge, N. Ito, P. R. Evans, C.-H. Teo, K. Nagai, *Nature* **1994**, 372, 432–438.
- [217] R. E. Botto, B. Coxon, *J. Am. Chem. Soc.* **1983**, 105, 1021–1028.
- [218] W. G. Scott, J. B. Murray, J. R. P. Arnold, B. L. Stoddard, A. Klug, *Science* **1996**, 274, 2065–2069.

Gemini Surfactants



General structure (top), four examples of gemini surfactants, and three (six?) Emory graduate students working in the field of geminis: Bessie Mbadugha (left), Kevin Caran (right), and Andrey Peresykin (bottom).

Gemini Surfactants

Fredric M. Menger* and Jason S. Keiper

How easy it is to dismiss the humdrum surfactant! After all, its structure is unglamorous by present-day norms. And the surfactant has been entrenched in so many areas of commerce for so many decades that its chemistry might seem old and tired. The purpose of this review is to persuade the reader otherwise, all the while focusing on a remarkable new surfactant, the gemini. Geminis, the common name for “bis-surfactants”, can self-assemble at concentrations

almost a hundredfold lower than for corresponding conventional surfactants. Surface activity can be improved a thousandfold. Geminis have already shown promise in skin care, antibacterial regimens, construction of high-porosity materials, analytical separations, and solubilization processes. Scores of patents dealing with geminis have appeared in the last few years. Indeed, geminis might well turn out, in the opinion of some, to be more useful to “l’homme de la rue” than crown

ethers or fullerenes. This review delves into such topics as synthesis, critical micellization concentration, aggregate size and shape, gels, vesicles, and films. The information comes from scientists all over the world; one might say that gemini research is bathed in a continuous sunlight or summer. No prior knowledge of colloid chemistry is presupposed in this article.

Keywords: colloids • micelles • surface chemistry • surfactants

1. Introduction

One of the last things chemistry needs is new and superfluous terminology. We felt justified in coining the expression “gemini surfactant” only because geminis have structures and properties that are unique to the world of surfactants.^[1, 2] Thus, a conventional surfactant has a single hydrocarbon tail connected to an ionic or polar headgroup. In contrast, a simple gemini has the arrangement shown in Figure 1. A

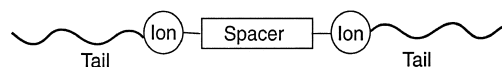


Figure 1. Schematic representation of the gemini surfactant structure.

specific example would be $C_{12}H_{25}N^+(CH_3)_2-CH_2-C_6H_4-CH_2-N^+(CH_3)_2C_{12}H_{25}$. Actually, related surfactants with more than two tails are now known, and we have absorbed them into the gemini classification even though this creates something of a misnomer. As far as the unique properties are concerned, geminis can be orders of magnitude more surface-active than comparable conventional surfactants.^[3] And

apparent critical micellization concentrations (where surfactants abruptly aggregate in water) can be larger for longer chains than for shorter chains (just the reverse of normal).^[2] It is the purpose of this review to inform the general reader about a remarkable set of compounds.

Before proceeding, things should be clarified historically. In 1991 we assigned the word “gemini” to bis-surfactants in which the spacer is rigid (e.g. a benzene or stilbene system).^[1] The idea was to examine how enforced separation of the two hydrocarbon chains within a surfactant molecule would affect its properties. It was only later that others extended the definition to include all such double-surfactants regardless of whether the spacers were rigid or not. The point here is that, under this more general definition, geminis were known long before our own entry in the area. Special mention should be made of Bunton et al., who studied the catalysis of nucleophilic substitutions by “dicationic detergents”;^[4] of Devinsky et al., who reported on the surface activity and micelle formation of some new “bisquaternary ammonium salts”;^[5] and of Okahara et al., who prepared and examined “amphiphilic compounds with two sulfate groups and two lipophilic alkyl chains”.^[6]

How quickly these days the summarizable becomes the unsummarizable! In the last half-dozen years, interest in geminis has soared and, along with it, so has the publication rate. Rosen and Tracy, in a particularly readable account appearing in a 1998 issue of *Journal of Surfactants and Detergents*, list over 60 journal articles and nearly 40

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patents.^[7] (Our own work, having been supported by two public agencies, the NIH and NSF, was never patented.) In front of us is a stack of virtually all papers on geminis. The nations from which the authors hail are the following: France (36); USA (25); Japan (21); India (11); Germany, Israel, The Netherlands, and Spain (7); Slovakia (6); United Kingdom (5); New Zealand (4); Italy (3); Belgium and Russia (2); Argentina, Greece, and Sweden (1). Adolf Wurtz once remarked that chemistry is a French science, and when it comes to gemini chemistry it must be admitted his country has played a leading role. However, we prefer to take a more global view and express pleasure that gemini research is an activity which is never denied sun nor summer.

A disadvantage of our particular style of review is that some contributors to the field are not mentioned and are, therefore, owed an apology. It may be only scant compensation that within our cited papers there appear reference lists that, taken collectively, include everybody's work.

2. Structure

The first task is to give a representative sampling of gemini structures that have already been synthesized, and Scheme 1 serves that purpose. Relevant structural features, many of which are illustrated in Scheme 1, include:

- All geminis possess at least two hydrophobic chains and two ionic or polar groups.
- A great deal of variation exists in the nature of the spacer, which can be short (2 methylene groups) or long (12 methylene groups); rigid (stilbene) or flexible (methylene chain); and polar (polyether) or nonpolar (aliphatic, aromatic).
- The polar group can be positive (ammonium), negative (phosphate, sulfate, carboxylate), or nonionic (polyether, sugar^[18]).

- Although the great majority of geminis have two identical polar groups and two identical chains, unsymmetric geminis are known.^[15, 19]
- "Geminis" with three or more polar groups or tails have been synthesized.^[20–23]

One can appreciate, from a brief inspection of Scheme 1, how colloid chemistry, for so long confined to conventional and commercially available surfactants, has been able to expand its outlook with fresh new possibilities.

3. Synthesis

Before physical characterization of a gemini can begin, the compound must, of course, be synthesized. Understandably, this is an impediment for those who are not inclined toward synthetic organic chemistry. Two factors ameliorate the problem. First, gemini synthesis and purification, while sometimes not trivial, are also not tedious by present-day standards. Second, those who are repelled by synthesis can often seek help from those who are not. We (meaning the students of Emory who seem to enjoy synthesis) have prepared over one hundred geminis, many of which have been sent worldwide to academic and industrial laboratories alike. (We are especially pleased when receipt of requested compounds is acknowledged with a brief letter of thanks.) Fruitful collaborations between groups of different expertise are common in the gemini arena.

Simple cationic geminis, $\text{RN}^+(\text{CH}_3)_2-(\text{CH}_2)_n-\text{N}^+(\text{CH}_3)_2\text{R}$ (R = a long chain) can be prepared as shown in Scheme 2 either by method A or B (with method B being preferable when $n = 2$). Typically, one boils under reflux a mixture of the reagents in dry ethanol for two or three days and purifies the product by recrystallization.^[8] Method A is attractive when the dibromide is reactive and commercially available (e.g. $\text{BrCH}_2\text{C}_6\text{H}_4\text{CH}_2\text{Br}$ to create an aromatic spacer).^[2] If the

Fredric M. Menger is the author of two previous reviews in Angewandte Chemie incorporating his group's other research interests in self-assembling systems and giant vesicles. He received his B.A. from the Johns Hopkins University in 1958 and his Ph.D. from the University of Wisconsin in 1963. He subsequently was Postdoctoral Research Fellow at the Northwestern University (1963–1965). A list of publications and his scientific credentials can be found on the Internet (<http://www.emory.edu/CHEMISTRY/faculty/menger.html>). Rather unknown are his diversions from the stress of chemical research: mountain climbing (the first American to ascend the highest peak in Russia); music (blues harmonica under a pseudonym); field work (water and road projects in Nicaragua); and biology (a forthcoming book on the evolution of the mind).

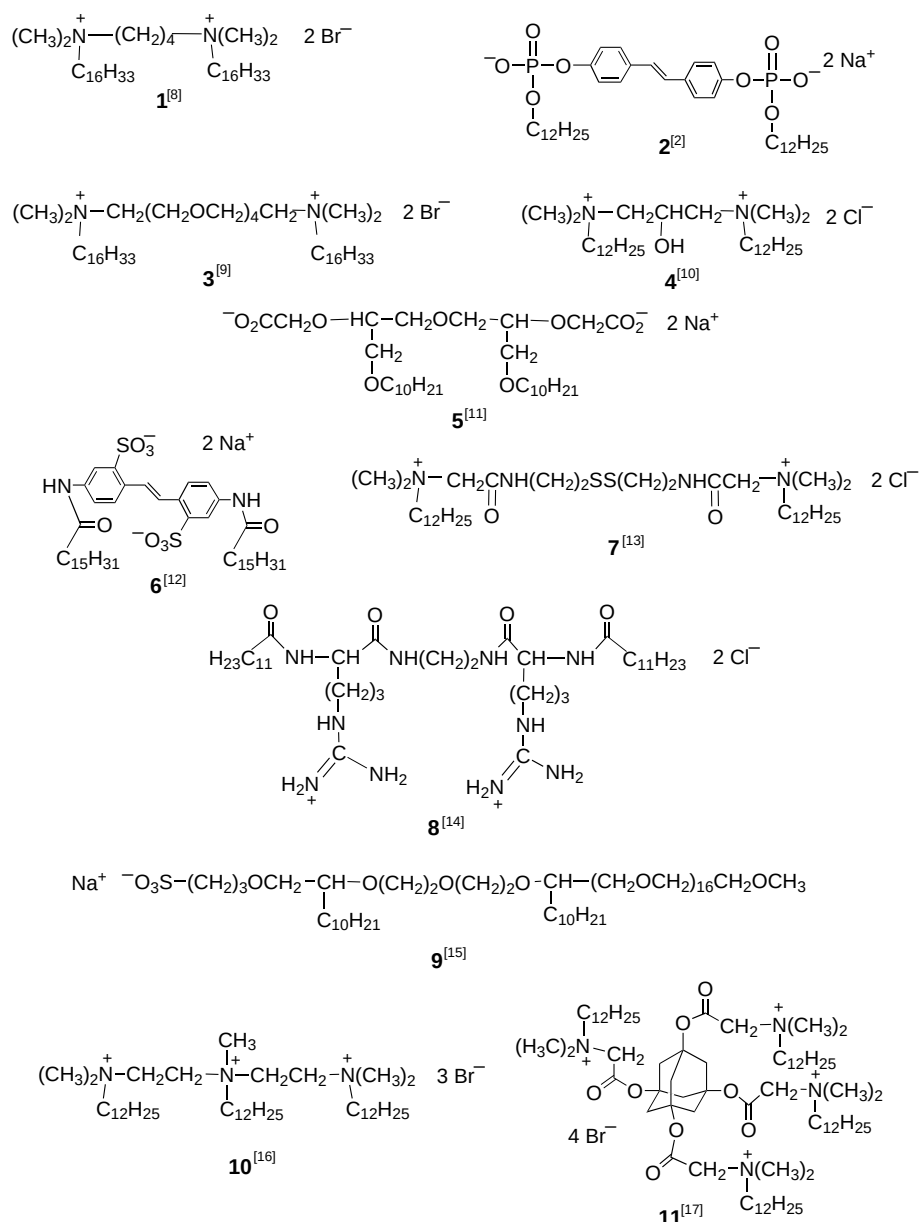
Jason S. Keiper is a graduate student in Professor Menger's group at Emory University. After this he will be pursuing postdoctoral studies with Professor Joseph DeSimone at the University of North Carolina. His interest in colloid chemistry was sparked during undergraduate work with Professor Larry Romsted at Rutgers University. While surfactants and lipids occupy the majority of his time, he finds solace in his tenor saxophone and stock cars.



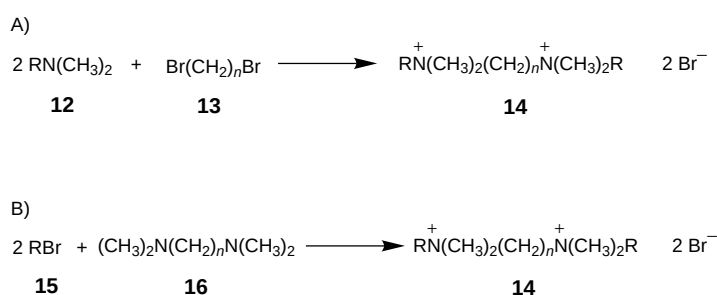
F. M. Menger (on the right)



J. S. Keiper



Scheme 1. Representative sampling of gemini structures that have already been synthesized.



Scheme 2. Synthesis of simple cationic geminis. Method B is preferred when $n = 2$.

dibromide is not purchasable, then it might be possible to synthesize it from the corresponding dialcohol (e.g. $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_3\text{CH}_2\text{OH}$ plus PBr_3 gives $\text{BrCH}_2(\text{CH}_2\text{OCH}_2)_3\text{CH}_2\text{Br}$ and, from there, a gemini with a polyoxyethylene

spacer).^[9] Variations on these themes are shown in the top part of Scheme 3; an alkylation of the tertiary amine occurs at the less substituted epoxide carbon atom, leading to **18**.^[10] A specific example of Method B is shown in the preparation of “trimeric” gemini **20**; over 40 h in 1-propanol at 100 °C, followed by column chromatography, were required for this reaction.^[22]

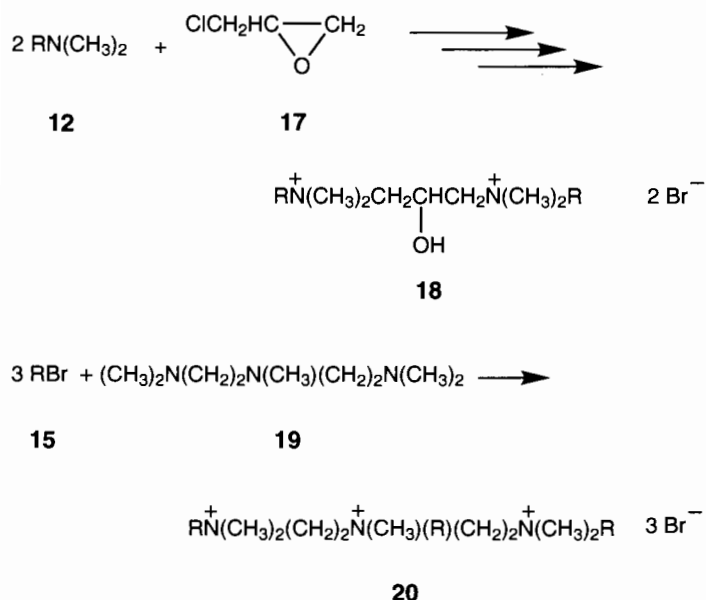
Anionic gemini **22**, a bis-phosphodiester with a flexible spacer, has been synthesized as detailed in Scheme 4 using well established chemistry.^[24] Synthesis of the corresponding gemini with a rigid stilbene spacer, **2**, also involved a multistep but routine procedure.^[2]

Geminis can be as complex as the desires and ambitions of the investigator allow. Four examples of more intricate geminis are given in Schemes 5–8. No particular lesson is intended here except, perhaps, that synthetic chemistry provides an opportunity for the colloid chemist to explore structure–activity relationships that are otherwise beyond reach. Virtually all known analytical methods have already been applied to micellar systems composed of conventional surfactants. This mode of research, milked to exhaustion, is being revived with wonderful new structures. To illustrate the diversity made possible through synthesis, we might mention a recently obtained gemini-type surfactant with four ionic groups and four chains tetrahedrally disposed about an adamantane nucleus (compound **11** in

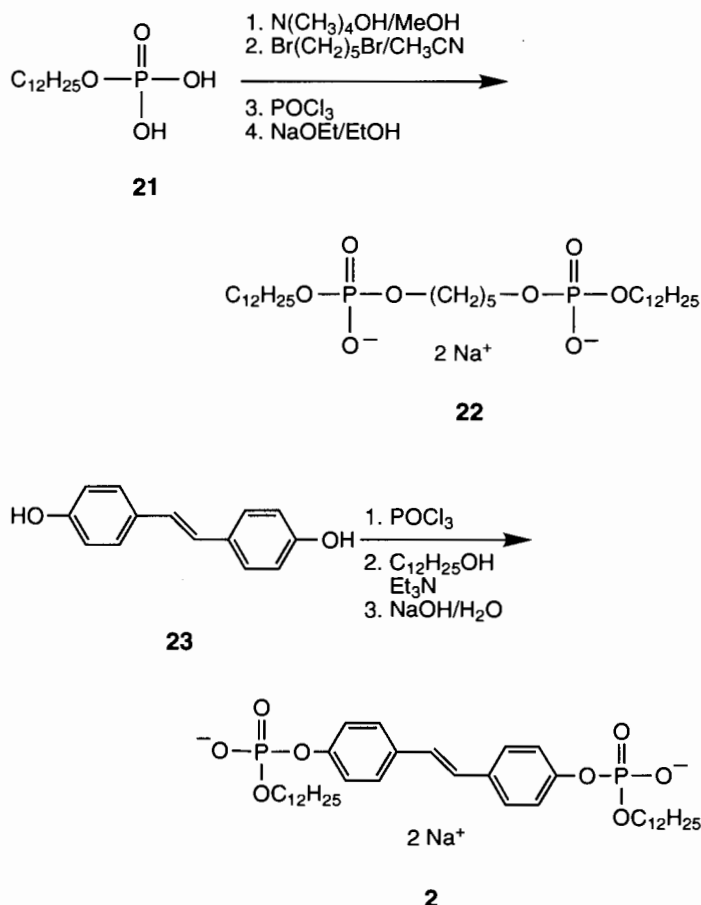
Scheme 1).^[17] At the time of this writing, we have no data, and few premonitions, concerning the colloidal properties of such a molecule. As with all surfactants, the purity of the geminis is of critical concern. This concern is reflected, for example, in diphosphate geminis (e.g. **2**), which we characterized by NMR spectroscopy (^1H , ^{13}C , ^{31}P), mass spectrometry (FAB), and elemental analysis.^[2] Single ^{31}P NMR signals were particularly satisfying indications of purity.

4. Critical Micellization Concentration

The colloid chemist has a host of experimental options when contemplating the characterization of a newly acquired surfactant: calorimetry, conductometry, ESR spectroscopy, goniometry, microscopy, light scattering, neutron scattering, NMR spectroscopy, rheometry, spectrophotometry, and ten-

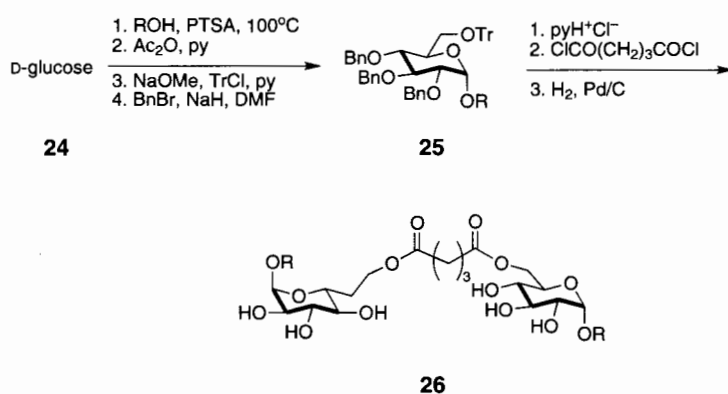


Scheme 3. Top: A variation to methods A and B (Scheme 2) for the synthesis of cationic geminis.^[10] Bottom: A specific example of method B for the synthesis of **20**.^[22]

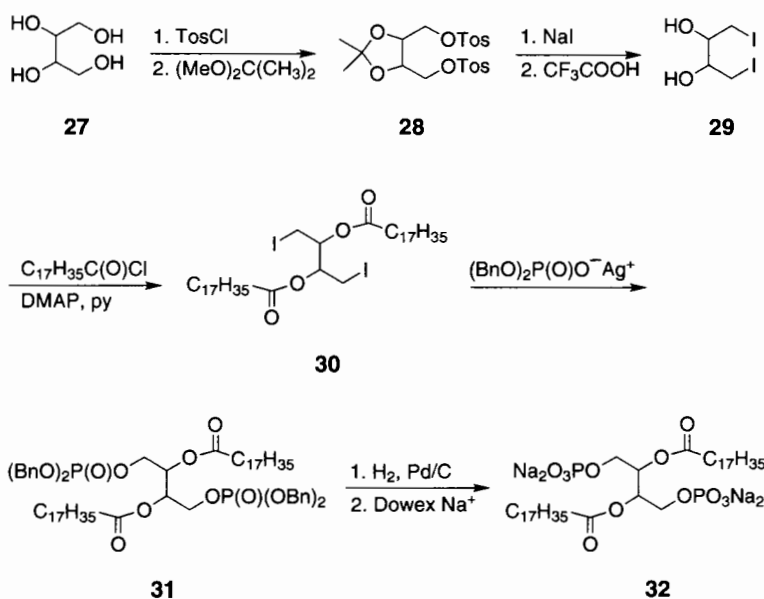


Scheme 4. Synthesis of **22**^[24] and **2**.^[2]

siometry to name a few. Information is thereby acquired on micelle formation and structure, surface activity, solubilization, adsorption, wetting, foaming, phase behavior, and thermotropic properties. One parameter, however, stands out as the mainstay, the keystone of all surfactant research: the critical micellization concentration (CMC). The CMC is the concentration above which monomeric surfactant mole-



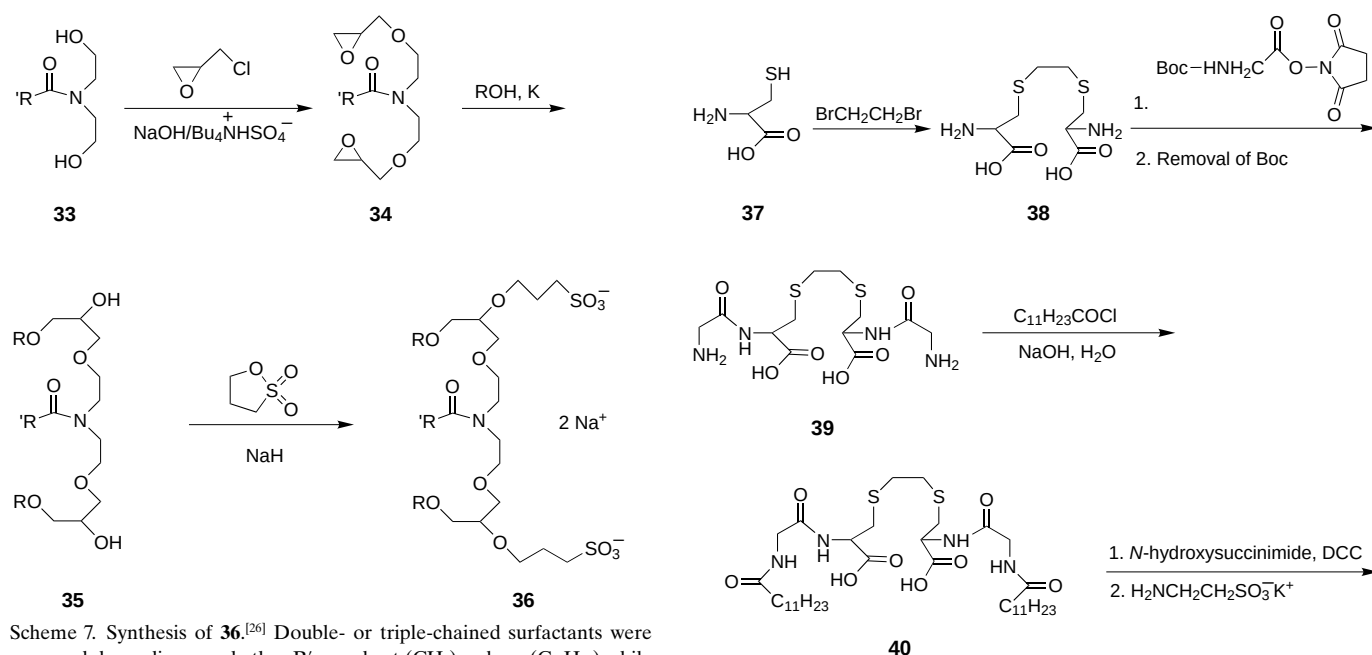
Scheme 5. Synthesis of **26**.^[18] The authors carried out the synthesis with R = butyl, but the sequence is applicable to longer chains as well. Bn = benzyl, PTSA = *p*-toluenesulfonic acid, py = pyridyl, Tr = triphenylmethyl (trityl).



Scheme 6. Synthesis of **32**.^[25] Three stereoisomers of the product (*R,R*, *S,S*, and *R,S*) were prepared. Tos = toluene-4-sulfonyl.

cules abruptly assemble into aggregates called micelles. "Abruptly" is, of course, a subjective term. If the micelles are small (e.g. 10 molecules), then the CMC may embody a concentration range. If, on the other hand, the micelles are a more usual size of 50–100 molecules, then the CMC is indeed sharply defined owing to the highly cooperative nature of the aggregation process. In any event, the CMC is useful in that it reveals the propensity of the surfactant to assemble in water. For example, it stands to reason that the longer a surfactant's tail, the lower its CMC. Hydrophobic forces, opposed by electrostatic repulsion among the ionic headgroups at the micelle surface, drive the micellization. Micelles are known to be disorganized assemblies with interiors consisting of mobile, nonlinear hydrocarbon chains.^[28]

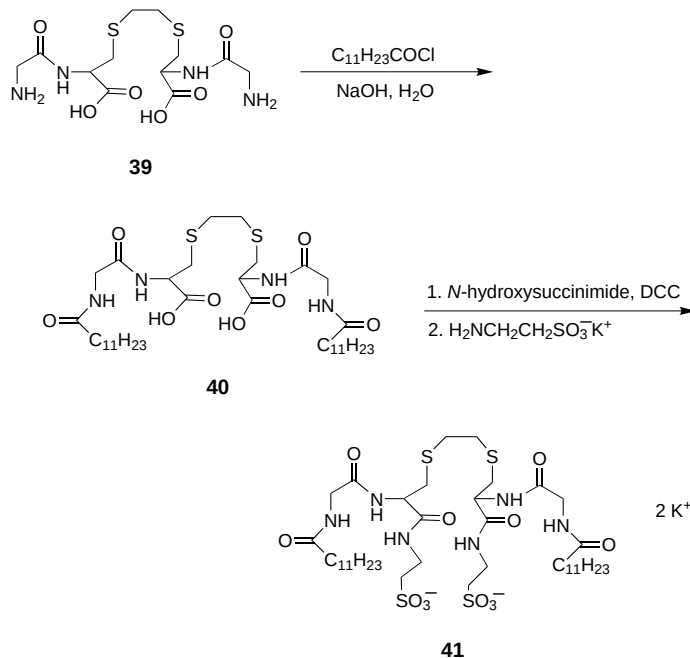
Surface tension is probably the most common means of determining the CMC. Below the CMC, surfactant molecules position themselves at the air/water interface and thus lower the surface tension. Above the CMC, any added surfactant prefers to join a micelle rather than to enter the interfacial film. Consequently, a plot of surface tension versus concentra-



Scheme 7. Synthesis of **36**.^[26] Double- or triple-chained surfactants were prepared depending on whether R' was short (CH₃) or long (C₁₁H₂₃) while keeping R long (C₁₀H₂₁).

tion decreases steeply below the CMC and levels off above it; the break in the plot is taken as the CMC.^[29] The method is fast, convenient, and nondestructive to the surfactant. The only cautionary note is that surface tension can be time-dependent, so that it is often advisable to make measurements on samples that have rested undisturbed for many hours.^[2]

Table 1 lists CMC values for 14 geminis (preceded by four conventional surfactants for comparison purposes). Depending upon how the data were obtained, CMCs can differ by twofold or more, but such an uncertainty is insignificant relative to the large variations evident. Interesting generalizations emerge from the numbers:



Scheme 8. Synthesis of **41**.^[27] Boc = *tert*-butoxycarbonyl, DCC = *N,N'*-dicyclohexylcarbodiimide.

- a) Geminis have remarkably low CMC values compared with corresponding surfactants of equivalent chain length. (The comparison is, justifiably, made on a “per ionic headgroup” basis while neglecting the spacer.) For example, the CMC in entry 5 is 16 times smaller than in entry 1, and the CMC in entry 18 is 80 times smaller than in entry 4.

Table 1. Critical micellization concentrations (CMC) of representative gemini surfactants.^[a]

Entry	Surfactant	CMC [mM]	Ref.
1	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₃ Br [−]	16	[7]
2	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₃ Cl [−]	22	[20]
3	C ₁₆ H ₃₃ N ⁺ (CH ₃) ₃ Br [−]	1	[30]
4	C ₁₂ H ₂₅ OSO ₃ [−] Na ⁺	8	[7]
5 ^[b]	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −(CH ₂) _n −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅ 2 Br [−]	1	[31]
6	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −(CH ₂) ₁₆ −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅ 2 Br [−]	0.12	[8]
7	C ₁₆ H ₃₃ N ⁺ (CH ₃) ₂ −(CH ₂) ₂ −N ⁺ (CH ₃) ₂ C ₁₆ H ₃₃ 2 Br [−]	0.003	[32]
8	C ₈ H ₁₇ N ⁺ (CH ₃) ₂ −(CH ₂) ₃ −N ⁺ (CH ₃) ₂ C ₈ H ₁₇ 2 Br [−]	55	[33]
9	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −(CH ₂) ₂ −O−(CH ₂) ₂ −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅ 2 Cl [−]	0.5	[7]
10	C ₁₆ H ₃₃ N ⁺ (CH ₃) ₂ −(CH ₂) ₅ −N ⁺ (CH ₃) ₂ C ₁₆ H ₃₃ 2 Br [−]	0.009	[34]
11	C ₁₆ H ₃₃ N ⁺ (CH ₃) ₂ −(CH ₂) ₂ −O−(CH ₂) ₂ −N ⁺ (CH ₃) ₂ C ₁₆ H ₃₃ 2 Br [−]	0.004	[34]
12	C ₁₆ H ₃₃ N ⁺ (CH ₃) ₂ −CH ₂ −(CH ₂ −O−CH ₂) ₃ −CH ₂ −N ⁺ (CH ₃) ₂ C ₁₆ H ₃₃ 2 Br [−]	0.02	[9]
13	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −CH ₂ −CH(OH)−CH ₂ −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅ 2 Br [−]	0.8	[10]
14 ^[c]	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −CH ₂ −C ₆ H ₄ −CH ₂ −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅	0.03	[29]
15	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −CH ₂ −CH(OH)−CH(OH)−CH ₂ −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅ 2 Br [−]	0.7	[35]
16	C ₁₂ H ₂₅ N ⁺ (CH ₃) ₂ −CH ₂ −CH(OH)−CH ₂ −N ⁺ (CH ₃) ₂ −CH ₂ −CH(OH)−CH ₂ −N ⁺ (CH ₃) ₂ C ₁₂ H ₂₅ 3 Cl [−]	0.5	[36]
17	C ₁₂ H ₂₅ OPO ₂ [−] −O−(CH ₂) ₆ −O−PO ₂ [−] OC ₁₂ H ₂₅ 2 Na ⁺	0.4	[34]
18	C ₁₀ H ₂₁ O−CH ₂ −CH(OSO ₃ [−])−CH ₂ −O−(CH ₂) ₂ −O−CH ₂ −CH(OSO ₃ [−])−CH ₂ −OC ₁₀ H ₂₁ 2 Na ⁺	0.01	[37]

[a] Entries 1–4 list conventional surfactants for comparison purposes. [b] *n* = 3–8. [c] In 0.1N NaCl.

- b) The CMC values are not sensitive to the polarity of short spacers (2–8 atoms). Attention is called to the C₁₂ geminis in entries 5, 9, 13, 15, and 16, all with diverse spacers but with CMC values within a factor of 2 of each other.
- c) A long hydrocarbon spacer of 16 methylene groups reduces the CMC almost tenfold relative to a short spacer of 3–8 methylene groups (compare entries 5 and 6). No doubt the long spacer contributes to the overall hydrophobicity of the gemini, reducing the monomer solubility and enhancing its tendency to self-assemble.
- d) Geminis are more responsive to tail length than conventional surfactants. Thus, increasing the tail length by four carbon atoms in a conventional cationic surfactant lowers the CMC 16-fold (entries 1 and 3). A corresponding tail lengthening in the gemini series lowers the CMC by two orders of magnitude (compare entries 5, 7, and 10).
- e) Other factors being more or less equal, anionic surfactants have somewhat lower CMC values than their cationic counterparts (compare entries 5 and 17).

5. Aggregation Numbers

A critical micellization concentration says nothing about the size or shape of a micelle. One suspects that gemini micelles are not of the small, “5–10 monomer” variety because plots of surface tension versus concentration show a sharp bend at the CMC.^[35] Small micelles would have produced a broad transition region. In any event, the question can be settled by determining the number of molecules per micelle (i.e., the aggregation number or AN) using one of several methods: light or neutron scattering and steady-state or time-resolved fluorescence quenching. Since light scattering according to P. Debye is tedious and requires extrapolation to low concentrations (owing to interfering micelle–micelle interactions), current use of the method is rare.^[38] Neutron scattering is more common but costly and not generally available. This leaves fluorescence methods involving a probe (often pyrene) and an appropriate quencher. Steady-state fluorescence quenching is especially popular, and it merits a brief description.

In the steady-state fluorescence method, one measures the emission of a probe (whose concentration is much smaller than the concentration of the micelles, [M]) as the quencher concentration, [Q], is increased from 0 to about twice that of the micelles. An ordinary spectrofluorometer works fine.^[39] The major assumptions are a) the probe fluorescence lifetime is much shorter than the residence times of the probe and the quencher within the micelle; b) quenching is much faster than probe decay; and c) Poisson statistics control the distribution of probe and quencher among the micelles. Under these circumstances, the delightfully simple Equation (1) is obeyed (where I_0 and I_Q are the fluorescence emission in the absence and presence of quencher, respectively).

$$\frac{I_0}{I_Q} = \exp\left(\frac{[Q]}{[M]}\right) \quad (1)$$

By plotting $\ln(I_0/I_Q)$ versus [Q] at a constant total concentration of surfactant, [S]_{total}, one obtains the concentration of

the micelles. Assuming that the concentration of the surfactant monomer at all times equals the CMC, then the AN can be calculated from Equation (2) for which all three terms to the right of the equal sign are now known.

$$AN = \frac{[S]_{\text{total}} - \text{CMC}}{[M]} \quad (2)$$

Before specifying AN values, it will be helpful to introduce a shorthand notation for the cationic geminis. A gemini with two C₁₂ tails and a C₃ spacer separating the quaternary nitrogen atoms (see entry 5 in Table 1) will, for example, be designated 12-3-12. The AN for 12-3-12 is 45 (determined by pyrene fluorescence quenching at 0.02 M surfactant).^[39] Small-angle neutron scattering data gave AN values of 35 and 74 for 10-3-10 (1 wt%) and 16-5-16 (2.5 mM), respectively.^[40, 41] Gemini micelles tend to expand at increased concentrations, with 16-5-16 growing from 74 at 2.5 mM to 138 at 50 mM.^[42] Of course, just as the CMC says nothing about the AN, the AN reveals little about micelle shape. This latter topic is covered in Section 6. For the moment it is sufficient to know that gemini aggregation numbers (as far as they are known) are usually not out of the ordinary relative to those of single-chained analogues.

6. Micelle Shape

Micelle shape is a complicated business, as it depends not only on surfactant structure but also upon solution conditions such as concentration, temperature, and ionic strength. Yet despite its complexities, shape is worth discussing because it affects rheological and solubilization properties, which are important issues from a practical standpoint. Spherical micelles, formed by many conventional surfactants at moderate concentrations, are favored by large headgroups which impair orderly packing. If there are two chains per headgroup, as in a phospholipid, then the headgroup and chains are of roughly equal diameter and the compound can pack into a parallel array (i.e., a bilayer). In addition to spherical micelles, one finds shapes that are oblate (disklike) or prolate (like a medicine capsule or rod). Nonspherical micelles can be formed even by single-chained surfactants; cetyltrimethylammonium bromide above 0.2 M, for example, assembles into micelles with an elongated shape.^[43]

Small-angle neutron scattering indicates that 16-3-16 at 2.5–10 mM and 30 °C forms disklike micelles.^[44] Upon heating to 45–70 °C, the disk converts into a rod (with a length of 500 Å and a radius of 27 Å) owing, probably, to subtle changes in the effective charge per monomer. With spacers of 5 or longer, micelles are prolate-shaped. Thus, spacer length controls micelle shape more than it does CMC. In summary, 16-3-16 behaves as if its headgroup were unusually small since the spacer is shorter than the equilibrium distance between charged headgroups within a conventional micelle; more orderly packing is thus possible. When a spacer length is increased to 5 and above, the effective headgroup area increases to a relatively normal value, and the micelle rearranges its shape closer to that assumed by a cetyltrimethylammonium bromide micelle.

Cryo-transmission electron microscopy studies on 12-*n*-12 and 16-*n*-16 geminis have produced photos with the following morphologies: a) giant, entangled, wormlike micelles with 12-2-12 and 12-3-12 (consistent with an observed viscoelasticity at higher concentrations); b) spherical micelles for 12-*n*-12 (*n* = 4, 8, 12); c) vesicles of 12-16-12; d) vesicles, membrane fragments, and wormlike micelles for a sample of 16-3-16 prepared from a 12 mM solution.^[45] This last observation is somewhat worrisome as it conflicts with the results from small-angle neutron scattering (SANS). Since both cryo-electron microscopy (cryo-EM) and SANS entail assumptions and/or potential artifacts, the source of the discrepancy is unclear.

In the absence of any added salt, solutions of less than 2 wt % 12-2-12 display low viscosities. Above 2 wt %, however, the viscosity rises abruptly due to formation of wormlike micelles.^[46] The average length of the worms, and hence the viscosity, is affected by two opposing forces: a) electrostatic repulsive energy among the cationic nitrogen atoms that favors scission of the worms; and b) “end-cap energy” that favors micelle growth by minimizing the number of high-energy termini. At elevated 12-2-12 concentrations, electrostatic interactions become screened, end-cap energy assumes a more dominant role, and the micelles grow into semiflexible worms. It is interesting that micelles can be forced to grow even at lower 12-2-12 concentrations by an applied shear that exceeds a certain critical shear rate.^[47] The tendency to form worms increases as the spacer length decreases and the chain length decreases.

Attention should be drawn to theoretical modeling of ionic and nonionic geminis, with both hydrophobic and hydrophilic spacers, using Monte Carlo simulations.^[48] Among the various conclusions, we cite the following:

- Short hydrophobic spacers give grossly nonspherical micelles (“threads”), whereas long hydrophobic spacers lead to rodlike micelles, in agreement with experiment.
- As the bending stiffness of the hydrophobic spacer increases, so does the CMC.
- Micelles with hydrophilic spacers are more or less spherical.
- Bending stiffness in a hydrophilic spacer decreases the CMC.
- The morphologies of ionic and nonionic geminis are identical regardless of whether the spacer is hydrophobic or hydrophilic. We are currently testing the “stiffness” predictions using acetylenic units in hydrophobic spacers and sugar units in hydrophilic spacers.

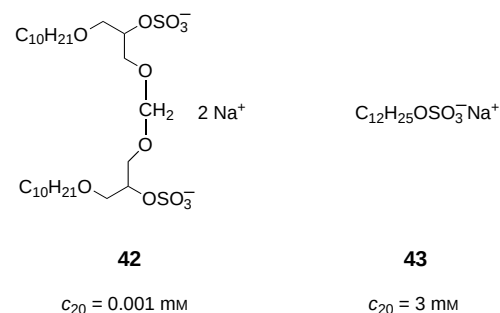
7. Surface Activity

The surface tension of water (72 mNm^{−1}) is typically reduced to 30–40 mNm^{−1} at the CMC of a surfactant. It is for this reason that surfactants are called “surface-active”. Surface activity has a close connection to the wide-ranging applications of surfactants in virtually every major industry (pharmaceutical, food, metal, paint, petroleum, polymer, etc.). Perhaps this is a suitable time to take a pause from the laborious exposition of physical properties and to quote from

a few abstracts of the many recent gemini patents. The objective here is to carry the reader (briefly, for we are academic types!) into the world of tangible reality:

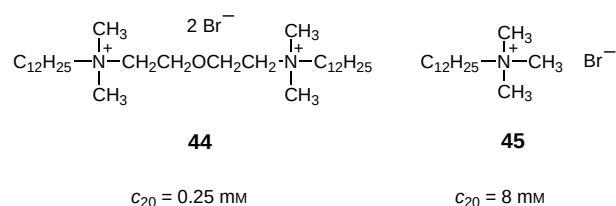
- 1) *Gemini-Type Betaine Surfactants from Amines and Aldehydes*: “Amphoteric amphiphilic compounds ... are manufactured by condensation of C₆–C₂₂ aldehydes with di-, oligo-, or polyamines, hydrogenation of the resulting condensation products, and carboxymethylation with ClCH₂COOH or ClCH₂COONa. [The geminis] ... can be combined with all current anionic, nonionic, cationic, and ampholytic surface-active agents and are particularly suitable for use as emulsifiers, antifoaming agents, detergents, dispersants, and hydrotropic agents in industry and in the home, e.g. in the metalworking field, in ore extraction, in the washing and cleaning of textiles, hard surfaces, the skin and the hair, and in cosmetics.”^[49]
- 2) *Gemini Polyhydroxy Fatty Acid Amide Surfactants for Detergents and Personal Care Compositions*: “Thus, D-glucose was reacted with 1,2-bis(3-aminopropoxy)ethane in the presence of Raney Ni to give a disugar diamine which was then amidated with methyl octanoate to give the gemini polyhydroxy fatty acid amide. The prepared gemini polyhydroxy fatty acid amide was suitable for use in dishwashing, detergents and laundry detergents (including fabric softening detergents).”^[50]
- 3) *Manufacture of Novel Gemini Surfactants*: “The title surfactants, useful as wetting agents, oil-in-water emulsifiers and thickeners, are available through condensation of alk(en)yl oligoglucosides with bisepoxides.”^[51]
- 4) *Amphoteric Surfactants Having Multiple Hydrophobic and Hydrophilic Groups*: “The title amphoteric gemini surfactants, mild and environmentally benign, ... are prepared.”^[52]
- 5) *Nonionic Gemini Surfactants and Their Blends*: “The title surfactants are extremely effective emulsifiers for oil-in-water emulsions that provide improved detergency at even low concentration levels.”^[53]

With this interlude behind us, we can now return to the question of surface activity. A favorite means of reporting surface activity is the *c*₂₀ value representing the surfactant concentration that reduces the surface tension by an arbitrary 20 mNm^{−1}.^[54] The *c*₂₀ values reflect the tendency of surfactants to adsorb at the air/water interface. Specific numbers for an anionic gemini and its single-chained counterpart are given in Scheme 9.^[6] It is seen that the gemini is three orders of

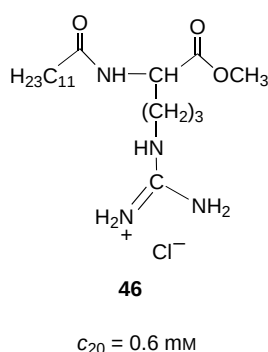
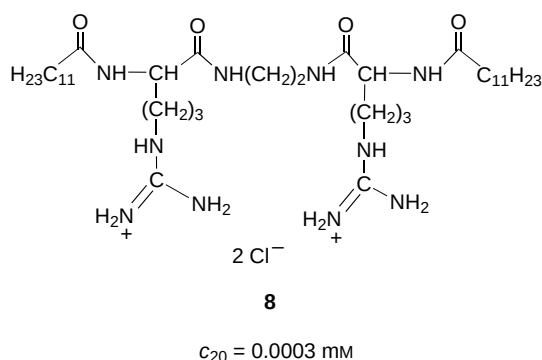


Scheme 9. The *c*₂₀ values for an anionic gemini and its single-chained counterpart.

magnitude more surface-active. A comparison between a cationic gemini and conventional surfactant also shows a significant difference in surface activity (Scheme 10).^[10, 35] A third example portraying the superiority of a gemini over an “ordinary” surfactant is shown in Scheme 11.^[14]



Scheme 10. Comparison of the c_{20} values for a cationic gemini and a conventional surfactant.



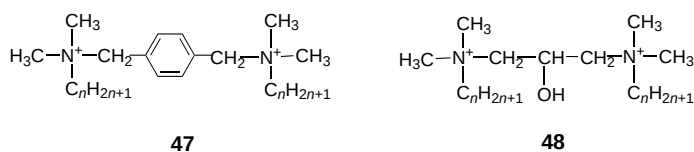
Scheme 11. Comparison of c_{20} values, showing the superiority of a gemini over an ordinary surfactant.

Why are geminis so prone to adsorb at the air/water interface? Rosen has ascribed the effect to a distortion of the water structure by the hydrophobic groups.^[3] Accordingly, two hydrophobic groups in a single molecule are more disruptive than individual chains (with the hydrophilic groups contributing little to the change in water structure). Migration to the air/water interface of a micelle is thereby promoted. In our view, interfacial packing effects probably dominate any selective changes in water structure. There is evidence, in fact, that geminis do indeed form a more coherent interfacial film. This evidence is based upon the area-per-molecule for surfactants adsorbed at a saturated air/water interface as determined from surface tension data and the Gibbs adsorption equation.^[31] A small cross-sectional area of the surfactant

indicates a tight packing at the interface and an efficient lowering of the surface tension. Thus, a gemini surfactant bearing two sulfonate groups was shown to adsorb at the air/water interface with an area-per-chain of only $26 \text{ \AA}^2$ compared to $43 \text{ \AA}^2$ for a corresponding single-chained sulfonate.^[55] Favorable packing was also found for bisquaternary ammonium geminis.^[13] Apparently, the gemini spacer forces the pair of ionic groups to reside in a less space filling geometry relative to that of two ordinary surfactants. Of course, intermolecular distances between individual gemini molecules may not differ much from normal.

Mention should be made of a specialized but useful “maximum bubble pressure” method for monitoring dynamic surface tension, that is, the variation of surface tension with time.^[56] (Adsorption of geminis at the air/water interface can sometimes be a slow process.^[2]) In brief, the measurement is carried out by forcing known pressures of N_2 gas through a capillary into a surfactant solution to form bubbles. Pressure is converted into surface tension by using pure liquids of known surface tension as a calibration. Since data can be acquired in 10 ms, rather fast processes at the N_2 /water interface can be observed. Typically, plots of dynamic surface tension versus time are divided into four stages: a) an induction period where there is little change; b) a region of rapid surface tension decrease; c) meso-equilibrium; and d) equilibrium regions.

Two geminis, one with a rigid, hydrophobic spacer and one with a flexible, more hydrophilic spacer (**47** and **48**, respectively), were examined by the maximum bubble pressure



method.^[57] It was found for all compounds that about two-thirds of the interface is covered at the end of the induction period just prior to the “fast-fall” region. In other words, there must be substantial surface coverage before an effect on the surface tension manifests itself. The time required to reach the fast-fall region depends heavily upon the chain length. In the aromatic series, for example, the C_{10} and C_{16} geminis have induction periods of about 0.2 and 10 s, respectively. Clearly, long chains inhibit diffusion-controlled organization into interfacial films. Induction times for geminis with a rigid spacer are shorter than for similar sized geminis with a flexible spacer. This is rather unexpected because one might have intuitively speculated that flexible geminis have greater diffusivity and, therefore, a smaller adsorption time. It may be that flexible spacers allow, for some reason, formation of cumbersome linear aggregates owing to chain–chain interactions. Viscosity data bear this out.^[57]

8. Solubilization

Many organic compounds that are normally insoluble in water, or only slightly soluble, dissolve to a greater extent in

the presence of micellar surfactant. The increased solubility (to form thermodynamically stable and isotropic solutions) is called “solubilization.” Solubilization merits attention owing to its considerable technological relevance (detergency and tertiary oil recovery being only two examples of solubilization-dependant phenomena). Not surprisingly, the pioneers of surfactant chemistry were highly interested in solubilization. Thus, McBain and Richards found that potassium laurate micelles solubilized 0.23 moles of cyclohexane per mole of soap.^[58] And Kleven reported that sodium dodecyl sulfate solubilized 0.87 moles of *n*-heptanol per mole of surfactant.^[59]

A few aspects of solubilization, necessary to give the reader a feel for the subject, are compiled here:

- The location within micelles of a solubilized compound (the “additive” component) depends upon its structure. Saturated hydrocarbons concentrate at the micellar core. A single functional group, however, shifts the additive’s solubilization site to the interfacial region made up of the head-groups, the first few methylene groups of the chains, and water molecules.^[60] Even nonpolar aromatic moieties prefer the interfacial region (especially with cationic micelles).
- Generally speaking, an increase in surfactant chain length increases the solubilization power of the system.
- Branching of the surfactant chain tends to diminish solubilization power.
- For a given chain length, solubilization by micelles of different charge types usually follows the sequence non-ionic > cationic > anionic.^[61]
- Introduction of a second ionic group to the polar end of a surfactant has been shown in one case to increase the solubilization of *n*-octanol, but to reduce the solubilization of *n*-octane.^[51] This latter observation illustrates the complexity of the situation: multiple interactions take place within an aggregate whose variable structure is affected by a variety of parameters including the presence of an additive itself.

Solubilization in gemini micelles has not been examined in detail. The one exception (that we know of) involved the determination of the solubility of toluene and *n*-hexane in aqueous solutions of cationic geminis.^[30] The experiments were carried out by shaking 3 mL of hydrocarbon with 30 mL of aqueous surfactant for many hours until clarity was achieved. Analysis of the water layer for hydrocarbon provided the solubilization power of the surfactant. It was found that, in the words of the authors, “the propensity of gemini micelles for oil solubilization is significantly better than of conventional surfactants; this is true on a molar basis as well as a weight basis”.^[30] For example, 12-2-12 gave a [toluene]/[surfactant] ratio of 3.8 compared to 0.78 for cetyltrimethylammonium bromide. The geminis showed a distinct preference for solubilizing toluene over *n*-hexane (owing, we presume, to ion–dipole interactions^[62]). Thus, 10-2-10 and 12-2-12 led to [n-hexane]/[surfactant] ratios of only 0.29 and 0.99, respectively. (Inexplicably, *n*-hexane and 14-2-14 have a value which is at least ten times greater than that for cetyltrimethylammonium bromide.) It is proposed that the enhanced solubilization power of geminis may be related to tubular shapes of their aggregates discussed earlier in Section 6. In any event, the solubilization data bode well for

future applications of geminis. Three useful applications that have already been published are given:

Geminis with a hydroxylated spacer (1,3-bis(dodecyl-*N,N*-dimethylammonium)-2-propanol dichloride (**4**, Scheme 1) and its C₁₄ analogue) were used to separate all members of a family of 17 ergot alkaloids by micellar electrokinetic capillary chromatography.^[63] Separation was achieved in less than 8 min using 20–40 mM surfactant in 50 mM phosphate buffer (pH 3.0) at 20 °C. Corresponding single-chained surfactants (e.g. cetyltrimethylammonium bromide) failed to give complete separation of all components in the complex mixture. The precise mechanism of the difference is not understood.

Polymerization of styrene into spherical and fairly mono-disperse “latex” particles (10–100 nm in diameter depending upon conditions) can be carried out in oil-in-water micro-emulsions. Before delving into how geminis can play a role in the process, it is worthwhile to define “microemulsions”. Oil-in-water microemulsions are thermodynamically stable, optically transparent mixtures of hydrocarbon and water in which tiny hydrocarbon droplets are dispersed in the water with the aid of (typically) a surfactant and a cosurfactant such as *n*-butanol or *n*-hexanol. It is amazing to watch 10 % or more of hexane dissolve into water in the presence of a surfactant/cosurfactant combination. It turns out, however, that a few surfactants will form “ternary globular microemulsions” in styrene without the assistance of any cosurfactant. Among these are the *n*-alkyltrimethylammonium halides. By polymerizing styrene as “oil droplets” stabilized only by a cationic surfactant (conventional or gemini), one can avoid the complications of a fourth component. Further simplicity can be gained by using γ -ray irradiation, rather than a chemical initiator, to effect polymerization.^[64] It was recognized that geminis are particularly interesting in such systems because surfactant shape, aggregation properties, and thus the interfacial spontaneous curvature can be easily “tuned” by changing the spacer length of the gemini. Spacer length contributes a new, easily adjustable structural parameter to the field of surfactant chemistry.

Geminis were found to be superior to conventional single-tailed surfactants in solubilizing styrene in water.^[64] Among the 12-*n*-12 geminis (*n* = 2, 4, 6, 8, 10, 12), all formed single-phase oil-in-water microemulsions with styrene (although for *n* = 2 the stable microemulsion region in the phase diagram is very small). Latex particle size showed a modest dependence upon spacer length with a maximum at *n* = 10. For example, polymerization (at 25 °C, surfactant-to-monomer ratio = 5, and 1,3-diisopropenylbenzene as a cross-linker) gave hydrodynamic radii ranging from 10 nm for *n* = 2 to 15 nm for *n* = 10. Weight-average molecular weights (at 25 °C, surfactant-to-monomer weight ratio = 2, no cross-linker) varied from about 3×10^5 for *n* = 2 and *n* = 12 to 1.8×10^6 for *n* = 6. The data were attributed without details to different interfacial curvatures resulting from varying surfactant shapes. Uncertainties notwithstanding, the work serves to alert the colloid community to the tunability of properties of geminis in matters of practical importance.

While on the subject of practicality, mention should be made of the antimicrobial properties of geminis. Amphiphilic

quaternary ammonium salts, such as benzyldodecyldimethylammonium bromide (BDDAB) and 2-ethoxycarbonylpentadecyltrimethylammonium bromide (EPTAB), are well-known disinfectants. The question was raised whether geminis of the general structure **49** (see Table 2) would display similar bioactivity.^[65] In an amazingly thorough study, *m* was varied from 6 to 16, and *n* from 2 to 8, for a total of 45 compounds. Minimum inhibitory concentrations (MICs, defined as the lowest concentration of antibacterial agent inhibiting visible growth after 24 h incubation at 37 °C) were determined for this set of geminis against *E. coli* bacteria. By way of calibration, MICs for single-chained BDDAB and EPTAB were found to be 625 and 78 μM, respectively. Apparently the lower the MIC, the better.

Table 2 lists select MIC values for the gemini series.^[65] The following generalizations emerge from the data: a) Short-chain compounds (*m* = 6) are not effective. b) Short spacers

Table 2. Minimum inhibitory concentrations (MICs) against *E. coli* for gemini surfactants **49**.

49

Gemini ^[a]		MIC [μM]
<i>m</i>	<i>n</i>	
6	2	21 000
12	2	6
16	2	5700
6	4	8100
12	4	25
16	4	2800
6	8	930
12	8	190
16	8	5200

[a] *m* = chain length, *n* = spacer length.

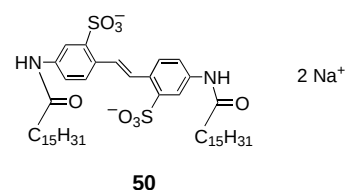
give lower MIC values. c) Lengthening the chain from *m* = 12 to *m* = 16 severely diminishes the activity; self-association effects in the compounds with *m* = 16 may be impairing interactions with the bacterial membranes. d) Optimal activity is achieved with *m* = 10 or 12. The gemini with *m* = 12 and *n* = 2 is one or two orders of magnitude more potent than the conventional single-chained disinfectants BDDAB and EPTAB. Surpassing the commercial compounds to this degree is a noteworthy achievement. One wonders about the bioactivity of the other classes of geminis, not only as disinfectants but as anti-viral agents and mediators of transfection.

9. Gemini Films

In Section 7 on surface activity, we alluded to the fact that water-soluble surfactants adsorb at the air/water interface and, in this manner, lower the surface tension. Water-insoluble amphiphiles, on the other hand, can often be spread onto a water surface in the form of a film only one molecule thick (an “insoluble monolayer”). With the aid of a device called a film balance, a monolayer can be compressed with a

moveable barrier while, simultaneously, recording the pressure generated by the film. If, for example, the film component is in a “gaseous” state, there will be little pressure change as the film is compressed. If the film molecules are highly compacted, then the pressure will rise steeply as the film is further compressed. Considerable information on the orientation and packing behavior of molecules at the air/water interface can thus be obtained by plotting surface pressure versus film area-per-molecule to give pressure-area (π -*A*) isotherms. This section is devoted to monolayer films of two gemini systems.

A gemini of interest, **50**, has two sulfonates attached directly to a photoactive stilbene spacer.^[12] The compound can be spread as a stable monolayer by depositing a solution

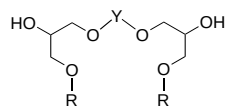


in chloroform onto pure water and allowing the chloroform to evaporate. The π -*A* isotherm showed a steep rise in surface pressure at around 140 Å² per molecule, indicating a close packing at this area. Further compression caused the film to buckle and collapse. Now the area of 140 Å² is much larger than the areas of the two chains (40 Å²). The area of 140 Å² reflects, therefore, the stilbene spacer lying parallel to the surface of the water with the sulfonate groups immersed in the water and the hydrophobic tails extended into the air. Upon irradiation of the film at 366 nm, the π -*A* isotherm changed drastically. Most notably, there ensued a large decrease in the surface area at constant pressure. This is consistent with a *trans*-to-*cis* isomerization of the stilbene, a conclusion supported by spectral properties of the film after being transferred to a quartz plate. Irradiation of the *cis* monolayer at 254 nm led to an increase in surface area as the *trans* isomer was regenerated.

The efficient *trans*-to-*cis* conversion was puzzling because the reaction was known to be strongly inhibited in condensed monolayers that resist extensive molecular reorganization. Fluorescence microscopy helped explain the unusual facility of the isomerization. Thus, *trans*-gemini was mixed with 2 % of a fluorescent dye prior to deposition upon the water. Upon compression of the film, dark nonfluorescent domains, representing regions of crystalline gemini that had “squeezed out” the dye, were evident under the fluorescence microscope. When the film was irradiated at 366 nm, the domains disappeared and the entire microscope field took on a uniform fluorescence. It was concluded that initially formed *cis* isomer provided a fluid medium in which *trans* gemini is sufficiently mobile to further isomerize. In the absence of such a photoinduced solid-to-fluid transition, reversible photoisomerization would have been difficult to achieve.

A second article dealing with pressure–area isotherms of gemini-type structures begins with the following sentence: “Because almost all the surfactants in general use consist

simply of one hydrophobic alkyl chain and one hydrophilic headgroup, there are limitations to modifying or improving the surface-active properties within the range of the above-mentioned structure.”^[66] We heartily agree with the sentiment;



51

geminis have added a new dimension to surfactant chemistry. In the paper just quoted, the focus is on the monolayer properties of compounds **51** ($R = C_{10}H_{21} - C_{18}H_{37}$, $Y = C_2H_4 - C_6H_{12}$). In this particular study, the free hydroxy groups were not further derivatized to sulfates or

other ionic groups because monolayer formation requires compounds that are reasonably insoluble in water. Figure 2 shows how films with $R = C_{10}H_{21}$ expand as the spacer is lengthened from $Y = C_2H_4$ to $Y = C_4H_8$ and $Y = C_6H_{12}$ (a, b,

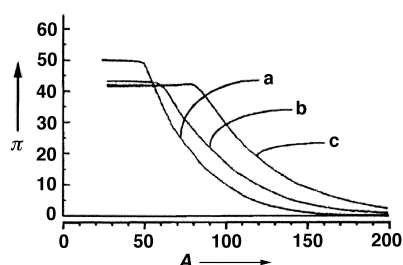


Figure 2. Effect of spacer length on pressure–area isotherms for 10-O-*n*-O-10 geminis where $n = 2$ (a), 4 (b), and 6 (c). Pressure π is in $mN m^{-1}$ and area A in $\text{\AA}^2$ per molecule. Taken from ref. [66].

and c, respectively). Monolayers packed more tightly as the chain length R was increased. This was seen, for example, from “liftoff areas” defined as the first point on the π – A isotherm showing a detectable resistance to compression by the moveable barrier. Since the liftoff area for $R = C_{10}H_{21}/Y = C_6H_{12}$ is $239 \text{ \AA}^2$, compared to only $138 \text{ \AA}^2$ for $R = C_{18}H_{37}/Y = C_6H_{12}$, the latter has a greater propensity to assemble into a condensed monolayer owing, no doubt, to favorable alignment of the chains. Limiting area, defined as the molecular area at zero pressure (obtained by extrapolation) showed the same thing. Limiting areas for $R = C_{10}H_{21}/Y = C_6H_{12}$ and $R = C_{18}H_{37}/Y = C_6H_{12}$ were found to be 174 and $130 \text{ \AA}^2$ per molecule, respectively.

The films studied in the above work can be contrasted with soluble films composed of simple 12-*n*-12 cationic geminis as examined experimentally^[31] and theoretically.^[67] It turns out that the area-per-surfactant molecule at the air/water interface (determined from surface tension data and the Gibbs equation) increases rapidly for short spacers, reaches a maximum at 10–12 methylene groups, and decreases with even longer spacers (Figure 3).^[31] The nonmonotonic dependence of molecular area on the spacer length is accounted for theoretically by the interplay of three factors: a) a simple geometrical effect in which area expands as does the spacer; b) a gemini–gemini interaction that tends to decrease the specific area beyond a certain spacer length; and c) an entropic effect of flexible spacers that accentuates the second factor.^[67] In our opinion, “hair-pin turns” among sufficiently long spacers could impart areas that are smaller than expected

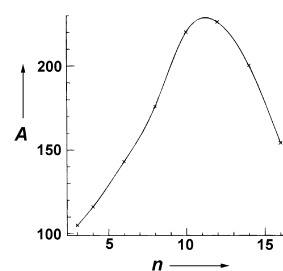


Figure 3. Plot of area A [$\text{\AA}^2$] per surfactant molecule at the air/water interface against spacer length n for 12-*n*-12. Taken from ref. [67].

and, therefore, account for the right-hand wing of the bell-shaped curve. According to the calculations, however, spacers do not depart from the air/water interface.^[67] If this is true, then our looping conformers would have to reside on the water surface. In connection with conformational issues, attention is drawn to selective decoupling ^{13}C and ^1H NMR studies which address the subject in detail.^[68]

10. New Materials

Introducing pores of controlled size and shape into silica and other common materials offers great opportunities to those interested in topics such as shape-selective catalysis, molecular sieving, chemical sensing, and selective adsorption.^[69] The fashioning of porous silica received a great impetus less than ten years ago when cationic surfactants were first used in the conversion of silicate anions into mesoporous molecular sieves.^[70] Presumably, polyanionic oligomeric silicate can ion-exchange with surfactant counterions (often bromide). Intermicellar repulsion is thereby reduced, and the resulting aggregates provide a template for the ensuing silica mesophase formation. One of the most promising materials made in this manner, called MCM-48, is cubic in structure and is characterized by a three-dimensional pore system. Since conventional alkyltrimethylammonium surfactants give hexagonal or lamellar structures rather than high-quality MCM-48, a search began for alternative surfactants.^[71] Cationic geminis, particularly those with long spacers such as 16-12-16 and 18-12-18, have been shown to promote the synthesis of excellent quality MCM-48 with high surface area and narrow pore-size distribution.^[72, 73] However, in order to help place things in proper context, it will be useful to first give directions for making MCM-48.^[69]

Gemini surfactant is dissolved in basic water and then mixed with $\text{Si}(\text{OEt})_4$ in a ratio of gemini/NaOH/ $\text{H}_2\text{O}/\text{Si}(\text{OEt})_4$ of 0.06/0.6/150/1. The mixture is stirred for 2 h at room temperature and then placed in an autoclave at 100°C for several days. The resulting white solid is removed by vacuum filtration, added to fresh water (20 g per one g solid), and autoclaved at 100°C once again. In the final step, the solid precursor is heated in ambient air (“calcined”) from room temperature to 550°C at a heating rate of 5 deg min^{-1} . With this procedure, production of high-quality MCM materials has been reduced from several weeks to a few days. Making MCM-48 is simplicity itself.^[69]

Both geminis 18-12-18 and 16-12-16 yield excellent MCM-48 with a surface area of 1200–1600 m²g⁻¹ and pore volumes exceeding 1.2 mL g⁻¹.^[69] Narrow pore-size distributions are obtained for MCM-48 with pore radii of 12 and 13 Å when using 16-2-16 and 18-2-18, respectively. The spacer length of the gemini surfactant determines the crystallographic phase that is formed, whereas the chain length determines the average pore size. VO_x has been grafted onto gemini-based MCM-48, demonstrating the potential of the material in heterogeneous catalysis.^[73] Gemini surfactants clearly have a promising future in materials technology.

11. Vesicles, Gels, and Phase Behavior

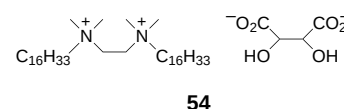
We end this review by citing, briefly in each case, examples of gemini surfactants that self-assemble into interesting structures. Compound **52** was called a “double-chain, double-head group surfactant” (an accurate but lengthy description of a gemini, making us glad we coined the euphemism)^[1, 2] that forms vesicles when sonicated in aqueous base.^[74] At pH 9.2, the vesicles have hydrodynamic diameters of about 360 Å. Stable vesicles, in which entrapped guests remain for months, can be made from triple-chained gemini **53**.^[75] Encapsulation is one of many potential applications of geminis that remain to be exploited.

Diastereomeric gemini surfactants **32** (Scheme 6), bearing two phosphate groups, form unilamellar vesicles upon sonication (150–250 Å for *S,S* and 500–1000 Å for *R,S*).^[76] Remarkably, when Ca²⁺ is added to the vesicles, the *S,S* isomer undergoes fusion, whereas the *R,S* isomer undergoes fission. The difference is attributed to a Ca²⁺ effect on headsize which increases with the *S,S* isomer but decreases with the *R,S* isomer.

A solution of 5% (w/w) 12-2-12 in water is viscous, a fact consistent with wormlike micelles. When *n*-hexanol is added, it is possible to induce a micelle-to-vesicle transition.^[77, 78]

Viscosity data, coupled to cryo-transmission electron microscopy, reveal the details of the process. Thus, at lower levels of *n*-hexanol the viscosity increases as the small OH headgroups of the alcohol decrease the spontaneous curvature of the micelles (i.e., flatten the interface) and thereby elongate the micelles. The viscosity ultimately decreases rapidly at higher *n*-hexanol concentrations; this is attributed to a spontaneous curvature close to zero where relatively flat vesicles and highly branched micelles are favored. At even greater *n*-hexanol concentrations, the vesicles assume microscopically visible tubulelike shapes hundreds of microns long.

Gemini surfactants composed of 16-2-16 with L- and D-tartrate counterions (**54**) have been reported to gelate organic solvents (CH₂Cl₂, CHCl₃, toluene, etc.) containing traces of



water.^[79] As little as 10 mM gemini can immobilize the solvent. Transmission electron microscopy reveals the presence of long, entangled helical fibers (right handed for L-tartrate, and left-handed for D-tartrate counterions). A proposed structure of the gel is shown in Figure 4, but other modes of bridging the cationic layers cannot be excluded. Layerlike liquid crystalline phases of “counterion-coupled gemini surfactants” or “cocogems” have been used as media for polymerizing acrylamide.^[80] The overall morphology of the resulting polymer gel is controlled by the anisotropy of the surfactant assembly.

Phase behavior is an important property of surfactants, assembling as they do into “lyotropic liquid crystalline mesophases” when mixed with water and into “thermotropic liquid crystalline phases” when heated. Discussions of the subject often refer to characteristic textures, to phase diagrams, and to mesophases such as H₁, V₁, L_α, and L_β. Polarized optical microscopy, calorimetry, X-ray diffraction, neutron scattering, and viscometry are favorite tools in the exploration of phase behavior. Clearly, the topic is too esoteric for the general reader. Suffice it to say here that geminis have a unique phase behavior and that those interested in learning more can consult the literature.^[81–84]

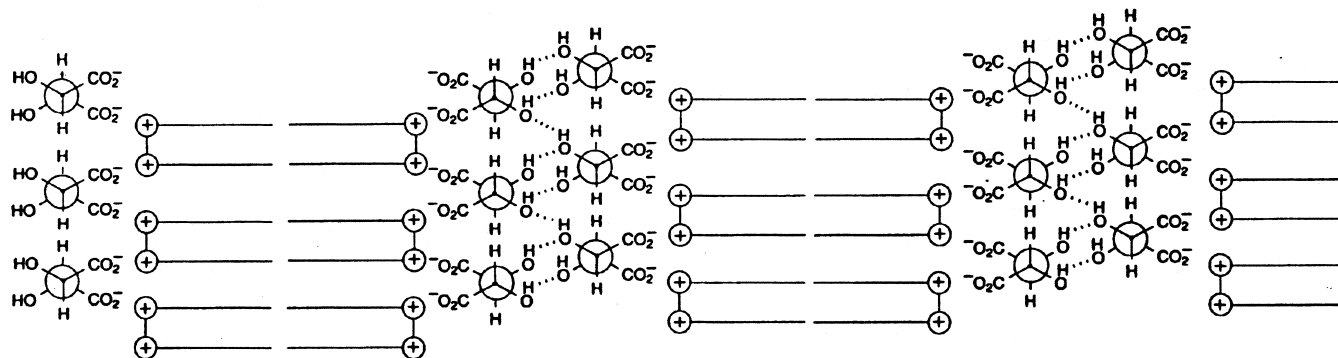


Figure 4. Proposed structure of aggregates of a gemini with tartrate counterions. Taken from ref. [79].

12. Concluding Remarks

Writing a review is something like preparing a buffet dinner. Multiple dishes are set forth, and the hungry can select or not select; consume as presented or enhance with spices and sauces; come back for more or resist the temptation. With both buffets and reviews, the hope is that the participant ends up reasonably content. There is, however, a philosophic difference between buffets and reviews. In a buffet, the diner will rather quickly become satiated and ready to leave. With a well-written review, at least a few readers might want to continue on, perhaps for many years. In the case at hand, this could involve the synthesis of new members of the gemini family (an intense interest of ours); or the development of new gemini-based materials; or the application of geminis to skin care and transdermal drug delivery; or the use of chiral geminis for separating enantiomers chromatographically; or the fabrication of new transfection agents. Indeed, geminis could well turn out to be more useful to the person in the street than (for example) crown ethers and fullerenes.^[85]

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- [1] F. M. Menger, C. A. Littau, *J. Am. Chem. Soc.* **1991**, *113*, 1451–1452.
- [2] F. M. Menger, C. A. Littau, *J. Am. Chem. Soc.* **1993**, *115*, 10083–10090.
- [3] M. J. Rosen, *CHEMTECH* **1993**, *23*, 30–33.
- [4] C. A. Bunton, L. Robinson, J. Schaak, M. F. Stern, *J. Org. Chem.* **1971**, *36*, 2346–2350.
- [5] F. Devínský, L. Masárová, I. Lacko, *J. Colloid Interface Sci.* **1985**, *105*, 235–239.
- [6] Y.-P. Zhu, A. Masuyama, M. Okahara, *J. Am. Oil Chem. Soc.* **1990**, *67*, 459–463.
- [7] M. J. Rosen, D. J. Tracy, *J. Surfactants Deterg.* **1998**, *1*, 547–554.
- [8] R. Zana, M. Benroau, R. Rueff, *Langmuir* **1991**, *7*, 1072–1075.
- [9] S. De, V. K. Aswal, P. S. Goyal, S. Bhattacharya, *J. Phys. Chem. B* **1998**, *102*, 6152–6160.
- [10] T.-S. Kim, T. Kida, Y. Nakatsuji, T. Hirao, I. Ikeda, *J. Am. Oil Chem. Soc.* **1996**, *73*, 907–911.
- [11] Y.-P. Zhu, A. Masuyama, Y. Kobata, Y. Nakatsuji, M. Okahara, M. J. Rosen, *J. Colloid Interface Sci.* **1993**, *158*, 40–45.
- [12] O. Karthaus, M. Shimomura, *J. Am. Chem. Soc.* **1996**, *118*, 9174–9175.
- [13] M. Diaz, A. Manresa, A. Pinazo, P. Erra, M. R. Infante, *J. Chem. Soc. Perkin Trans. 2* **1994**, 1871–1876.
- [14] L. Pérez, A. Pinazo, M. J. Rosen, M. R. Infante, *Langmuir* **1998**, *14*, 2307–2315.
- [15] P. Renouf, C. Mioskowski, L. Lebeau, D. Hebrault, J.-R. Desmurs, *Tetrahedron Lett.* **1998**, *39*, 1357–1360.
- [16] R. Zana, *Colloids Surf. A* **1997**, *123–124*, 27–35.
- [17] F. M. Menger, V. A. Migulin, unpublished results.
- [18] M. J. L. Castro, J. Kovensky, A. F. Cirelli, *Tetrahedron Lett.* **1997**, *38*, 3995–3998.
- [19] R. Oda, I. Huc, S. J. Candau, *Chem Commun.* **1997**, 2105–2106.
- [20] R. Zana, H. Levy, D. Papoutsi, G. Beinert, *Langmuir* **1995**, *11*, 3694–3698.
- [21] M. J. Rosen, T. Gao, Y. Nakatsuji, A. Masuyama, *Colloids Surf. A* **1994**, *88*, 1–11.
- [22] K. Esumi, M. Goino, Y. Koide, *J. Colloid Interface Sci.* **1996**, *183*, 539–545.
- [23] We have synthesized a series of dendrimeric surfactants with as many as 20 tails. F. M. Menger, A. V. Peresypkin, unpublished results.
- [24] F. L. Duivenvoorde, M. C. Feiters, S. J. van der Goost, J. B. F. N. Engberts, *Langmuir* **1997**, *13*, 3737–3743.
- [25] N. A. J. M. Sommerdijk, T. H. L. Hoeks, M. Synak, M. C. Feiters, R. J. M. Nolte, B. Zwanenburg, *J. Am. Chem. Soc.* **1997**, *119*, 4338–4344.
- [26] Y.-P. Zhu, A. Masuyama, Y. Kirito, M. Okahata, *J. Am. Oil Chem. Soc.* **1991**, *68*, 539–543.
- [27] K. Jennings, I. Marshall, H. Birrell, A. Edwards, N. Haskins, O. Soderman, A. J. Kirby, P. Camilleri, *Chem. Commun.* **1998**, 1951–1952.
- [28] F. M. Menger, R. Zana, B. Lindman, *J. Chem. Ed.* **1998**, *75*, 115.
- [29] L. D. Song, M. J. Rosen, *Langmuir* **1996**, *12*, 1149–1153.
- [30] T. Dam, J. B. F. N. Engberts, J. Karthäuser, S. Karaborni, N. M. van Os, *Colloids Surf. A* **1996**, *118*, 41–49.
- [31] E. Alami, G. Beinert, P. Marie, R. Zana, *Langmuir* **1993**, *9*, 1465–1467.
- [32] J. Zhao, S. D. Christian, B. M. Fung, *J. Phys. Chem. B* **1998**, *102*, 7613–7618.
- [33] M. Frindi, B. Michels, H. Levy, R. Zana, *Langmuir* **1994**, *10*, 1140–1145.
- [34] F. Devínský, I. Lacko, F. Bitterová, L. Tomecková, *J. Colloid Interface Sci.* **1986**, *114*, 314–322.
- [35] M. J. Rosen, L. Liu, *J. Am. Oil Chem. Soc.* **1996**, *73*, 885–890.
- [36] T.-S. Kim, T. Kida, Y. Nakatsuji, I. Ikeda, *Langmuir* **1996**, *12*, 6304–6408.
- [37] Y.-P. Zhu, A. Masuyama, M. Okahara, *J. Am. Oil Chem. Soc.* **1990**, *67*, 459–463.
- [38] A. L. Underwood, E. W. Anacker, *J. Colloid Interface Sci.* **1984**, *100*, 128–135.
- [39] R. G. Alargova, I. I. Kochijashky, M. L. Sierra, R. Zana, *Langmuir* **1998**, *14*, 5412–5418.
- [40] H. Hirata, N. Hattori, M. Ishida, H. Okabayashi, M. Frusaka, R. Zana, *J. Phys. Chem.* **1995**, *99*, 17778–17784.
- [41] V. K. Aswal, S. De, P. S. Goyal, S. Bhattacharya, R. K. Heenan, *Phys. Rev. E* **1998**, *57*, 776–783.
- [42] S. De, V. K. Aswal, P. S. Goyal, S. Bhattacharya, *J. Phys. Chem.* **1996**, *100*, 11664–11671.
- [43] F. Reiss-Husson, V. Luzzati, *J. Phys. Chem.* **1964**, *68*, 3504–3511.
- [44] V. K. Aswal, S. De, P. S. Goyal, S. Bhattacharya, R. K. Heenan, *J. Chem. Soc. Faraday Trans.* **1998**, *94*, 2965–2967.
- [45] D. Danino, Y. Talmon, R. Zana, *Langmuir* **1995**, *11*, 1448–1456.
- [46] V. Scmitt, F. Schosseler, F. Lequeux, *Europhys. Lett.* **1995**, *30*, 31–36.
- [47] R. Oda, P. Panizza, M. Schmutz, F. Lequeux, *Langmuir* **1997**, *13*, 6407–6412.
- [48] P. K. Maiti, D. Chowdhury, *J. Chem. Phys.* **1998**, *109*, 5126–5133.
- [49] K. Kwetkat (Hüls AG), WO-B 9731890, **1997** [*Chem. Abstr.* **1997**, *127*, 249754r].
- [50] J. J. Scheibel, D. S. Conner, Y. C. Fu (Proctor and Gamble Co.), US-A 5534197, **1996** [*Chem. Abstr.* **1996**, *125*, 171553m].
- [51] O. Rhode, H.-C. Rath (Henkel K.G. a.A.), DE-B 19645189, **1998** [*Chem. Abstr.* **1998**, *128*, 323164n].
- [52] D. J. Tracy, R. Li, J. Yang (Rhodia Inc.), US-A 5789371, **1998** [*Chem. Abstr.* **1998**, *129*, 177210b].
- [53] D. J. Tracy, R. Li (Rhône-Poulenc Inc.), WO-B 9823365, **1998** [*Chem. Abstr.* **1998**, *129*, 55770p].
- [54] M. J. Rosen, *Surfactants and Interfacial Phenomena*, 2nd ed., Wiley, New York, **1989**, chap. 2, 3, 5.
- [55] Y.-P. Zhu, A. Masuyama, T. Nagata, M. Okahara, *Yukagaku* **1991**, *40*, 473–477.
- [56] X. Y. Hua, M. J. Rosen, *J. Colloid Interface Sci.* **1988**, *124*, 652–659.
- [57] M. J. Rosen, L. D. Song, *J. Colloid Interface Sci.* **1996**, *179*, 261–268.
- [58] J. W. McBain, P. H. Richards, *Ind. Eng. Chem.* **1946**, *38*, 642–646.
- [59] H. B. Kleven, *Chem. Rev.* **1950**, *47*, 1–74.
- [60] D. Myers, *Surfactants Science and Technology*, VCH, New York, **1988**, pp. 156–158.
- [61] S. Saito, *J. Colloid Interface Sci.* **1967**, *24*, 227–234.
- [62] S. Mecozzi, A. P. West, Jr., D. A. Dougherty, *J. Am. Chem. Soc.* **1996**, *118*, 2307–2308.
- [63] K. Chen, D. C. Locke, T. Maldacker, J.-L. Lin, S. Aawasiripong, U. Schurrath, *J. Chromatogr. A* **1998**, *822*, 281–290.

- [64] M. Dreja, B. Tieke, *Langmuir* **1998**, *14*, 800–807.
- [65] M. Pavlíková, I. Lacko, F. Devínský, D. Mlynářek, *Collect. Czech. Chem. Commun.* **1995**, *60*, 1213–1228.
- [66] Y. Sumida, A. Masuyama, T. Oki, T. Kida, Y. Nakatsuji, I. Ikeda, M. Nojima, *Langmuir* **1996**, *12*, 3986–3990.
- [67] H. Diamant, D. Andelman, *Langmuir* **1994**, *10*, 2910–2916.
- [68] N. Hattori, A. Yoshino, H. Okabayashi, C. J. O’Conner, *J. Phys. Chem. B* **1998**, *102*, 8965–8973.
- [69] P. Van Der Voort, M. Mathieu, F. Mees, E. F. Vansant, *J. Phys. Chem. B* **1998**, *102*, 8847–8851.
- [70] C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature* **1992**, *359*, 710–712.
- [71] Q. Huo, D. I. Margolese, G. D. Stucky, *Chem. Mater.* **1996**, *8*, 1147–1160.
- [72] S. Manne, T. E. Schäffer, Q. Huo, P. K. Hansma, D. E. Morse, G. D. Stucky, I. A. Askey, *Langmuir* **1997**, *13*, 6382–6387.
- [73] P. Van Der Voort, M. Morey, G. D. Stucky, M. Mathieu, E. F. Vansant, *J. Phys. Chem. B* **1998**, *102*, 585–590.
- [74] D. J. Jaeger, E. L. G. Brown, *Langmuir* **1996**, *12*, 1976–1980.
- [75] Y. Sumida, A. Masuyama, H. Maekawa, M. Takasu, T. Kida, Y. Nakatsuji, I. Ikeda, M. Nojima, *Chem. Commun.* **1998**, 2385–2386.
- [76] N. A. J. M. Sommerdijk, T. H. L. Hoeks, M. Synak, M. C. Feiters, R. J. M. Nolte, B. Zwanenburg, *J. Am. Chem. Soc.* **1997**, *119*, 4338–4344.
- [77] R. Oda, L. Bourdieu, M. Schmutz, *J. Phys. Chem. B* **1997**, *101*, 5913–5916.
- [78] R. Oda, I. Huc, S. J. Candau, *Chem. Commun.* **1997**, 2105–2106.
- [79] R. Oda, I. Huc, S. J. Candau, *Angew. Chem.* **1998**, *110*, 2835–2838; *Angew. Chem. Int. Ed.* **1998**, *37*, 2689–2691.
- [80] M. Antonietti, C. Göltner, H.-P. Hentze, *Langmuir* **1998**, *14*, 2670–2676.
- [81] K. M. Layn, P. G. Debenedetti, R. K. Prud’homme, *J. Chem. Phys.* **1998**, *109*, 5651–5658.
- [82] E. Buhler, E. Mendes, P. Boltzenhagen, J. P. Munch, R. Zana, S. J. Candau, *Langmuir* **1997**, *13*, 3096–3102.
- [83] S. Fuller, N. N. Shinde, G. J. T. Tiddy, *Langmuir* **1996**, *12*, 1117–1123.
- [84] M. Dreja, S. Gramberg, B. Tieke, *Chem. Commun.* **1998**, 1371–1372.
- [85] Recent work from our own group on gemini surfactants not mentioned in this review includes: F. M. Menger, V. A. Migulin, *J. Org. Chem.* **1999**, *64*, 8916–8921; A. V. Peresypkin, F. M. Menger, *Org. Lett.* **1999**, *1*, 1347–1350; F. M. Menger, J. S. Keiper, V. Azov, *Langmuir* **2000**, *16*, 2062–2067.

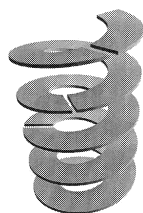
Syntheses of Functionalized and Aggregating Helical Conjugated Molecules**

Thomas J. Katz*

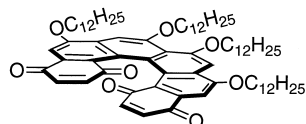
Although almost 100 years have passed since Meisenheimer and Witte prepared the first helicenes—helical molecules comprised of *ortho*-fused aromatic rings—it wasn't until Newman pointed out their chirality and in 1955, with Lednicer synthesized the first in nonracemic form that they attracted general interest. Many helicenes were prepared after Martin showed that all that was required was to expose diaryl-ethylenes to light and oxidants.^[1] Nevertheless, although their helical structures could be imagined to endow unusual and useful physical and chemical properties, in the half-century after Newman's synthesis, almost no application was found for helicenes. The reason was probably that the photochemical procedures gave neither appreciable amounts nor materials with significant functional groups.

However, during the past few years the combination of bis enol ethers of diacetyl aromatic species with *p*-benzoquinone,^[2] supplemented by an efficient procedure for effecting the required resolutions,^[3] has provided large quantities of helicenes with five to eight fused carbocyclic and heterocyclic rings. Their availability in turn has led to two kinds of discoveries that promise to make helicenes useful. One relates to the idea that, like planar aromatic compounds surrounded by alkyl chains, appropriate helicenes also might stack into long columns in the liquid phase, in solution, or as solids (see structure 1). However, unlike the columnar discotic liquid-crystal phases formed by planar aromatic

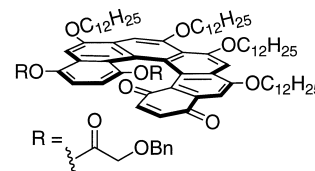
species, those of nonracemic helicenes should twist into large corkscrew configurations. The first examples were structures 2 and 3.^[3a, 4] In the case of 2, the twisted columnar configuration makes the pure material exhibit a unique, long fibrous morphology, very large circular dichroisms and specific rotation. Because of the mobile electrons and noncentrosymmetry of 2, a large second-order nonlinear optical response is



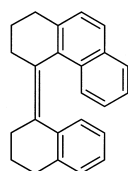
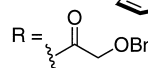
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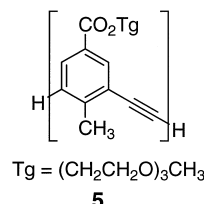
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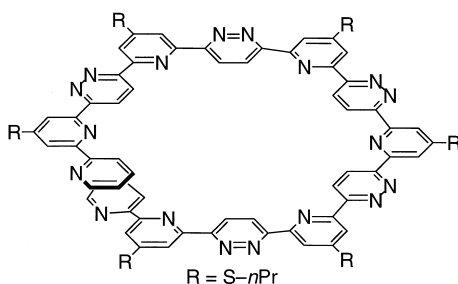


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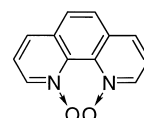


5

Tg = (CH₂CH₂O)₃CH₃



6



7

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observed.^[5] In the case of 3, the organization into columns has made it the first helically twisted columnar discotic liquid crystal.^[4] The other application for which helicenes have shown promise is in asymmetric catalysis. Although only two helicene-based catalysts are now known,^[6] the availability of functionalized helicenes in large amounts might be

expected to stimulate the design, synthesis, and application of others.

A number of recent efforts have been directed at finding other ways to obtain helical, conjugated molecules but only a few can be mentioned here. One interesting and previously unexplored way uses the trimerization of acetylene to prepare helicenes.^[7] Another dispenses with helicenes and instead ingeniously twists a polyacene around its long axis.^[8] Other methods eliminate parts of the helicene framework. Derivatives of **4** are examples, some of which have been shown to exhibit remarkable properties as switches and unidirectional molecular rotors.^[9] Still others use cyclophane scaffolds to bind and twist stacks of substituted benzenes.^[10] Finally, some ways eliminate completely the need for a rigid structure to construct unsaturated molecules designed to adopt a helical conformation.^[11, 12] Of these last methods, that of J.-M. Lehn^[11c] and his coworkers, is discussed in greater detail below. This work is related to that of Moore et al., who, in a superb series of papers, showed that solutions of oligo-(*m*-phenylene ethynylene) **5** (and related structures) in an appropriate solvent (acetonitrile) adopt helical conformations and that the direction of the helical winding can be determined by chiral centers in the molecules.^[11a,b]

The idea that Lehn et al. have pursued in a number of recent publications is based on the principle that 2,2'-bipyridine adopts an *s-trans* conformation. Lehn attributes this preference to three effects: attraction between the nitrogen lone pairs and adjacent C–H atoms, repulsion between nitrogen-centered dipoles, and repulsion between the 6,6'-C–H atoms. The consequence, as illustrated in structure **6**, is that if the carbon atoms adjacent to the nitrogen atom of pyridines are united with the carbon atoms adjacent to the nitrogen atoms of various diazenes, the resulting structures are oligomeric derivatives of 2,2'-bipyridine. Because the bonds to the pyridines are in a *meta* relationship (as in **5**), the oligomers are bent; because the nitrogen atoms of the 2,2'-bipyridine substructures prefer to be *trans* orientated, the conformation adopted should be helical.^[13] That the structure of **6** is helical is evidenced by the positions of the NMR signals corresponding to the outermost protons of the end pyridine rings. These signals are at higher fields than in simple pyridines, which can be explained if the protons are subject to the ring currents of cofacially disposed benzene rings.

Similar, although smaller, structures with similar properties have been reported before.^[11c] New in the most recent paper is that the helical oligomers aggregate, supposedly into the kinds of columnar stacks (structure **1**) that are formed by the more rigid helicenes **2** and **3**. Moreover, pairs or triplets of columns are thought to twist about one another. Evidence that the molecules aggregate in solution is good: Solutions in CH₂Cl₂ and in pyridine are viscous and gel, the molecular weights (according to vapor pressure osmometric analyses) of the molecules as 2–18 mM CHCl₃ solutions^[14] are 2.5 times that of monomeric **6**, electron micrographs of freeze-fractured gels show fibrous structures, and the chemical shifts of the aromatic protons in solutions of CHCl₃ change with concentration. Evidence that the aggregates are columnar and that the columns twist about one another is less strong. For the

columnar structure, this evidence is that the direction of these NMR shifts are all upfield, which is the same direction that aromatic proton resonances of planar discotic molecules shift upon aggregation.^[15] For the columnar twist, the evidence is features in the electron micrographs of the freeze-fractured gels but these are not clearly related to the proposed structural model. Evidence for the twisted aggregate structures from other methods, such as electron- or X-ray diffraction, absorption or emission spectroscopy, polarized-light microscopy, or scanning-probe microscopy is, as yet, not available. However, the most interesting results to be anticipated would be from studies that could be carried out when the flexible structures are obtained in a nonracemic form: How circular dichroisms, specific rotations, transport properties, and other properties depend on concentration. Lehn draws particular attention to the transport properties because the diameter of the central cavity in **6** is large (8 Å). The channels formed by stacks of derivatives of **6** could therefore be similarly large. Moreover, their pitch should be more flexible than in aggregates of helicenes such as **2** or **3**.

A completely different approach to functionalized helical conjugated structures is suggested by the recent syntheses of 1,10-phenanthroline-*N,N'*-dioxides (**7**).^[16] Even though the structures are simple, it was only during the past three years that they were synthesized. Although it should be possible to prepare them by oxidizing 1,10-phenanthrolines, an effective oxidizing agent remained unknown until Rozen recently showed that HOF·CH₃CN was effective. This novel oxidant is formed when fluorine is passed into aqueous acetonitrile. Previously, the only way to obtain the structure was a clever method by Antkowiak and Antkowiak that, following the precedents for preparing 4,5-disubstituted phenanthrenes, constructed the fully aromatic structure only after the nitrogen atoms of a dihydro- (and therefore nonplanar) derivative of phenanthroline had been oxidized.^[17] The 1,10-phenanthroline-*N,N'*-dioxides have within their helical structures possibly useful functional groups that are polar and that should ligate to metals. It remains, however, for these dioxides to be obtained, as 4,5-dimethylphenanthrene has been,^[18] in nonracemic form and for the direct oxidation to be applied to higher helicenes, such as 1,16-diaza[6]helicene.^[19]

[1] W. H. Laarhoven, W. J. C. Prinsen, *Top. Curr. Chem.* **1984**, *125*, 63.

[2] S. D. Dreher, D. Weix, T. J. Katz, *J. Org. Chem.* **1999**, *64*, 3671, and references therein.

[3] a) C. Nuckolls, T. J. Katz, G. Katz, P. J. Collings, L. Castellanos, *J. Am. Chem. Soc.* **1999**, *121*, 79, and references therein; b) T. Thongpanchang, K. Paruch, T. J. Katz, A. L. Rheingold, K.-C. Lam, L. Liable-Sands, *J. Org. Chem.* **2000**, *65*, 1850, and references therein.

[4] C. Nuckolls, T. J. Katz, *J. Am. Chem. Soc.* **1998**, *120*, 9541.

[5] Moreover, since this response arises in essence entirely from the material's chirality, a structure comprised of alternating layers of the *unpoled* enantiomers, in which each layer is one coherence length thick, gives rise to second harmonics that are quasi-phase matched (B. Busson, M. Kauranen, C. Nuckolls, T. J. Katz, A. Persoons, *Phys. Rev. Lett.* **2000**, *84*, 79, and references therein).

[6] a) M. T. Reetz, E. W. Beuttenmüller, R. Goddard, *Tetrahedron Lett.* **1997**, *38*, 3211; b) A. Terfort, H. Görls, H. Brunner, *Synthesis* **1997**, 79; c) S. D. Dreher, T. J. Katz, K.-C. Lam, A. L. Rheingold, *J. Org. Chem.* **2000**, *65*, 815.

[7] I. G. Stará, I. Starý, A. Kollárovič, F. Teplý, Š. Vyskočil, D. Šaman, *Tetrahedron Lett.* **1999**, *40*, 1993, and references therein.

- [8] X. Qiao, D. M. Ho, R. A. Pascal, Jr., *Angew. Chem.* **1997**, *109*, 1588; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1531.
- [9] B. L. Feringa, N. P. M. Huck, A. M. Schoevaars, *Adv. Mater.* **1996**, *8*, 681.
- [10] a) S. Misumi in *Cyclophanes, Vol. II* (Eds.: P. M. Keehn, S. M. Rosenfeld), Academic, New York, **1983**, p. 573, and references therein; b) J. Harren, A. Sobanski, M. Nieger, C. Yamamoto, Y. Okamoto, F. Vögtle, *Tetrahedron: Asymmetry* **1998**, *9*, 1369, and references therein; c) S. Grimme, J. Harren, A. Sobanski, F. Vögtle, *Eur. J. Org. Chem.* **1998**, 1491.
- [11] a) M. S. Gin, T. Yokozawa, R. B. Prince, J. S. Moore, *J. Am. Chem. Soc.* **1999**, *121*, 2643, and references therein; b) M. S. Gin, J. S. Moore, *Org. Lett.* **2000**, *2*, 135; c) L. A. Cuccia, J.-M. Lehn, J.-C. Homo, M. Schmutz, *Angew. Chem.* **2000**, *112*, 239; *Angew. Chem. Int. Ed.* **2000**, *39*, 233, and references therein.
- [12] A short review: A. E. Rowan, R. J. M. Nolte, *Angew. Chem.* **1998**, *110*, 65; *Angew. Chem. Int. Ed.* **1998**, *37*, 63.
- [13] Lehn, using a vitalistic vocabulary, attributes the helical conformation of **6** to a “helicity codon” rather than to a directing effect.
- [14] The precise meaning of these figures is unclear.
- [15] A. S. Shetty, J. Zhang, J. S. Moore, *J. Am. Chem. Soc.* **1996**, *118*, 1019.
- [16] S. Rozen, S. Dayan, *Angew. Chem.* **1999**, *111*, 3682; *Angew. Chem. Int. Ed.* **1999**, *38*, 3471.
- [17] R. Antkowiak, W. Z. Antkowiak, *Tetrahedron Lett.* **1997**, *38*, 1857; R. Antkowiak, W. Z. Antkowiak, *Heterocycles* **1998**, *47*, 893.
- [18] R. N. Armstrong, H. L. Ammon, J. N. Darnow, *J. Am. Chem. Soc.* **1987**, *109*, 2077.
- [19] H. A. Staab, M. Diehm, C. Krieger, *Tetrahedron Lett.* **1994**, *35*, 8357.

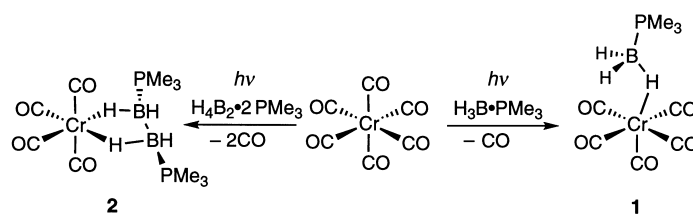
B–H Bond Activations in the Alkane Analogues $\text{H}_3\text{B} \cdot \text{PR}_3$

Warren E. Piers*

The selective, catalytic functionalization of normally inert C–H bonds has been called one of the “Holy Grails” of chemistry.^[1] Consequently, much effort has been expended towards understanding the mechanism of C–H bond activation by homogeneous transition metal compounds. In contrast, comparatively little has been done on the activation of bonds in compounds which are isoelectronic to alkanes. The relationship between BH_4^- and CH_4 has been noted, but analogies between complexes formed through ligation of BH_4^- and the more elusive σ complexes of CH_4 are tenuous, based on the obvious difference in charge for these two species. For instance, with the exception of a few examples, BH_4^- tends to bind metals in an η^2 fashion, while CH_4 has been computationally predicted to assume an η^1 , end-on bonding posture.^[2] Thus, while the borohydride complexes $[(\text{CO})_4\text{M}(\eta^2\text{-BH}_4)]^-$ ($\text{M} = \text{Cr}, \text{W}$) are known,^[3] they serve as poor structural models for the methane complexes $[(\text{CO})_5\text{M} \cdot \text{CH}_4]$ observed when $[\text{M}(\text{CO})_6]$ is photolyzed in a methane matrix.^[4]

To eliminate this difference and provide a more useful chemical analogy, Shimoi et al. have recently examined the photolysis of $[\text{M}(\text{CO})_6]$ in the presence of $\text{H}_3\text{B} \cdot \text{L}$ ($\text{L} = \text{PMe}_3, \text{PPh}_3, \text{NMe}_3$),^[5] methane analogues which are both isoelectronic and neutral. The products isolated, in excellent yield, are remarkable η^1 -borane complexes of the $\text{M}(\text{CO})_5$ fragment that contain an unsupported M–H–B interaction (exemplified by **1**; Scheme 1). The increased stability of these alkane-analogue σ complexes allowed for complete characterization, including four examples crystallographically.

All the structural and spectroscopic data for $[(\text{CO})_5\text{M}(\eta^1\text{-BH}_3 \cdot \text{L})]$ support a bonding picture in which σ donation



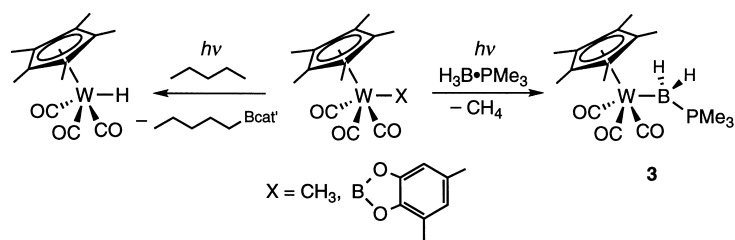
Scheme 1.

from a B–H bond into the a_1 orbital of $\text{M}(\text{CO})_5$ is the dominant feature of the interaction. Molecular orbital calculations show that π back donation from $\text{M}(\text{CO})_5$ to $\text{H}_3\text{B} \cdot \text{L}$ is negligible, a combination of the poor π donating ability of the former and the high energy of the B–H σ^* orbitals. These complexes are more stable than the alkane complexes due to the greater degree of polarization and electron richness inherent in the B–H bond in $\text{H}_3\text{B} \cdot \text{L}$ as opposed to the C–H bonds in CH_4 . Interestingly, η^2 complexes are not observed upon further photolysis, although the same authors have shown that related species, for example **2**, are formed when $[\text{M}(\text{CO})_6]$ is photolyzed in the presence of diborane $\text{B}_2\text{H}_4 \cdot 2\text{PMe}_3$ (Scheme 1).^[6] Taken together, the experimental and computational data on the η^1 complexes provide convincing support for a valid and valuable analogy between complex **1** and the methane adducts $[(\text{CO})_5\text{M} \cdot \text{CH}_4]$.^[2, 4] The nature of these adducts also has implications for the reaction pathway for B–H bond activation, which was investigated in a subsequent paper by these same authors.^[7]

Photolysis of a metal carbonyl that contains a somewhat more electron rich metal center, and also a sacrificial alkyl ligand, in the presence of the borane–phosphane alkane analogues leads to formation of Lewis base stabilized boryl compounds. Photolytic loss of CO from $[\text{Cp}^*\text{M}(\text{CO})_3\text{CH}_3]$ ($\text{M} = \text{Mo}, \text{W}$; $\text{Cp}^* = \text{C}_5\text{Me}_5$) generates “[$\text{Cp}^*\text{M}(\text{CO})_2\text{CH}_3$]”, a highly reactive molecular fragment capable of interacting with X–H σ bonds. When those bonds are the B–H bonds of

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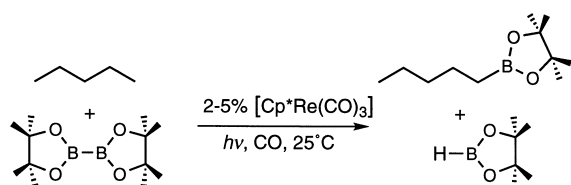
$\text{H}_3\text{B} \cdot \text{L}$, methane loss ensues with production of a phosphane-stabilized boryl species (exemplified by **3**; Scheme 2). Unlike the extensive chemistry known for the parent alkyl ligand CH_3 , there are no known compounds containing an unligated BH_2 ligand; even base-stabilized examples are exceedingly



Scheme 2.

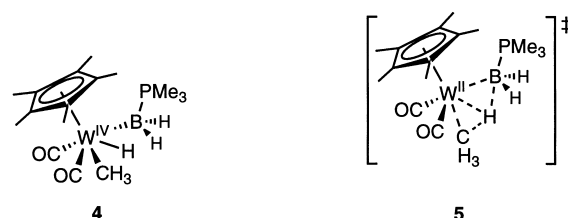
rare.^[8] In **3**, the W–B bond is the longest known at 2.476(7) Å, presumably because the stabilizing phosphane ligand precludes π bonding between W and B. The relatively low stretching frequencies for the CO ligands, however, suggest that the boryl ligand is a stronger σ donor than the methyl group of the starting materials.

As Scheme 2 further suggests, this chemistry is closely related to the stoichiometric alkane functionalization reactions reported by Hartwig and Waltz in 1997,^[9] a discovery which stands as an important milestone in the search for this particular Holy Grail. In this instance, it is a boryl ligand which assumes the role of the sacrificial species while the alkane solvent is activated. However, one crucial difference is notable: B–C bond formation is observed with elimination of R–Bcat' rather than H–Bcat' (cat' = 3,5-dimethylcatechol), allowing for further functionalization of the alkane fragment. Very recently, the Hartwig group has reported a catalytic version of this reaction, employing a rhenium-based catalyst and a diborane(4) compound as shown in Scheme 3.^[10]



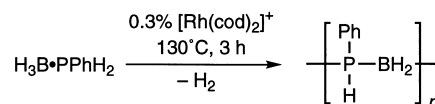
Scheme 3.

Mechanistically, the reactions shown in Schemes 2 and 3 bring to light a conundrum for the key steps in the reactions: oxidative addition or σ -bond metathesis? This question has also arisen within the context of C–H activation reactions.^[11] Clearly, in the reaction producing compound **3**, the B–H bond is in some way activated; the experimental data does not, however, distinguish between full oxidative addition (producing intermediate **4**) or a concerted methane elimination process (via transition state **5**). Distinguishing these mechanistic possibilities experimentally is nontrivial and computational studies^[12] have proven helpful for the cationic iridium system of Bergman and Arndtsen.^[11] Probably the choice between the two pathways will depend on the variables



inherent to the system in question, that is, metal, ligands, and bond being activated. For formation of **3**, the authors speculate, quite reasonably, that σ bond metathesis is more likely given the high energy of the B–H σ^* orbitals, which would need to be populated in a mechanism based on oxidative addition. Whatever the mechanism of methane loss, the initial work with $\text{M}(\text{CO})_5$ (Scheme 1) provides a firm foundation for the notion that $\eta^1\text{-H}_3\text{B} \cdot \text{L}$ adducts would be the initial products in $[\text{Cp}^*\text{M}(\text{CO})_3\text{CH}_3]$ chemistry.

The work of Shimoi et al.^[5–7] provides an excellent model system for alkane activation, but it also has implications for chemistry aimed at the activation and functionalization of the $\text{H}_3\text{B} \cdot \text{L}$ adducts themselves. The Manners group has recently reported the dehydropolymerization of $\text{H}_3\text{B} \cdot \text{PPhH}_2$ to high molecular weight poly(phosphinoborane) at 130 °C using Rh^{I} -based catalyst precursors (Scheme 4).^[13] By performing the



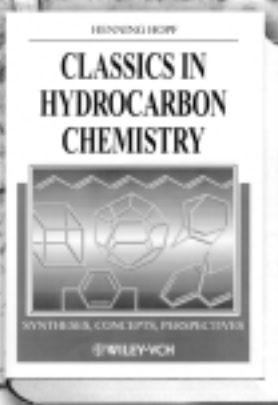
Scheme 4. cod = 1,5-cyclooctadiene.

reaction in neat monomer, molecular weights of 31 000, corresponding to a degree of polymerization of 254, were achieved. The authors point out that this polymer is a structural analogue of polystyrene, which raises intriguing questions about its properties. Little mechanistic investigation has been presented to date, but clearly the results of Shimoi et al. have a bearing on possible mechanistic steps in the polymerization process. The apparent lack of B–B or P–P bond formation in this reaction suggests that, despite the higher temperatures in the Rh-catalyzed reaction, the borane–phosphane adduct reacts without dissociation into its constituents. Since Rh^{I} is involved, an oxidative addition of a B–H or P–H bond is a plausible first step in the polymerization reaction; the work of Shimoi and co-workers points to the former. Subsequent B–P bond formation could then occur by a σ -bond metathesis reaction involving P–H bonds. Of course, there are several possible mechanistic scenarios which await further investigation.

One of the joys of chemistry is the seemingly endless games one can play with the periodic table. In the various papers reported here, the close relationship between alkanes and borane–phosphane adducts has been exploited to reveal some remarkable chemistry. The work addresses some mechanistic questions and raises others concerning the fundamental bond activation chemistry involved, but also has exciting prospects for new materials and practical alkane functionalization processes.

- [1] B. A. Arndtsen, R. G. Bergman, T. A. Mobley, T. H. Peterson, *Acc. Chem. Res.* **1995**, 28, 154.
 [2] S. Zaric, M. B. Hall, *J. Chem. Phys. A* **1997**, 101, 4646.
 [3] a) S. W. Kirtly, M. A. Andrews, R. Bau, G. W. Grynkeiwich, T. J. Marks, D. L. Tipton, B. R. Whittlesey, *J. Am. Chem. Soc.* **1977**, 99, 7154; b) M. Y. Darensbourg, R. Bau, M. W. Marks, R. R. Burch, Jr., J. C. Deaton, S. Slater, *J. Am. Chem. Soc.* **1982**, 104, 6961.
 [4] a) R. N. Perutz, J. J. Turner, *J. Am. Chem. Soc.* **1975**, 97, 4791; b) C. E. Brown, Y. Ishikawa, P. A. Hackett, D. M. Raynor, *J. Am. Chem. Soc.* **1990**, 112, 2536.
 [5] M. Shimoi, S. Nagai, M. Ichikawa, Y. Kawano, K. Katoh, M. Uruichi, H. Ogino, *J. Am. Chem. Soc.* **1999**, 121, 11704.
 [6] M. Shimoi, K. Katoh, H. Ogino, *J. Chem. Soc. Chem. Commun.* **1990**, 811.
 [7] Y. Kawano, T. Yasue, M. Shimoi, *J. Am. Chem. Soc.* **1999**, 121, 11744.
 [8] G. J. Irvine, M. J. G. Lesley, T. B. Marder, N. C. Norman, C. R. Rice, E. G. Robins, W. R. Roper, G. R. Whittell, L. J. Wright, *Chem. Rev.* **1998**, 98, 2685.
 [9] K. M. Waltz, J. F. Hartwig, *Science* **1997**, 277, 211.
 [10] H. Chen, J. F. Hartwig, *Angew. Chem.* **1999**, 111, 3597; *Angew. Chem. Int. Ed.* **1999**, 38, 3391.
 [11] B. A. Arndtsen, R. G. Bergman, *Science* **1995**, 270, 1970.
 [12] a) D. L. Strout, S. Zaric, S. Niu, M. B. Hall, *J. Am. Chem. Soc.* **1996**, 118, 6068; b) M.-D. Su, S.-Y. Chu, *J. Am. Chem. Soc.* **1997**, 119, 5373.
 [13] H. Dorn, R. A. Singh, J. A. Massey, A. J. Lough, I. Manners, *Angew. Chem.* **1999**, 111, 3540; *Angew. Chem. Int. Ed.* **1999**, 38, 3321.

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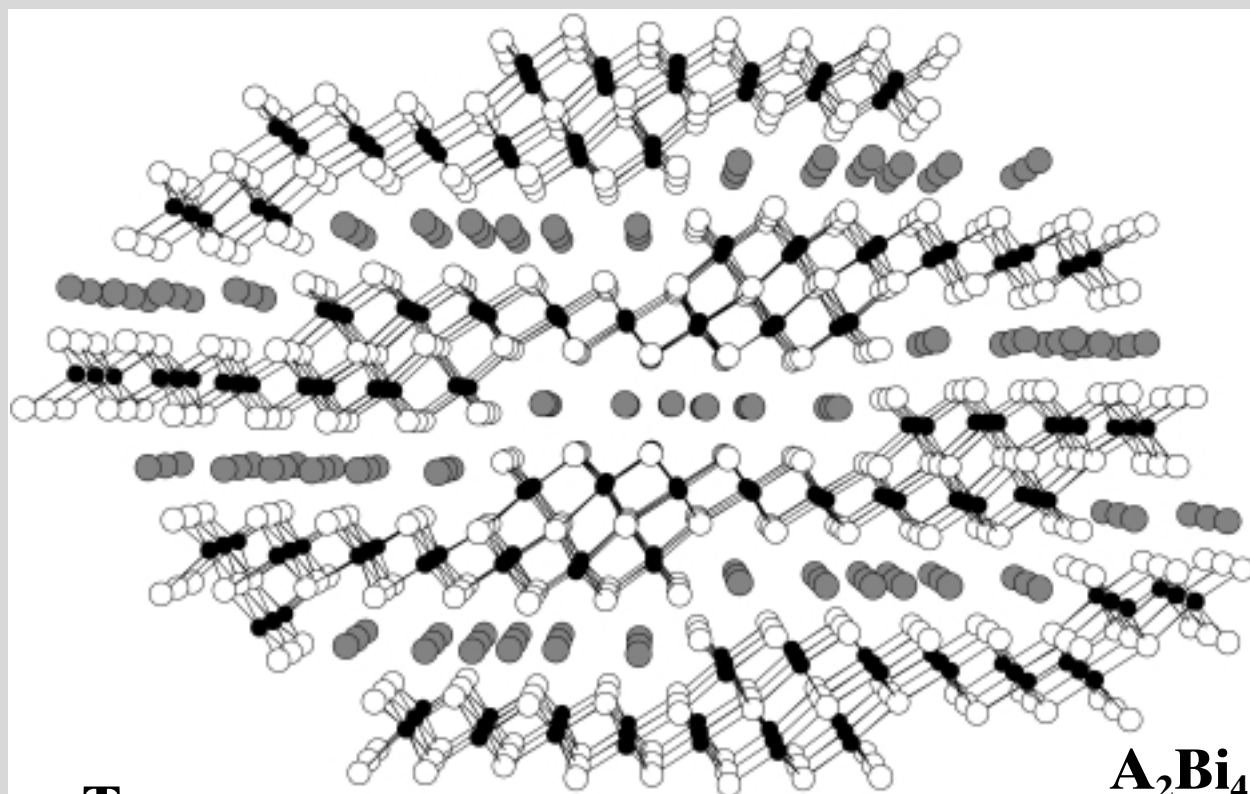
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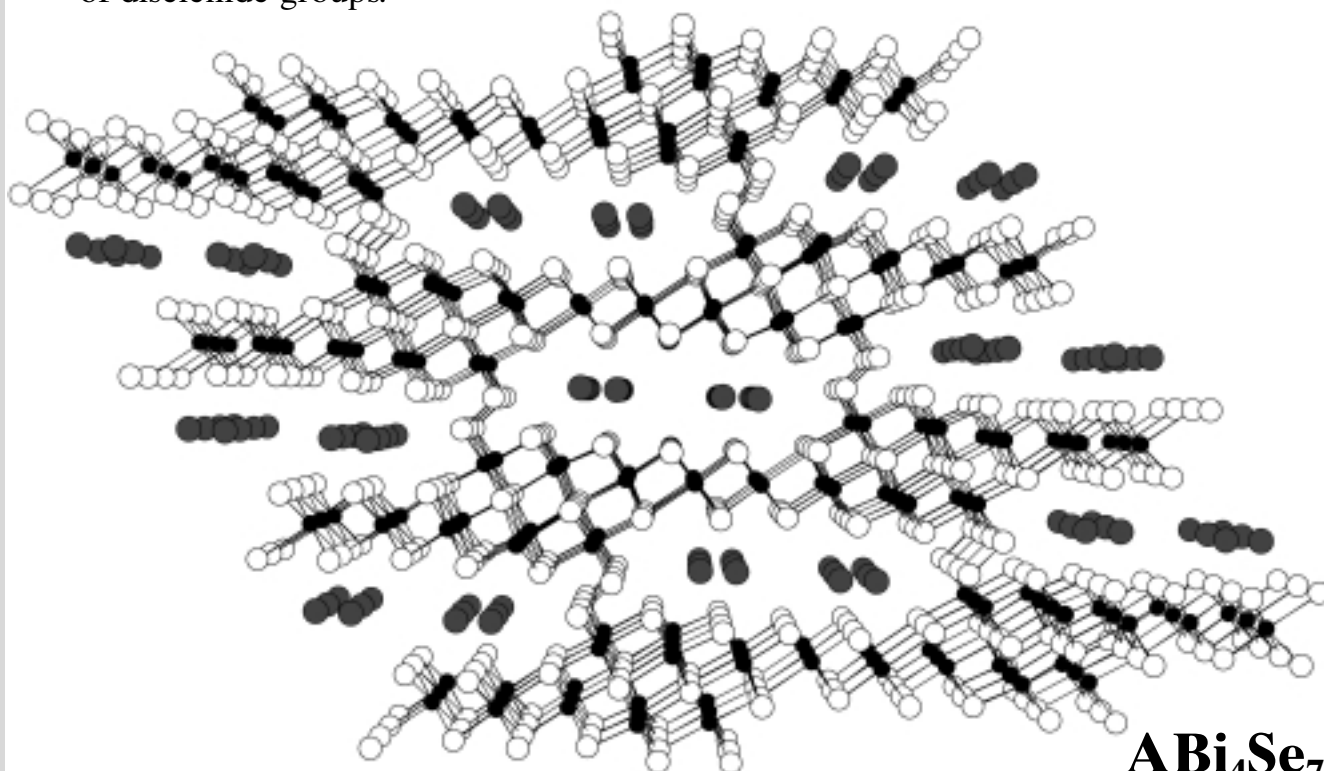
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Redox-Induced “Zipper” Action in $\text{Rb}_2\text{Bi}_4\text{Se}_7$ and $\text{Cs}_2\text{Bi}_4\text{Se}_7$: Coupling of Slabs to a Three-Dimensional Framework through Single-Crystal to Single-Crystal Conversion**

Lykourgos Iordanidis and Mercouri G. Kanatzidis*

The number of known topotactic single-crystal to single-crystal solid-state transformations is limited. Most of the reported cases involve the dimerization or polymerization of organic or organometallic^[1] molecules by heat or light, or loss of solvent from coordination complexes^[2] or rigid framework structures^[3] without the collapse of the rest of the structure. One well-studied solid-state example is the topotactic reduction of $\gamma\text{-MnO}_2$ to $\text{MnO}(\text{OH})$ with hydrazine.^[4] Our interest in exploring new solid-state chemistry in the A/Bi/Se (A = alkali metal) systems led us to the discovery of the new layered isostructural phases $\text{Rb}_2\text{Bi}_4\text{Se}_7$ and $\text{Cs}_2\text{Bi}_4\text{Se}_7$. The behavior of these phases is very different from any other A/Bi/Se phases studied by us before^[5] in that they exhibit a remarkable redox transformation in the solid state. This conversion, of which we know of no other examples, involves the topotactic oxidative coupling of entire rows of Se^{2-} ions to give Se_2^{2-} groups in a cooperative way reminiscent of the closing action of a zipper. This remarkable process involves the rapid expulsion of alkali ions from the crystals and results in the daughter compounds RbBi_4Se_7 and CsBi_4Se_7 , in which the original layers are stitched into a three-dimensional framework. The facility with which the zipperlike action transforms the starting compounds is attested by the clean and quick conversion of whole single crystals from one phase to the next without loss of crystallinity.

$\text{Rb}_2\text{Bi}_4\text{Se}_7$ is a two-dimensional (2D) layered structure made of Bi_2Te_3 -type and CdI_2 -type blocks (Figure 1 a).^[6] The Rb atoms reside between the layers in trigonal prismatic sites. The $[\text{Bi}_4\text{Se}_7]^{2-}$ framework consists of edge-sharing distorted Bi octahedra. A key characteristic of the structure is that adjacent layers come in close, nonbonding contact through the Se5 atoms, which are at a distance of 3.336(6) Å. As seen in Figure 1 b the Se5 atoms in each layer form parallel rows that approach one another side by side so that the Se atoms in one row are staggered with respect to those in the other row. Rb1 has a bicapped trigonal prismatic coordination with bonds varying between 3.445(5) and 3.733(4) Å. Rb2 and Rb3 have similar trigonal prismatic coordination and are disordered over certain sites in the space provided between the layers.

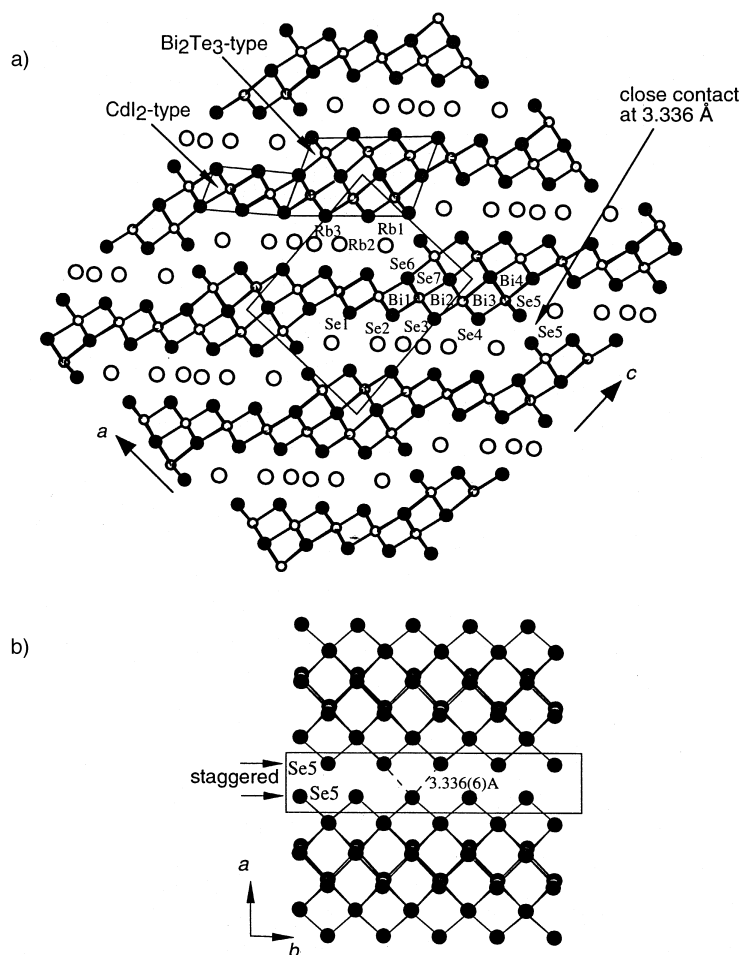


Figure 1. Projection of the structure of $\text{Rb}_2\text{Bi}_4\text{Se}_7$ down the b axis. The Bi1 and Bi2 octahedra are distorted with Bi–Se bond lengths between 2.870(3)–3.061(3) Å. The octahedra around Bi3 and Bi(4) are more distorted with Bi–Se bond lengths varying between 2.808(4) and 3.205(3) Å. In each Bi octahedron there is a short bond *trans* to a long bond. For example, in the Bi4 octahedra there are two short Bi–Se bonds of 2.792(2) Å *trans* to two long bonds of 3.205(3) Å. The bond angles for Bi1 and Bi2 octahedra are more regular, varying between 86.74(8)–92.58(9)° for Bi1 and 86.55(8)–93.47(9)° for Bi2, while they are less regular in the Bi3 and Bi4 octahedra varying between 82.06(8)–94.64(9) and 82.05(8)–97.8(1)°, respectively.

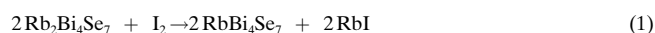
Preparations of $\text{Rb}_2\text{Bi}_4\text{Se}_7$ and $\text{Cs}_2\text{Bi}_4\text{Se}_7$ give homogeneous-looking samples with thin, long plate morphology. We noticed that fresh batches of the compound tended to become “wet” and stuck to the vial walls when stored in air, yet no obvious decomposition of the compound was evident. We monitored the X-ray powder diffraction pattern of fresh samples of $\text{Rb}_2\text{Bi}_4\text{Se}_7$ and found them to be changing with time (small but significant shifts in the positions of a few reflections) upon exposure of the sample to ambient conditions. We concluded that an oxidative, probably topotactic, transformation was under way. Surprisingly, the structure of the “aged” products could be solved from data collected on the transformed crystals. Thus, we discovered the presence of diselenide (Se_2^{2-}) groups and a composition of RbBi_4Se_7 not $\text{Rb}_2\text{Bi}_4\text{Se}_7$. This process occurs also in $\text{Cs}_2\text{Bi}_4\text{Se}_7$ regardless of the crystal size. It is important to note that the structures of ABi_4Se_7 (A = Rb, Cs) were determined employing the same specimens used to determine the structures of $\text{A}_2\text{Bi}_4\text{Se}_7$.

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The two structure types are closely related,^[7] which proves that their redox interconversion is fully topotactic. The structure of ABi_4Se_7 contains the same $[\text{Bi}_4\text{Se}_7]^{2-}$ slabs of $\text{A}_2\text{Bi}_4\text{Se}_7$, but now they are connected through Se–Se bonds to form a 3D structure (Figure 2a). Compared to $\text{A}_2\text{Bi}_4\text{Se}_7$, the slabs in ABi_4Se_7 have shifted with respect to each other so that the Se5 atoms in two adjacent rows move from a staggered to an eclipsed position to form an infinite row of single Se5–Se5 bonds with a length of 2.354(9) Å (Figure 2b). This process resembles a zipperlike closing action. The Rb atoms in RbBi_4Se_7 show a diffuse character (high-temperature factors) as a result of the transformation process. Rb1 and Rb2 are now the only alkali atoms remaining in the tunnels and spread over the entire available space. The Cs analogue behaves correspondingly. A schematic representation of the “zipper” action is shown in Figure 3.

The interconversion of the pair $\text{A}_2\text{Bi}_4\text{Se}_7/\text{ABi}_4\text{Se}_7$ can be readily effected with the proper oxidants and reductants. Whereas the reaction with ambient oxygen is difficult to control and depends on the relative humidity, the reaction with I_2 in wet CH_3CN is facile and transforms the samples, usually within one hour [Eq. (1)]. The formation of Se–Se bonds can also be confirmed by Raman spectroscopy through observation of its symmetric stretching mode at 233 cm^{-1} .



To reverse the process (“zipper” open), ABi_4Se_7 can be heated at moderate temperatures (200–300 °C) with a stoichiometric amount of alkali metal to give back $\text{A}_2\text{Bi}_4\text{Se}_7$.

This rare example of very long-range topotactic chemistry in the solid state is exceptional because the individual Se atoms that become diselenide groups are not actually accessible to oxygen for steric reasons. The fact that the oxidative coupling occurs in the presence of water suggests that the process is electrochemical in nature. The fact that all pertinent Se^{2-} atoms couple to give Se_2^{2-} groups even though the $\text{A}_2\text{Bi}_4\text{Se}_7$ phases are dense, and not porous, supports the electrochemical route. For “zipper” closing, electrons must be removed through the conductive $[\text{Bi}_4\text{Se}_7]^{2-/1-}$ framework,

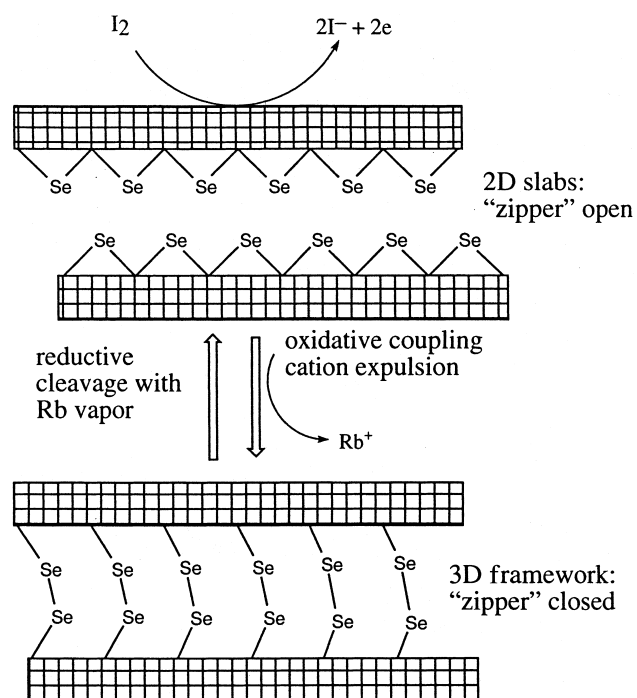


Figure 3. Schematic diagram showing the oxidative zipperlike couples of two rows of Se atoms belonging to two adjacent $[\text{Bi}_4\text{Se}_7]^{2-}$ layers into a single row of diselenide groups. This process happens for all such rows over the entire single crystal.

generating holes, which apparently migrate to the Se_5^{2-} atoms and cause them to form Se–Se bonds across the layers. These bonds therefore represent a form of hole localization in these materials. Alkali metal atoms are simultaneously expelled to maintain electroneutrality. The narrow energy band gaps present in $\text{A}_2\text{Bi}_4\text{Se}_7$ and ABi_4Se_7 (<0.55 eV) allow adequate electron transport to complete the transformation.

In conclusion, an unparalleled all solid-state, topotactic oxidative coupling has been discovered, which generates rows of Se–Se bonds, a process that resembles the closing action of a zipper. The process is reversible as the “zipper” can also open by reduction with alkali metal vapor. $\text{A}_2\text{Bi}_4\text{Se}_7$ have the unique characteristic of oxidatively transforming to produce

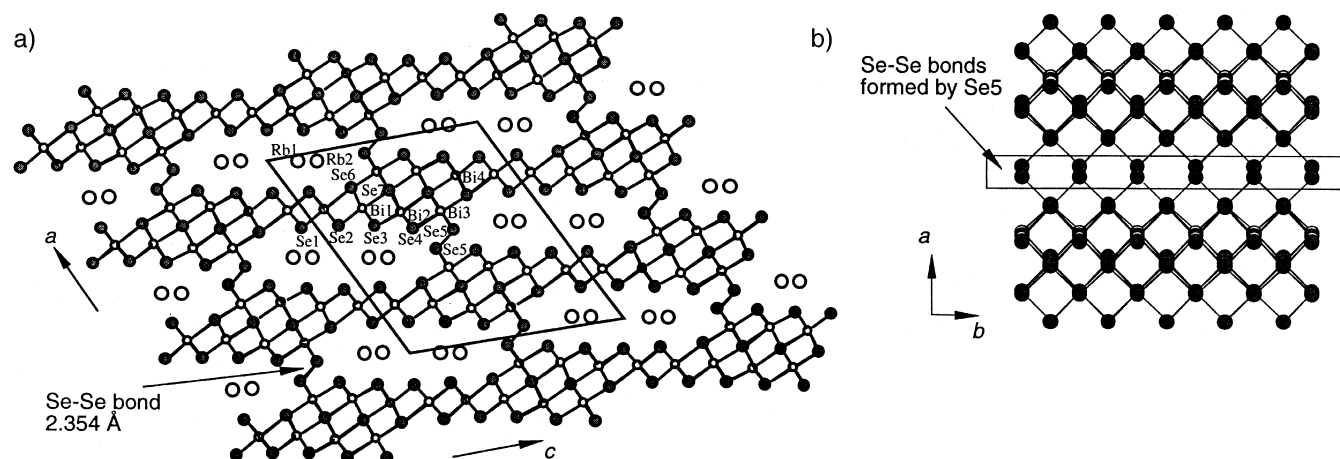


Figure 2. Projection of the structure of RbBi_4Se_7 down the *b* axis. Atoms Bi1 and Bi4 have bond lengths between 2.703(5)–3.260(5) and 2.883(4)–3.050(4) Å, respectively, while their angles are closer to that of a normal octahedron varying between 88.1(1)–92.0(1)° for Bi1 and between 88.4(1)–91.9(1)° for Bi4. In the case of Bi2 and Bi3 the Bi–Se bond lengths vary between 2.801(4)–3.097(4) and 2.776(4)–3.173(4) Å, respectively. The angles around Bi2 and Bi3 are between 87.2(1)–96.1(1) and 83.43(9)–96.5(1)°, respectively.

ABi₄Se₇ in a single-crystal to single-crystal fashion. We anticipate that the zipperlike action in these materials will also be feasible using electrochemical means.

Experimental Section

Rb₂Bi₄Se₇: A mixture of Rb₂Se (0.060 g, 0.240 mmol) and Bi₂Se₃ (0.314 g, 0.480 mmol) was transferred to a carbon-coated silica tube which was flame-sealed under vacuum. The tube was heated at 810 °C for 6 d, then cooled to 510 °C at 7 °C h⁻¹ and to 50 °C over 10 h. The product consisted of silver-gray long laths. Semiquantitative energy-dispersive analysis (EDS) using a scanning electron microscope (SEM) on several needles gave an average composition of Rb₂Bi₄Se_{6.8}. **Cs₂Bi₄Se₇:** The procedure was similar to the one above but using a mixture of Cs₂Se (0.060 g, 0.174 mmol) and Bi₂Se₃ (0.228 g, 0.348 mmol). A single-phase product was obtained. The optical band gaps of these two compounds were measured spectroscopically and are 0.53 and 0.56 eV, respectively.

ABi₄Se₇ (A = Rb, Cs): Rb₂Bi₄Se₇ (0.1 g, 0.064 mmol) was ground into powder and added to a 2.5 mm solution of I₂ in degassed, wet CH₃CN (100 mL) at 23 °C. The mixture was continuously stirred under an N₂ atmosphere. The oxidation to ABi₄Se₇ was complete in less than 1 h. The optical band gaps of both compounds are ~0.4 eV.

A Bruker SMART Platform CCD diffractometer using graphite monochromatic MoK α radiation (λ = 0.71073 Å) was used for data collection in all cases. Several different sets of frames, covering a random area of the reciprocal space, were collected using 0.3° steps in ω . The SMART^[8a] software was used for data acquisition and the SAINT^[8a] program for data extraction. The absorption correction was carried out with SADABS^[8a] and the structure solution (direct methods) and refinement (full-matrix least squares on F^2) were done with the SHELXTL^[8a] and/or the SHELX97^[8b] package of crystallographic programs.

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- [1] a) K. Cheng and B. M. Foxman, *J. Am. Chem. Soc.* **1979**, *99*, 8102; b) K. Honda, F. Nakanishi, N. Feeder, *J. Am. Chem. Soc.* **1999**, *121*, 8246; c) M. Leibovitch, G. Olovsson, J. R. Scheffer, J. Trotter, *J. Am. Chem. Soc.* **1998**, *120*, 12755, and references therein; d) M. Leibovitch, G. Olovsson, J. R. Scheffer, J. Trotter, *J. Am. Chem. Soc.* **1997**, *119*, 1462; e) T. Suzuki, *Pure Appl. Chem.* **1996**, *68*, 281.
- [2] a) S. Rabe, U. Müller, *Z. Anorg. Allg. Chem.* **1999**, *625*(8), 1367; b) U. Englert, B. Ganter, T. Wagner, W. Klauel, *Z. Anorg. Allg. Chem.* **1998**, *624*(6), 970.
- [3] a) C. J. Kepert, M. J. Rosseinsky, *Chem. Commun.* **1999**, 375; b) H. Li, M. Eddaoudi, M. O'Keefe, O. M. Yaghi, *Nature* **1999**, *402*, 276.
- [4] W. Feitknecht, H. R. Oswald, U. Feitknecht-Steinmann, *Helv. Chim. Acta* **1960**, *43*, 1947.
- [5] a) T. J. McCarthy, S.-P. Ngeyi, J.-H. Liao, D. C. DeGroot, J. Schindler, C. R. Kannewurf, M. G. Kanatzidis, *Chem. Mater.* **1993**, *5*, 331; b) D.-Y. Chung, K.-S. Choi, L. Iordanidis, J. L. Schindler, P. W. Brazis, C. R. Kannewurf, B. Chen, S. Hu, C. Uher, M. G. Kanatzidis, *Chem. Mater.* **1997**, *9*, 3060; c) D.-Y. Chung, L. Iordanidis, K.-S. Choi, M. G. Kanatzidis, *Bull. Korean Chem. Soc.* **1998**, *19*, 1283.
- [6] a) Rb₂Bi₄Se₇: Crystal dimensions 0.65 × 0.025 × 0.025 mm³, space group $P2_1/m$, a = 13.062(2), b = 4.2072(5), c = 15.301(2) Å, β = 93.137(2)°, V = 839.61(17) Å³, Z = 2, ρ_{calcd} = 6.169 g cm⁻³, $2\theta_{\text{max}}$ = 50.18°, T = 173(2) K, 3887 reflections collected, 1689 independent, 1201 observed [$I > 2\sigma(I)$], μ = 62.69 mm⁻¹, $T_{\text{max/min}}$ = 0.064/0.029, 85 parameters, final R indices [$I > 2\sigma(I)$]: $R1$ = 0.066, $wR2$ = 0.16, max/min residual electron density 6.60/−3.15 e Å⁻³. b) Cs₂Bi₄Se₇: Crystal dimensions 0.5 × 0.025 × 0.015 mm³, space group $P2_1/m$, a = 13.227(1), b = 4.1857(4), c = 15.556(2) Å, β = 94.592(2)°, V = 858.49(14) Å³, Z = 2, ρ_{calcd} = 6.400 g cm⁻³, $2\theta_{\text{max}}$ = 56.92°, T = 173.1(1) K, 5978 reflections collected, 2224 independent, 1975 observed [$I > 2\sigma(I)$], μ = 59.86 mm⁻¹, $T_{\text{max/min}}$ = 1.00/0.43, 86 parameters, final R indices [$I > 2\sigma(I)$]: $R1$ = 0.036, $wR2$ = 0.094, max/min residual electron density 3.79/−3.03 e Å⁻³.
- [7] RbBi₄Se₇: Data on this compound were collected on two different crystals. One of them was the same crystal (now transformed) initially used to determine the structure of Rb₂Bi₄Se₇. The other was selected from a converted batch of sample: Crystal dimensions 0.35 × 0.025 ×

0.025 mm³, space group $C2/m$, a = 22.286(5), b = 4.1760(8), c = 19.794(4) Å, β = 116.89(3)°, V = 1642.9(6) Å³, Z = 4, ρ_{calcd} = 5.960 g cm⁻³, $2\theta_{\text{max}}$ = 50.44°, T = 293(2) K, 4127 reflections collected, 1651 independent, 1186 observed [$I > 2\sigma(I)$], μ = 61.13 mm⁻¹, $T_{\text{max/min}}$ = 1.00/0.51, 80 parameters, final R indices [$I > 2\sigma(I)$]: $R1$ = 0.082, $wR2$ = 0.209, max/min residual electron density 5.64/−3.75 e Å⁻³. CsBi₄Se₇: Crystal dimensions 0.42 × 0.02 × 0.02 mm³, space group $C2/m$, a = 22.224(7), b = 4.178(1), c = 19.742(6) Å, β = 116.384(1)°, V = 1642.2(9) Å³, Z = 4, ρ_{calcd} = 6.154 g cm⁻³, $2\theta_{\text{max}}$ = 57.16°, T = 173.1(1) K, 7393 reflections collected, 2154 independent, 1201 observed [$I > 2\sigma(I)$], μ = 60.39 mm⁻¹, $T_{\text{max/min}}$ = 1.00/0.46, 80 parameters, final R indices [$I > 2\sigma(I)$]: $R1$ = 0.053, $wR2$ = 0.123, max/min residual electron density 6.11/−3.59 e Å⁻³. Further details on the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository numbers CSD-411071, -411072, -411073, and -411074.

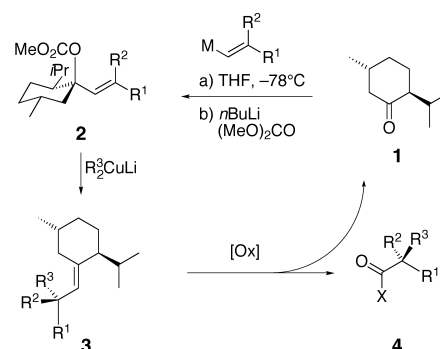
- [8] a) SMART versions 4 and 5 (1996–1998), SAINT versions 4, 5, and 6 (1994–1999), SADABS, SHELXTL V-5, Bruker Analytical X-ray Systems Inc. Madison, Wisconsin 53719 USA; b) G. M. Sheldrick, University of Göttingen, Germany.

A Novel Method To Generate Chiral Quaternary Carbon Centers of High Enantiomeric Purity Using a Highly Stereoselective Addition of Vinylalanes to a Chiral Aldehyde**

Claude Spino* and Christian Beaulieu

In memory of Larry Weiler

We recently reported on a novel alternative to the alkylation of chiral enolates using the S_N2' addition of cuprates to chiral carbonates **2** derived from menthone (**1**, Scheme 1).^[1] Oxidative cleavage of **3** furnished aldehydes, carboxylic acids, or alcohols having a tertiary α -chiral center (R^2 = H) with enantiomeric purities greater than 99 %.



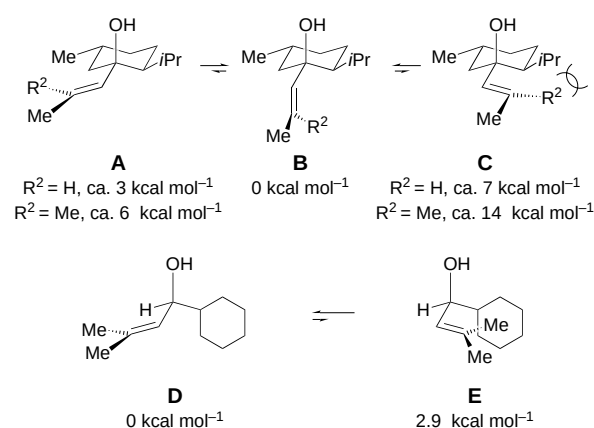
Scheme 1. Generation of tertiary chiral carbon centers from menthone (**1**).

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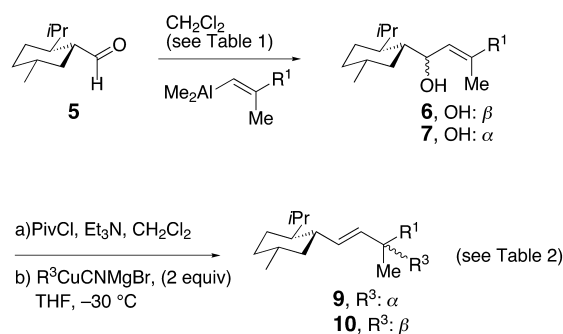
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

Quaternary centers of high optical purity are notoriously difficult to construct. Several ingenious strategies have helped to surmount this synthetic obstacle, but many compromise on the generality for the benefit of selectivity.^[2–4] The prospect of making quaternary carbon centers using the approach described above (Scheme 1, $R^2 = \text{Me}$) was appealing because the cuprate addition step should be selective regardless of the substitution on the allylic moiety.^[5] However, trisubstituted carbonates such as **2** ($R^2 = \text{Me}$) did not react with cuprate reagents because of the high energy required to attain one of the two reactive conformations **A** or **C** (Scheme 2, top).



Scheme 2. Calculations on the reactive conformations for models of **2** (**A**, **B**, **C**) and **11** (**D**, **E**).

We considered the chiral aldehyde **5** as a surrogate auxiliary (Scheme 3). It was prepared in two easy steps from menthone (Wittig olefination with $\text{ClPh}_3\text{PCH}_2\text{OMe}$ followed by hydrolysis with HCl /chloroform). Calculations on a model allylic



Scheme 3. Generation of quaternary chiral carbon centers from **5**. Piv = pivaloyl = 2,2-dimethylpropyl.

system indicated a 3 kcal mol⁻¹ energy difference between the two reactive conformations **D** and **E** (Scheme 2, bottom), which should provide enough bias to allow the *anti*-selective cuprate addition to take place on conformer **D** only. However, this change in chiral auxiliary (from menthone to **5**) created some uncertainties concerning issues crucial to the success of the method. Would the addition of vinyl anions to aldehyde **5** proceed stereoselectively? How could we ensure an $\text{S}_{\text{N}}2'$ addition of the cuprate reagent on the corresponding carbo-nate or ester?

The answer to the first question was initially a resounding no. Not surprisingly, di- or trisubstituted vinylmagnesium and

vinylolithium reagents gave, each time, approximately 2:1 mixtures of two easily separable diastereomeric alcohols. However, upon adding aldehyde **5** directly to a reaction mixture containing the carboalumination^[6] intermediate of 1-hexyne obtained from a zirconium-catalyzed reaction, we obtained a surprising ratio of 13:1 of two easily separable alcohols **6** and **7** in 65 % nonoptimized yield. The major alcohol was shown to have the configuration corresponding to the Felkin–Anh^[7] addition product **6f** (Scheme 3, Table 1) by

Table 1. Addition of vinylalanes to aldehyde **5**.

Entry	8	R^1	Yield [%] ^[a]	6:7 ^[b]
1	a	cyclohexyl	75	15:1
2	b	PhCH_2	76	12:1
3	c	Ph	63	20:1
4	d	$-(\text{CH}_2)_3\text{OTBS}$	68	8:1
5	e	$-\text{CMe}_2\text{CH}_2\text{OTBS}$	0	–
6	f	$-(\text{CH}_2)_3\text{CH}_3$	65	13:1
7	f ^[c]	$-(\text{CH}_2)_3\text{CH}_3$	93	17:1 ^[d]

[a] Yields of isolated products. [b] Ratios of the isolated products. [c] Addition on PhMeCHCHO . [d] Ratio determined by NMR integration of crude mixtures.

converting it, and its minor diastereomer **7f**, into the corresponding Mosher esters. This ratio was later reproduced with other vinylalanes prepared in a similar way. Also, the addition of vinylalane **8f** to phenylpropanal gave a 93 % yield of a mixture of diastereomeric alcohols in a 17:1 ratio based on NMR integration of the crude mixture. Preliminary results therefore indicate that the preference of vinylalanes for the Felkin–Anh mode of addition on α -chiral aldehydes could be general. Instances of increased Felkin–Anh selectivity with increasing size of the metal have been noted before.^[7] If the selectivity obtained here was a surprise, the chemical yields of these additions were also noteworthy. We are aware of only a few reports of vinylalanes being added directly to ketones or aldehydes, and usually in only modest yields (30–50 %).^[8–12] Trialkylalanes have been added selectively to β -ketosulfoxides and chiral β -ketoamides.^[13,14] Alkenylalanes prepared from hydro- or carboalumination of alkynes are usually treated with other electrophiles^[6] or converted into the iodide or bromide first or transmetalated directly with zinc, copper,^[15] lithium (ate complex), or other metals before addition to carbonyl electrophiles.^[16–18] The scope and use of this protocol to make diastereomerically enriched allylic alcohols is currently being investigated.

On the basis of the work of Goering and co-workers we expected that derivatization of alcohols **6** into the corresponding pivalates **11** (pivalate = 2,2-dimethylpropanoate) and the use of cuprate reagents of the type RCuCNMgBr should lead to highly regioselective reactions in favor of the $\text{S}_{\text{N}}2'$ product.^[19] This was indeed the case, while other types of cuprate reagents (for example, R_2CuLi) or other leaving groups (for example, OAc , OCO_2Me) gave unsatisfactory mixtures of regioisomers. Also, the use of a catalytic amount of CuCN led to the recovery of starting material only.^[19] Reactions using Goering's procedure with a range of alkyl cuprates proceeded to give a good yield of the cross-coupling products **9** and **10** with excellent stereoselectivities (Table 2).

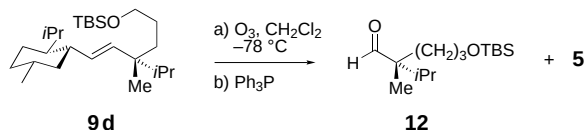
Table 2. S_N2' displacements of cuprates on the pivalates of alcohols **6**.

Entry	11	R^1	R^3	9	Yields [%]	d.e. ^[a] [%]
1	a	cyclohexyl	<i>i</i> Pr	a	95	> 98
2	b	PhCH ₂	<i>i</i> Pr	b	92	> 98
3	c	Ph	<i>i</i> Pr	c	90	91
4	d	-(CH ₂) ₃ OTBS	<i>i</i> Pr	d	95	> 98
5	d	-(CH ₂) ₃ OTBS	Et	e	89	> 98 ^[b]
6	e	<i>n</i> Bu	<i>n</i> C ₇ H ₁₅	f	90	> 95 ^[c]

[a] Determined by comparison of the HPLC traces with authentic mixtures of **9** and **10**. [b] Inferred from the optical rotation in comparison with literature value. [c] d.e. value based on differences in ¹³C NMR signals.

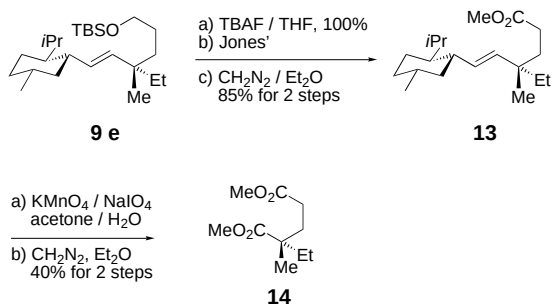
tert-Butyl, phenyl, and benzyl cyanocuprates were not reactive enough to take part in the reaction. The phenyl and benzyl groups could nonetheless be introduced starting from phenyl- or benzylacetylene. However, neopentyl alkynes could not be carboaluminated efficiently. Also, the stereoselectivity in the cuprate addition was slightly lower in the case of phenyl-substituted allyl pivalates **6c** and **7c**. This decrease was also observed in our first generation system with aryl-substituted allyl carbonates (Scheme 1, $R^1 = Ar$).^[1b]

The disubstituted double bonds in adducts **9** were easily cleaved with ozone or with KMnO₄/NaIO₄ leading to good yields of aldehydes, carboxylic acids, or alcohols depending on the work-up performed. For example, **9d** was cleaved with O₃ in dichloromethane to yield 65% of the aldehyde **12** (Scheme 4). The chiral auxiliary was recovered in 80% yield



Scheme 4. Oxidative cleavage of **9d**. TBS = *tert*-butyldimethylsilyl.

after chromatography and could be re-used. The conversion of **9e** into **14** (Scheme 5) was confirmed by comparison of its optical rotation ($[\alpha]_D$) with that of its enantiomer reported in the literature ($[\alpha]_D = -9.7^\circ$, CHCl₃, $c = 0.3$; literature $[\alpha]_D = +9.8^\circ$, $c = 0.3$).^[20] This observation indicated that the stereochemistry of **9e** results from an *anti*-selective cuprate addition on the configuration and conformation corresponding to **D** (Scheme 2).



Scheme 5. Proof of the absolute configuration of **9e**. TBAF = tetrabutylammonium fluoride.

The present method should prove a valuable addition to the arsenal of asymmetric reactions available for the synthesis of natural or unnatural chiral compounds.

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- [1] a) C. Spino, C. Beaulieu, *J. Am. Chem. Soc.* **1998**, *120*, 11 832–11 833; b) C. Beaulieu, C. Spino, *Tetrahedron Lett.* **1999**, *40*, 1637–1640.
- [2] K. Fuji, *Chem. Rev.* **1993**, *93*, 2037–2066.
- [3] E. J. Corey, A. Guzman-Perez, *Angew. Chem.* **1998**, *110*, 402–415; *Angew. Chem. Int. Ed.* **1998**, *37*, 388–401.
- [4] K. A. Lutomski, A. I. Meyers, *Asymmetric Synthesis. Stereodifferentiating Reactions, Part B, Vol. 3* (Ed.: J. D. Morrison), Academic Press, New York, **1984**, p. 213.
- [5] T. Ibuka, N. Akimoto, M. Tanaka, S. Nishii, Y. Yamamoto, *J. Org. Chem.* **1989**, *54*, 4055–4061.
- [6] For leading references, see E.-i. Negishi, D. Y. Kondakov, *Chem. Soc. Rev.* **1996**, 417–426, and references therein.
- [7] For a review on the stereoselectivity of addition to chiral carbonyl groups, see A. Mengel, O. Reiser, *Chem. Rev.* **1999**, *98*, 1191–1223, and references therein.
- [8] H. Newman, *Tetrahedron Letters* **1971**, *47*, 4571–4572.
- [9] P. Garner, J. M. Park, E. Malecki, *J. Org. Chem.* **1988**, *53*, 4395–4398.
- [10] R. S. Coleman, A. J. Carpenter, *Tetrahedron Lett.* **1992**, 1697–1700.
- [11] T. Tsuda, T. Yoshida, T. Kawamoto, T. Saegusa, *J. Org. Chem.* **1987**, *52*, 1624–1627.
- [12] T. Tsuda, T. Yoshida, T. Saegusa, *J. Org. Chem.* **1988**, *53*, 1037–1040.
- [13] T. Fujisawa, A. Fujimura, Y. Ukaji, *Chem. Lett.* **1988**, 1541–1544.
- [14] M. Taniguchi, H. Fujii, K. Oshima, K. Utimoto, *Tetrahedron Lett.* **1992**, *33*, 4353–4356.
- [15] P. Wipf, *Synthesis* **1993**, 537–557.
- [16] E.-i. Negishi, D. Y. Kondakov, D. Choueiry, K. Kasai, T. Takahashi, *J. Am. Chem. Soc.* **1996**, *118*, 9577–9588.
- [17] K. Maruoka, H. Yamamoto, *Tetrahedron* **1988**, *44*, 5001–5032.
- [18] For a review on organoaluminum reagents, see J. R. Hauske, *Additions to C-X π -Bonds, Part 1, Vol. 1* (Ed.: S. L. Schreiber), Pergamon, Exeter, **1991**, pp. 49–75.
- [19] C. C. Tseng, S. D. Paisley, H. L. Goering, *J. Org. Chem.* **1986**, *51*, 2884–2891.
- [20] A. S. C. Prakasa Rao, V. K. Bhalla, U. R. Nayak, S. Dev, *Tetrahedron* **1973**, *29*, 1127–1130.

Exceptional Deshielding of ⁵⁹Co Caused by Deuteration of the Hydrogen Bonds in Cobaloximes**

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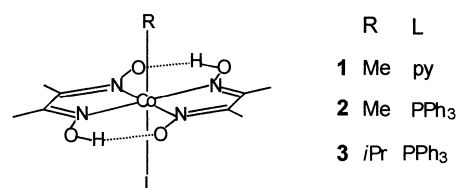
Transition metal shieldings are useful probes of structure and reactivity for coordination compounds, since they allow even tiny variations at the coordination site to be detected.^[1] The isotope ⁵⁹Co exhibits the largest known shielding range. Its NMR properties have been extensively studied for

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cobaloximes $[\text{RCo}(\text{Hdmg})_2\text{L}]$ (Hdmg = dimethylglyoximate)^[2] and are excellent candidates for the investigation of structural changes. For example, in these molecules the ^{59}Co chemical shift increases with increasing steric bulk of R and with increasing $\text{Co}-\text{C}$ bond length.^[2] Here we discuss the effects of hydrogen-bond deuteration and temperature variation on the ^{59}Co chemical shift in cobaloximes.

Usually for compounds of transition metals with d^6 configuration, the chemical shift increases with increasing bond length. A temperature increase implies bond extensions consistent with the higher population of the excited rovibrational states. The dependence of chemical shift on temperature can be used either to study how the shielding changes with bond length^[3] or, conversely, how the bonds lengthen with increasing temperature. For **1** (py = pyridine) and **2** the



^{59}Co chemical shift is an almost linear function of temperature, with slopes of 1.0 and 1.3 ppm K^{-1} , respectively. These sensitivities are rather low in comparison with those of other hexacoordinate cobalt complexes (1.38 ppm K^{-1} for $[\text{Co}(\text{CN})_6]^{3-}$, 1.42–1.68 ppm K^{-1} for $[\text{Co}(\text{NH}_3)_6]^{3+}$ and 2.85–3.04 ppm K^{-1} for $[\text{Co}(\text{NO}_2)_6]^{3-}$).^[4]

The mass dependence of the chemical shift, measured as the NMR isotope effect, is also related to the change in nuclear shielding with increasing bond length. Usually, the introduction of a heavier isotope into a ligand induces a decrease in bond lengths and a consequent decrease in chemical shift for the coordinated metal center. The magnitude of these effects increases with increasing chemical shift range of the observed nucleus, and for cobalt they are quite large: $[\text{Co}(\text{NH}_3)_6]^{3+}$ and $[\text{Co}(\text{en})_3]^{3+}$ complexes (en = ethylenediamine) exhibit a shift variation of about $\Delta\delta = -5$ for each deuterium substituent.^[5] For cobaloximes, H/D replacement at each hydrogen bond, three bonds remote from the metal, causes changes in ^{59}Co chemical shift that are remarkably large and of unexpected sign ($\Delta\delta = +25$ in **1** (Figure 1); $\Delta\delta = +27$ in **2** and **3**).^[6] This

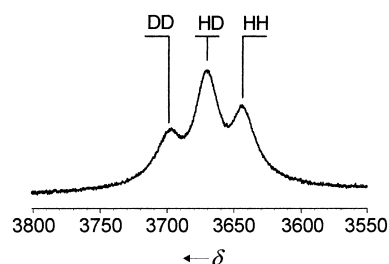


Figure 1. ^{59}Co spectrum of **1** in $[\text{D}_6]\text{acetone}$ upon addition of D_2O . DD = $[\text{MeCo}(\text{Ddmg})_2\text{py}]$, HD = $[\text{MeCo}(\text{Hdmg})(\text{Ddmg})\text{py}]$, HH = $[\text{MeCo}(\text{Hdmg})_2\text{py}]$.

reflects significant geometrical variations at the coordination site. Given the dependence of cobalt shielding on bond lengths for $[\text{Co}(\text{CN})_6]^{3-}$,^[3,7] the isotope effect observed in cobaloximes may correspond to a lengthening by some tenths of a picometer of the $\text{Co}-\text{N}$ bonds on the side of the deuterated hydrogen bond.

The exceptional isotope effect can be explained by the peculiarities of the hydrogen bond in cobaloximes. In a $\text{O}-\text{H}\cdots\text{O}$ bridge, the proton is shared by two O atoms, and the potential energy depends, to a first approximation, on the $\text{O}-\text{H}$, $\text{H}\cdots\text{O}$ and $\text{O}\cdots\text{O}$ distances and is a multidimensional double well. It reduces to a single well for very strong hydrogen bonds, in which for the vibrational ground state the highest probability density of the proton is at the midpoint.^[8]

^1H and ^2H NMR spectroscopy has been used profitably for the investigation of hydrogen bonding. The ^1H chemical shift, which increases with increasing $r(\text{O}-\text{H})$ and concomitant shortening of the $\text{H}\cdots\text{O}$ distance, is considered an index of the hydrogen-bond energy.^[9] The values of $\delta = 18.65$ for **1**, $\delta = 18.41$ for **2**, and $\delta = 18.18$ for **3** at 300 K are typical of fairly strong interactions. The primary isotope effect, that is, $^p\Delta = \delta(\text{H}) - \delta(\text{D})$, is the most important and reliable probe of the shape of the potential curve.^[8,10] The $^p\Delta$ value is large and positive (about +380 ppb for **1** and +410 ppb for **2**), does not depend on temperature,^[11] (Figure 2) and fits well to the

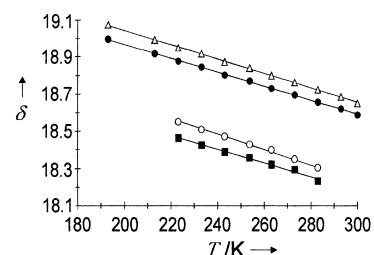


Figure 2. Temperature dependence of the hydrogen-bridge hydron chemical shift for the isotopomers of **1**. ^1H for HH (Δ), ^1H for HD ($\bullet$), ^2H for HD ($\circ$), and ^2H for DD ($\blacksquare$).

correlation with ^1H chemical shift found for molecules having intramolecular hydrogen bonds.^[10] A large positive $^p\Delta$ is characteristic of a double-well potential with a low central barrier and high anharmonicity. Here, upon substitution of D for H, the hydron fundamental vibrational wavefunction is modified so that $\langle r(\text{O}-\text{hydron}) \rangle$ is smaller and $\langle r(\text{O}\cdots\text{O}) \rangle$ is larger (secondary geometric effect).^[8] A double minimum is consistent with the $r(\text{O}\cdots\text{O})$ values found in cobaloximes, which cluster about 2.49 Å,^[12] provided the $\text{O}-\text{H}-\text{O}$ angle is less than 180° , as was previously proposed on the basis of IR data for $[\text{Ni}(\text{Hdmg})_2]$.^[13] The magnitude of $^p\Delta$ leads to an estimated secondary geometric effect of about 1–2 pm^[14] (note that 3 pm is the maximum secondary geometric effect reported so far^[15]). Hence, the increase in $\text{Co}-\text{N}$ bond lengths upon H/D substitution, suggested by the cobalt chemical shift, can be explained by the increase of the $\text{O}\cdots\text{O}$ distance inferred from $^p\Delta$.

Further insight into the expansion of the coordination site is provided by the secondary isotope effect observed at the opposite hydrogen bond, six bonds away. The $^s\Delta$ value for the

proton ($\delta(\text{H})_{[\text{RCo}(\text{Hdmg})(\text{Ddmg})\text{L}]}$ – $\delta(\text{H})_{[\text{RCo}(\text{Hdmg})_2\text{L}]}$), as well as that for the deuteron ($\delta(\text{D})_{[\text{RCo}(\text{Ddmg})_2\text{L}]}$ – $\delta(\text{D})_{[\text{RCo}(\text{Hdmg})(\text{Ddmg})\text{L}]}$) is about –70 ppb. The two hydrogen bonds are coupled^[16] in a cooperative way: not only the hydrogen bond where deuteration occurs is weakened, but also the other. The deuteration of either one or two hydrogen bonds results in further long-range secondary isotope shifts $n\Delta$ (n is the number of intervening bonds). The equatorial C=N carbon atoms of **1** are 64 ppb less shielded for $[\text{MeCo}(\text{Ddmg})_2\text{py}]$ than for the undeuterated complex. The same $^3\Delta$ value is observed on the side of the D bridge for $[\text{MeCo}(\text{Hdmg})(\text{Ddmg})\text{py}]$, where the inequivalence of the C=N carbon atoms clearly evidences the symmetry decrease caused by deuteration of one bridge. A long-range effect with opposite sign ($^5\Delta = -4$ ppb) was observed for the equatorial methyl protons in **2**. The axial methyl protons show a $^5\Delta$ of about +14 ppb for each deuteration step in both **1** and **2**.

The dependence of the ^1H and ^2H chemical shift of the hydrogen bond on temperature is linear with a negative slope (-0.004 ppm K^{-1} for **1** (Figure 2) and **2**), as was already found for enolates,^[17] which have the same kind of potential shape. It is noteworthy that such a negative dependence is also shared with the important class of hydrogen bonds whose lowest vibrational energy is higher than the potential barrier and which are characterized by negative $^2\Delta$ and negative secondary geometric effects.^[18]

From the above results, it can be concluded that the hydrogen bonds of cobaloximes have double-well potentials with a low central barrier and high anharmonicity and are subject to a positive secondary geometric effect upon deuteration. The latter is accompanied by a lengthening and weakening of the Co–N bonds that is large enough to drastically increase the ^{59}Co chemical shift.

Experimental Section

All the compounds were prepared according to literature procedures.^[19] NMR spectra were recorded on a 400 MHz JEOL EX-400 spectrometer on $[\text{D}_6]\text{acetone}$ solutions. For ^{59}Co spectra, a pulse width of 45°, a relaxation delay of 0.1 s, a sweep width of 60240 Hz, 8192 points, 10000 scans, and a broadening factor of 30 were used, and the data were zero-filled once before Fourier transformation. The solvent signal was used as internal standard at $\delta = 2.00$ for ^1H and ^2H , and at $\delta = 30.30$ for ^{13}C . The ^{59}Co chemical shift data were referred to $[\text{K}_3\text{Co}(\text{CN})_6]$ at 300 K. The isotopic substitution was accomplished by adding a few microliters of D_2O to the $2 \times 10^{-2}\text{ M}$ solutions of the cobaloximes in $[\text{D}_6]\text{acetone}$.

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- [1] W. von Philipsborn, *Chem. Soc. Rev.* **1999**, 28, 95–105.
- [2] a) C. Tavagnacco, G. Balducci, G. Costa, K. Täschler, W. von Philipsborn, *Helv. Chim. Acta* **1990**, 73, 1469–1479; b) F. Asaro, R. Gobetto, L. Liguori, G. Pellizer, *Chem. Phys. Lett.* **1999**, 300, 414–420; c) A. Medek, V. Frydman, L. Frydman, *Proc. Natl. Acad. Sci. USA* **1997**, 94, 14237–14242.
- [3] C. J. Jameson, D. Rehder, M. Hoch, *J. Am. Chem. Soc.* **1987**, 109, 2589–2594.
- [4] G. B. Benedek, R. Engelman, J. A. Armstrong, *J. Chem. Phys.* **1963**, 39, 3349–3363.
- [5] a) J. G. Russell, R. G. Bryant, M. M. Kreevoy, *Inorg. Chem.* **1984**, 23, 4565–4567; b) R. K. Harris, *Nuclear Magnetic Resonance Spectro-*

scopy. A Physicochemical View, Longman Scientific & Technical, Harlow, **1983**, p. 194.

- [6] The isotope effects on ^{59}Co were measured in the temperature range from 273 to 313 K, where the signals are narrow enough (linewidths at half height of about 2000–3500 Hz). The linewidth increases with decreasing temperature.
- [7] N. Godbout, E. Oldfield, *J. Am. Chem. Soc.* **1997**, 119, 8065–8069.
- [8] L. J. Altman, D. Laungani, G. Gunnarsson, H. Wennerström, S. Forsén, *J. Am. Chem. Soc.* **1978**, 100, 8264–8266.
- [9] G. A. Kumar, M. A. McAllister, *J. Org. Chem.* **1998**, 63, 6968–6972.
- [10] S. Mazzini, L. Merlini, R. Mondelli, G. Nasini, E. Ragg, L. Scaglioni, *J. Chem. Soc. Perkin Trans. 2* **1997**, 2013–2021.
- [11] The isotope effects on the shifts of ^1H , ^2H , and ^{13}C were determined at low temperature, where the peaks are narrower because the effects of scalar coupling to cobalt are averaged out by the faster cobalt relaxation and because the H/D exchange is slower.
- [12] L. Randaccio, S. Geremia, R. Dreos-Garlatti, G. Tauzher, F. Asaro, G. Pellizer, *Inorg. Chim. Acta* **1992**, 194, 1–8.
- [13] R. Blinc, D. Hadzi, *J. Chem. Soc.* **1958**, 4536–4540.
- [14] C. Engdahl, A. Gogoll, U. Edlund, *Magn. Reson. Chem.* **1991**, 29, 54–62.
- [15] P. W. R. Bessonette, M. A. White, *J. Chem. Phys.* **1999**, 110, 3919–3925.
- [16] S. S. Smirnov, N. S. Golubev, G. S. Denisov, H. Benedict, P. Schah-Mohammadi, H.-H. Limbach, *J. Am. Chem. Soc.* **1996**, 118, 4094–4101.
- [17] M. J. T. Robinson, K. M. Rosen, J. D. B. Workman, *Tetrahedron* **1977**, 33, 1655–1661.
- [18] M. Garcia-Viloca, R. Gelabert, A. González-Lafont, M. Moreno, J. M. Lluch, *J. Am. Chem. Soc.* **1998**, 120, 10203–10209.
- [19] H. A. O. Hill, K. G. Morallee, *J. Chem. Soc. A* **1969**, 554–559.

Enantiopure Simple Analogues of Annonaceous Acetogenins with Remarkable Selective Cytotoxicity towards Tumor Cell Lines**

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Annonaceous acetogenins, a relatively new class of natural products so far only found in Annonaceae, have been attracting worldwide attention in recent years because of their potent biological activities, especially as growth inhib-

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itors of certain tumor cells.^[1] They have been shown to function by blocking complex I in mitochondria^[2] as well as ubiquinone-linked NADH oxidase in the cells of specific tumor cell lines, including some multidrug-resistant^[3] ones. These features make the acetogenins excellent leads for new antitumor agents. However, due to the scant natural resources, the substantial amounts of enantiomerically pure samples required for further biological and clinical studies appear to be attainable only by means of chemical synthesis. Unfortunately, all the total syntheses of annonaceous acetogenins which have so far appeared in the literature^[4] still require more than 10 reaction steps, which makes the scale-up very difficult. Herein we wish to report a potential solution to the problem, which relies on structure simplification while maintaining all essential functionalities of the acetogenins.

It has been postulated that the antitumor activities of the acetogenins are associated with their ionophoric ability.^[5] Therefore, the hydroxy and ethereal oxygen atoms in the acetogenins appear to be essential for the biological activities. The THF rings, however, do not seem to be essential. A straightforward means of structural simplification is hence to remove the ethylene bridge in the THF rings,^[6] which eliminates two chiral centers and greatly simplifies the synthesis. Thus, the bis-THF annonaceous acetogenin isodesacetylvaricin **1** and its isomers may be reduced to the linear^[7] mimics **2** (Figure 1). In the latter case, four chiral

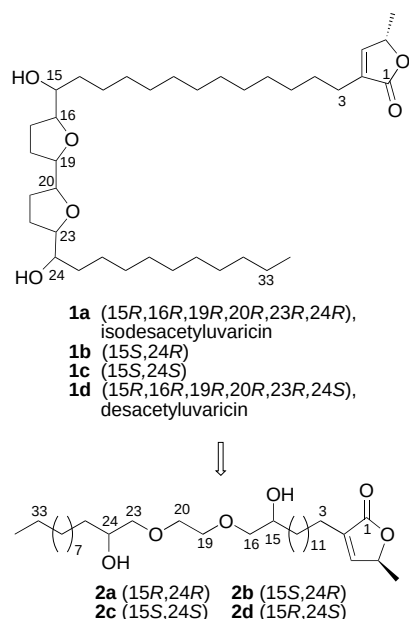


Figure 1. Representation of the general idea of simplifying bis-THF natural annonaceous acetogenins into linear analogues.

centers are removed from the molecule. This greatly reduces the difficulty encountered in the synthetic endeavors and thus promises easier scale-up in the future.

In order to explore the potential influence of the configuration of the hydroxy groups on the biological activities, we planned from the beginning to synthesize all four possible isomers of the simplified analogues (Figure 2) for biological

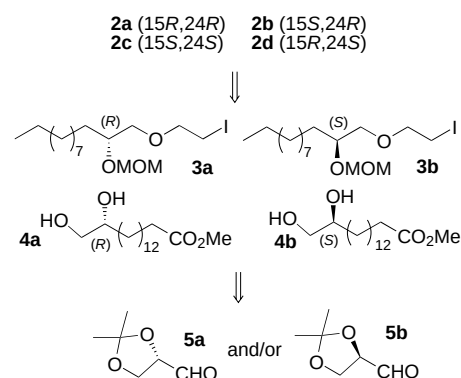
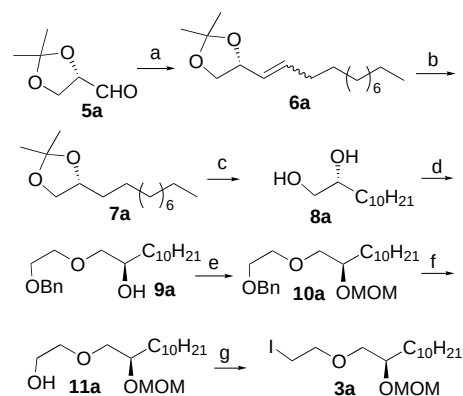


Figure 2. The chiral centers adjacent to the ethereal links in the targets can be attained from proper combination of **3a**, **3b**, **4a**, and **4b**, which are derived from **5a** and/or **5b** or from L-lactate.

screening, using different combinations four components **3a**, **3b**, **4a**, and **4b**, which were in turn derived from one of the protected glyceraldehydes **5a** or **5b**.

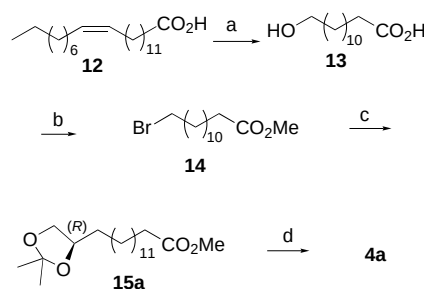
The synthesis is exemplified with preparation of **2a**, which starts with **5a** derived from L-ascorbic acid following a literature procedure.^[7] After chain extension^[8] at the carbonyl end by Wittig reaction and hydrogenation, the acetone **7a** was converted into diol **8a** and combined with 2-benzyloxyethyl iodide through a cyclic stannate (Scheme 1).^[9] The



Scheme 1. a) $C_8H_{17}CH=PPh_3$, 90%; b) H_2 , EtOH, Pd-C, 95%; c) HCl, MeOH; d) 1) Bu_3SnO , $CHCl_3$:MeOH (10:1), reflux, 2) CsF , ICH_2CH_2OBn , DMF, 80% over 2 steps; e) MOMCl, iPr_3NH , CH_2Cl_2 , 96%; f) H_2 , EtOH, Pd-C, 90%; g) I_2 , imidazole, PPh_3 , 82%. Bn = benzyl, MOM = methoxymethyl.

hydroxy group was masked as a MOM ether and the terminal benzyl protective group was removed by hydrogenation. The newly released primary hydroxyl functionality was then transformed into the desired iodide **3a**.

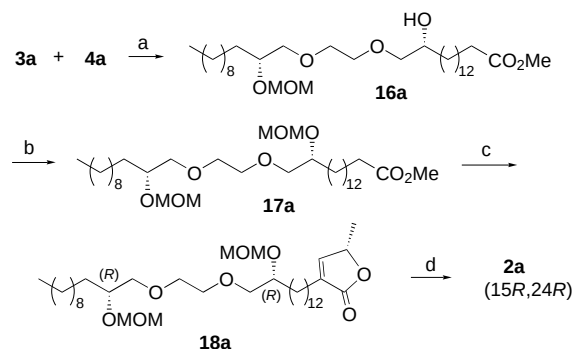
The other fragment required for the construction of target molecule **2a**, is the diol **4a**, which was prepared from *cis*-erucic acid **12** as shown in Scheme 2. The C–C double bond was first cleaved with ozone following a literature procedure^[10] to give the hydroxy acid **13**, which was transformed to corresponding methyl ester in methanol in the presence of $SOCl_2$. Smooth bromination with $CBBr_4$ in the presence of PPh_3 provided the bromo ester **14** as an immediate precursor to the corresponding phosphonium salt. In preparative runs, formation of **14**



Scheme 2. a) 1) O_3 , $0-5^\circ\text{C}$, $\text{EtOH}:\text{cyclohexane}$ (1:5), 2) KBH_4 ; b) 1) MeOH , SOCl_2 , 87% from **12**, 2) CBr_4 , PPh_3 , C_6H_6 , 87%; c) 1) PPh_3 , 2) $t\text{BuOK}$, then **5a**, 3) H_2 , EtOH , Pd-C , 67% over 3 steps; d) H^+ , MeOH , 91%.

could be combined with the following treatment with PPh_3 without separation, to give the desired phosphonium salt at elevated temperature in a one-pot manner. The subsequent Wittig reaction with **3a**, after hydrogenation of the C=C double bond and removal of the acetonide protective group, led to **4a**.

The coupling of **3a** and **4a** (Scheme 3) was achieved using a protocol similar to the one employed in the chain extension of **8a** above. The newly formed hydroxyl group was protected as



Scheme 3. a) 1) Bu_2SnO , $\text{CHCl}_3:\text{MeOH}$ (10:1), reflux, 2) CsF , DMF , 75% over 2 steps; b) MOMCl , $i\text{Pr}_3\text{NH}$, CH_2Cl_2 , 100%; c) 1) LDA , $(S)\text{-Me}(\text{OTHP})\text{CHCHO}$, 2) 9% H_2SO_4 , THF , 3) $(\text{F}_3\text{CCO})_2\text{O}$, NEt_3 , CH_2Cl_2 ; d) HCl , MeOH , 45–52% from **17a**. LDA = lithium diisopropylamide.

a MOM ether before the chiral center in the butenolide was introduced by an aldol condensation. After removal of the THP protective group with concurrent lactone ring closure, the hydroxyl was converted into trifluoroacetate and eliminated to give the C=C double bond. Finally, the MOM protective group was hydrolyzed using HCl in MeOH to afford the target molecule **2a**. It should be noted that in the present context, this strategy for construction of the lactone moiety after completion of the chain extension^[11] gave much better yields than the previously adopted one, where the lactone was formed at early stages of the synthesis.^[12]

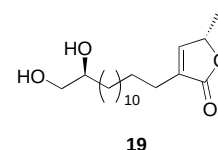
Other target molecules^[13] were synthesized in a similar manner by choosing proper forms of precursors **3** and **4**. Thus, coupling of **3a** and **4b** led to **2b**, while reaction of **3b** with **4a**, and **3b** with **4b**, yielded **2c** and **2d**, respectively.

The preliminary results (Table 1) of the in vitro tests against several human solid tumor cell lines show interesting cell line selectivity. Practically no activity was found with all the four

Table 1. The preliminary results of in vitro tests (using the MTT method) against KB, A2780, HCT-8, and HT-29 human solid tumor cell lines.

Compound	EC_{50} [$\mu\text{g mL}^{-1}$]			
	KB	A2780	HCT-8	HT-29
2a	>1	>1	0.066	0.272
2b	>1	>1	0.097	1.12
2c	>1	>1	0.032	0.11
2d	>1	>1	0.065	7.83
19	>1	>1	>1	>1

analogues against KB and A2780 cell lines. However, impressive EC_{50} values were observed with HCT-8, which all are on the nm order. Other interesting results are obtained with HT-29 cell line, different combinations of the chiral moieties show remarkable difference in the activity. Corresponding EC_{50} values for **19** are also listed in Table 1 for comparison.



In conclusion, we have developed a class of structurally simplified analogues of natural annonaceous acetogenins. The synthetic routes are significantly shortened. The remaining stereogenic centers are derived from the easily accessible chiral building blocks **5a** and/or **5b** or from L-lactate. All four analogues show remarkable activity against the HCT-8 cell line, while better differentiation between the four analogues is found with HT-29 cell line. Further work along this line is currently in progress in our laboratory.

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- Reviews: a) J. K. Rupprecht, Y. H. Hui, J. L. McLaughlin, *J. Nat. Prod.* **1990**, 53, 237; b) X. P. Fang, M. J. Rieser, Z. M. Gu, J. L. McLaughlin, *Phytochem. Anal.* **1993**, 4, 27; c) L. Zeng, Q. Ye, N. H. Oberlies, G. E. Shi, Z. M. Gu, K. He, J. L. McLaughlin, *Nat. Prod. Rep.* **1996**, 275; d) A. Cave, B. Figadère, A. Laurens, D. Cortes, *Prog. Chem. Org. C* **1997**, 70, 81.
- M. C. Gonzalez, J. R. Tormo, A. Bermejo, M. C. Zafra-polo, E. Estornell, D. Cortes, *Bioorg. Med. Chem. Lett.* **1997**, 7, 1113, and references therein.
- N. H. Oberlies, V. L. Croy, M. L. Harrison, J. L. McLaughlin, *Cancer Lett.* **1997**, 115, 73.
- For recent reviews, see: a) G. Cassiraghi, F. Zanardi, L. Battistini, G. Rassu, G. Aappendino, *Chemtracts: Org. Chem.* **1998**, 11, 803; b) A. Tanaka, T. Oritani, *J. Org. Synth. Chem. Soc. Jpn.* **1997**, 55, 877.
- a) S. Sasaki, K. Muruta, H. Naito, H. Sugihara, K. Hiratani, M. Maeda, *Tetrahedron Lett.* **1995**, 36, 5571; b) S. Sasaki, K. Muruta, H. Naito, R. Maemura, E. Kawahara, M. Maeda, *Tetrahedron* **1998**, 54, 2401.
- Our efforts along this line started in the early 90's Y.-L. Wu, Z.-J. Yao and Q. Yu, **1997**, CN-A 97 1 06368.0).
- During the preparation of this manuscript, Grée et al. published a synthetic endeavor aimed at similar target molecules: Y. L. Huérrou, J. Doyon, R. L. Grée, *J. Org. Chem.* **1999**, 64, 6782. However, they have currently only finished construction of the two chiral centers adjacent to the ethereal links, without introduction of the butenolide moiety.
- Y.-F. Wang, Y.-L. Wu, *Hecheng Huaxue* **1993**, 1, 215 (*Chem. Abstr.* **1994**, 121, 133755p).
- a) N. Nagashima, M. Ohno, *Chem. Lett.* **1987**, 141; b) H.-S. Byun, E. R. Kumar, R. Bittman, *J. Org. Chem.* **1994**, 59, 2630; c) R. Martínez-Bernhardt, P. P. Castro, G. Godjoian, C. G. Gutiérrez, *Tetrahedron* **1998**, 54, 8919.
- Y.-C. Wang, F.-C. Liu, *Synth. Commun.* **1994**, 24, 1265.

- [11] Similar strategies have been employed in the total synthesis of natural acetogenins. For example, see: a) J. Marshall, H.-J. Jiang, *J. Org. Chem.* **1998**, 63, 7066; b) S. Hanessian, T. A. Grillo, *J. Org. Chem.* **1998**, 63, 1049.
- [12] For example, see: Q. Yu, Y.-K. Wu, Y.-L. Wu, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1183, and references therein.
- [13] ¹H NMR data (CDCl₃, 600 MHz) and optical rotations for **2a–d**. Compound **2a**: δ = 6.98 (d, *J* = 1.2 Hz, 1H), 4.99 (dq, *J* = 7.2, 1.2 Hz, 1H), 3.83–3.74 (m, 2H), 3.72–3.60 (m, 4H), 3.53 (dd, *J* = 9.6, 10.2 Hz, 2H), 3.32 (dd, *J* = 9.6, 8.4 Hz, 2H), 2.26 (br. t, *J* = 7.8 Hz, 2H), 1.55 (quint, *J* = 6.6 Hz, 2H), 1.40 (d, *J* = 7.2 Hz, 3H), 1.50–1.20 (m, 40H), 0.88 (t, *J* = 6.9 Hz, 3H); [α]_D = +3.4 (*c* = 2.31 in CHCl₃). Compound **2b**: δ = 6.98 (br. s, 1H), 4.99 (br. q, *J* = 6.6 Hz, 1H), 3.82–3.74 (br. m, 2H), 3.72–3.60 (br. m, 4H), 3.54 (br. d, *J* = 9.6 Hz, 2H), 3.32 (br. t, *J* = 9.0 Hz, 2H), 2.26 (br. t, *J* = 7.5 Hz, 2H), 1.54 (quint, *J* = 7.2 Hz, 2H), 1.40 (d, *J* = 6.6 Hz, 3H), 1.48–1.16 (br. m, 40H), 0.88 (t, *J* = 7.2 Hz, 3H); [α]_D = –6.9 (*c* = 0.4 in CHCl₃). Compound **2c**: δ = 6.97 (d, *J* = 1.2 Hz, 1H), 4.98 (dq, *J* = 7.2, 1.2 Hz, 1H), 3.81–3.75 (m, 2H), 3.71–3.61 (m, 4H), 3.52 (dd, *J* = 9.6, 10.2 Hz, 2H), 3.31 (dd, *J* = 9.6, 8.4 Hz, 2H), 2.50 (br. s, 2H, OH), 2.26 (t, *J* = 7.8 Hz, 2H), 1.53 (quint, *J* = 7.8 Hz, 2H), 1.40 (d, *J* = 6.6 Hz, 3H), 1.48–1.20 (m, 40H), 0.87 (t, *J* = 7.2 Hz, 3H); [α]_D = 16.3 (*c* = 2.17 in CHCl₃). Compound **2d**: δ = 6.98 (d, *J* = 1.2 Hz, 1H), 4.99 (dq, *J* = 1.2, 6.6 Hz, 1H), 3.78 (m, 2H), 3.72–3.62 (m, 4H), 3.53 (dd, *J* = 2.4, 9.6 Hz, 2H), 3.32 (dd, *J* = 9.6, 8.4 Hz, 2H), 2.26 (br. t, *J* = 7.8 Hz, 2H), 2.15 (br. s, 2H, OH), 1.55 (quint, *J* = 7.2 Hz, 2H), 1.40 (d, *J* = 6.6 Hz, 3H), 1.48–1.20 (m, 40H), 0.88 (t, *J* = 6.9 Hz, 3H); [α]_D = –87.4 (*c* = 0.35 in CHCl₃).

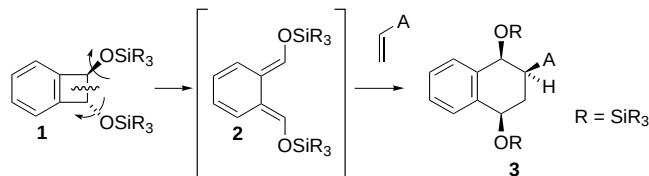
Thermal Intermolecular Hetero Diels–Alder Cycloadditions of Aldehydes and Imines via *o*-Quinone Dimethides**

Martin F. Hentemann, John G. Allen, and Samuel J. Danishefsky*

In memory of Wolfgang Oppolzer

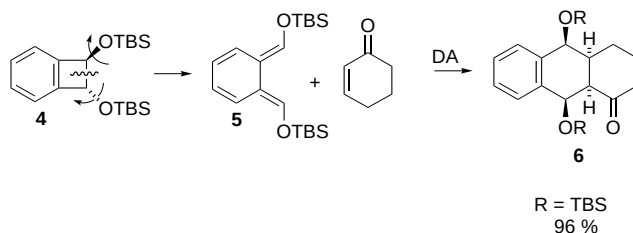
The value of the Diels–Alder (DA) reaction in chemical synthesis can scarcely be overstated.^[1] Our research group has been continuing to pursue its long-term interest in such cycloadditions, especially using suitable benzocyclobutenes.^[2] In particular, we have focused on 1,2-*trans*-disubstituted disilyloxy compounds of the type **1** and have found that such systems partake in intermolecular DA cycloadditions with suitable dienophiles under remarkably mild conditions.^[3] These results strongly suggest that **1** undergoes facile bond

reorganization to produce *o*-quinone dimethides (5,6-dimethylenecyclohexa-1,3-diene derivatives, such as **2**), which function as dienes in DA reactions to produce products of type **3** (Scheme 1).



Scheme 1. A = activator.

While this kind of cycloaddition is well known, its primary applications have been at the intramolecular level. Thus, many of the elegant sequences that have traditionally been used to generate *o*-quinone dimethides as a prelude to cycloaddition work well only when the putative dienophile is tethered to the diene.^[4] A particularly impressive illustration of our recently discovered “*trans*-1,2-bissiloxy effect” was seen in the uncatalyzed, near-quantitative formation of **6** from the cycloaddition of **4** with 2-cyclohexen-1-one (a notoriously sluggish dienophile) at 40 °C! (Scheme 2).^[5] It was subsequently discovered that **4** will also effect cycloaddition with 2-cyclohexen-1-one even at ambient temperatures, presumably via **5**.



Scheme 2. TBS = *tert*-butyldimethylsilyl.

We subsequently noted that benzocyclobutene **4** exhibits thermochromic behavior, a characteristic that simplifies the monitoring of these types of reactions. Compound **4** exists as a colorless oil, either neat, or in benzene solution at temperatures below 0 °C. However, it becomes yellow upon being warmed to room temperature. This color is discharged upon recooling. The color also disappears following exposure to a dienophile. Accordingly, it seems reasonable^[6] to interpret our findings in terms of a low equilibrium concentration of **5**, although in insufficient amount for detection by NMR analyses.

The initial successes with carbon dienophiles prompted us to expand the scope of our inquiry by focusing on heterodienophiles. In an extensive series of investigations in the 1980s our research group discovered and developed the first examples of diene–aldehyde and diene–imine condensations in cases where the formal heterodienophile was not especially activated.^[7] However, in those studies it was necessary to take recourse to Lewis acid catalysis to accomplish such reactions with general, as opposed to specially activated, heterodieno-

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philes. Herein we demonstrate formal cycloadditions between benzocyclobutenes and general aldehydes, as well as selected imines, under strictly thermal conditions.

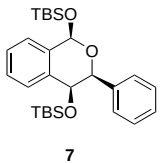
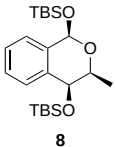
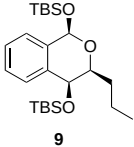
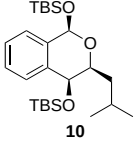
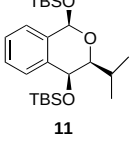
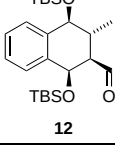
Reactions of **4** were carried out with various potential heterodienophiles in benzene or toluene and monitored by NMR spectroscopy. Uncatalyzed cycloadditions occurred under remarkably mild conditions in 2–8 h with complete stereospecificity. Particularly striking was the observation that the cycloaddition reactions proceed smoothly, albeit more slowly, even at room temperature. Reactions occurred well even with aliphatic aldehydes sterically encumbered by α and β branching (Table 1, entries 5 and 4, respectively). With the severely hindered heterodienophile pivalaldehyde, however, no reaction occurred even upon application of higher temperatures. To the best of our knowledge these reactions constitute the first examples of completely stereodefined [4+2] intermolecular cycloadditions of dienes with unactivated aldehydes in the absence of catalysis.^[8] We note that cycloaddition with crotonaldehyde (entry 6) affords exclusively the product resulting from cycloaddition at the olefin group.

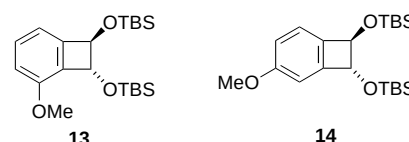
The stereochemistry of the adducts formed with aldehyde dienophiles was determined by NOE experiments. The complete *endo* specificity is intriguing, as it is exhibited even in the DA reactions of aliphatic aldehydes that have no obvious potential for stabilizing secondary orbital interactions during the course of the cycloaddition (Table 1, entries 2–5). This phenomenon, which awaits full explanation, is in contrast to previous stereochemical observations made in Lewis acid catalyzed hetero DA reactions.^[9]

We reported^[3] that incorporation of an *ortho* methoxy group in the benzocyclobutene **13** resulted in substantially decreased, but still exploitable, cycloaddition chemistry in the “all carbon” DA reaction. This attenuation of reactivity can be attributed to the electrocyclic ring-opening step, or to the cycloaddition reaction itself being hindered by steric constraints. Apropos of the first argument, we note that **13** is colorless at room temperature.

This weakened activity of **13** is even more obvious in the heterodienophilic cases. Thus, **13** does not undergo cycloaddition with any of the simple aldehydic DA dienophiles described above. However, placement of the methoxy group at the *meta* position, (see substrate **14**^[10] and Table 2) restores

Table 1.

Entry	Dienophile	Time [h]	Product	Yield [%]
1	benzaldehyde	2		92
2	acetaldehyde	2		90
3	butyraldehyde	8		88
4	isovaleraldehyde	8		90
5	isobutyraldehyde	13		89
6	crotonaldehyde	1.5		82



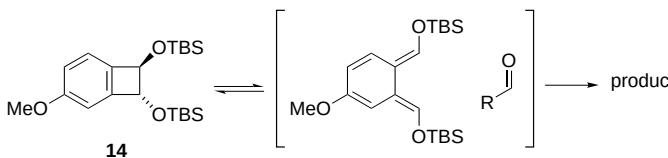
the reactivity towards cycloaddition. Qualitatively, the rates of cycloaddition of **14** are even greater than those of **3**.^[11]

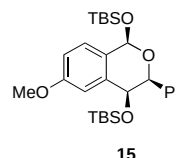
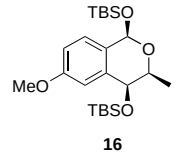
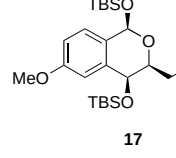
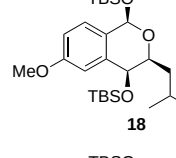
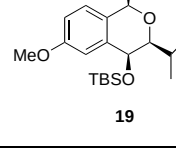
In addition to complete stereospecificity, the DA reactions of **14** with aldehydes occur with complete regioselectivity (Table 2). This observation is in striking contrast to the behavior of compounds **13** and **14** towards “all carbon” dienophiles, where mixtures of regioisomers may be produced.^[12] It is worthy of note that the more electron-rich **14** reacts more readily with isobutyraldehyde (Table 2, entry 5) than does the demethoxy derivative **4** (Table 1, entry 5). This effect may be the result of an accelerating effect of the methoxy group in **14** on the conrotatory electrocyclic reorganization step, or on the cycloaddition itself.

This new chemistry was extended to selected imines. Thus, reaction with *p*-toluenesulfonyl cyanide and *N*-benzylidenebenzenesulfonamide also proceeded smoothly with **4** and **14**, to provide adducts **20**–**23**. The slightly diminished yields reflected partial hydrolysis of the silyl ethers during purification, particularly for the 4-OMe derivatives (Table 3). The DA reaction of **4** and **14** with azadienophiles provides an excellent entry into the area of functionalized isoquinolines.

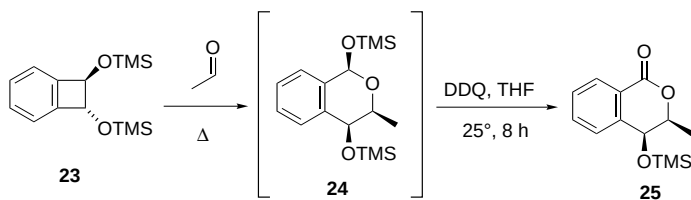
The Diels–Alder adducts can undergo post-cycloaddition processing to afford differentially oxidized products. For instance, cycloaddition of acetaldehyde and the bis(TMS-protected) benzocyclobutene derivative **23** gives adduct **24**, which was not isolated but rather treated with excess DDQ in

Table 2.



Entry	Dienophile	Time [h]	Product	Yield [%]
1	benzaldehyde	2		90
2	acetaldehyde	2		82
3	butyraldehyde	8		73
4	isovaleraldehyde	3		90
5	isobutyraldehyde	3		83

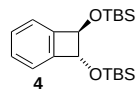
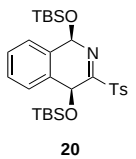
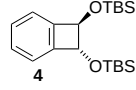
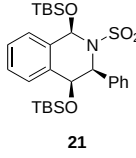
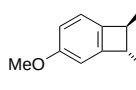
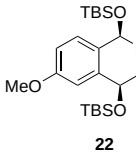
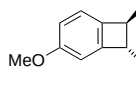
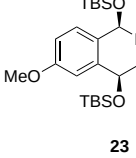
THF^[13] at 25 °C to give mono-oxidized product **25** in 82 % yield (Scheme 3). This structural motif is present in nature^[14] and the chemistry described above would provide a mild and concise route to other targets of this type.



Scheme 3. TMS = trimethylsilyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

In summary, stereocontrolled cycloadditions have been developed that proceed in the absence of catalyst. These interesting results, while establishing the power of the bis(siloxy) effect in providing synthetically valuable access to highly reactive, yet well behaved 1,2-quinone dimethides

Table 3.

Benzocyclobutene	Dienophile	Time [h]	Product	Yield [%]
	toluenesulfonyl cyanide	1		73
	N-benzylidene-phenylsulfonamide	4		68
	toluenesulfonyl cyanide	1		63
	N-benzylidene-phenylsulfonamide	3		57

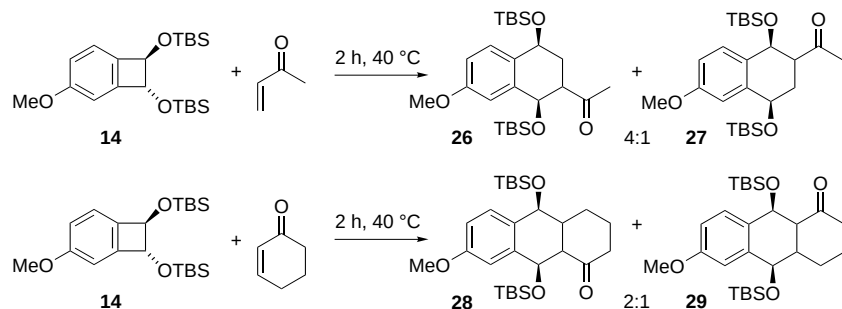
Ts = toluene-4-sulfonyl.

for cycloaddition reactions, raise interesting issues of mechanism, scope, and possibilities for catalysis. It seems likely that these advances will spur applications to heterocyclic synthesis, including target structures that are of biological interest.

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- [1] For many recent examples, see K. C. Nicolaou, E. J. Sorenson, *Classics in Total Synthesis*, VCH, Weinheim, **1996**, and references therein.
- [2] J. L. Segura, N. Martin, *Chem. Rev.* **1999**, 99, 3199, and references therein.
- [3] J. G. Allen, M. F. Hentemann, S. J. Danishefsky, *J. Am. Chem. Soc.* **2000**, 122, 571.
- [4] a) W. Oppolzer, *Synthesis* **1978**, 793; b) T. Kametani, *Pure Appl. Chem.* **1979**, 51, 747; c) K. C. Nicolaou, W. E. Barnette, P. Ma, *J. Org. Chem.* **1980**, 45, 1463; d) T. Kametani, H. Nemoto, *Tetrahedron* **1981**, 37, 3; e) P. Magnus, T. Gallagher, P. Brown, P. Pappalardo, *Acc. Chem. Res.* **1984**, 17, 35.
- [5] a) F. Fringuelli, A. Taticchi, E. Wenkert, *Org. Prep. Proced. Int.* **1990**, 22, 131; b) P. D. Barlett, G. F. Woods, *J. Am. Chem. Soc.* **1940**, 62, 2933; c) F. Fringuelli, F. Pizzo, A. Taticchi, T. D. J. Halls, E. Wenkert, *J. Org. Chem.* **1982**, 47, 5057.
- [6] W. Choy, H. Yang, *J. Org. Chem.* **1988**, 53, 5796.
- [7] a) J. F. Kerwin, S. J. Danishefsky, *Tetrahedron Lett.* **1982**, 23, 3739; b) S. J. Danishefsky, *Chemtracts* **1989**, 2, 273; c) S. J. Danishefsky, M. T. Bilodeau, P. H. Seeberger, *Aldrichimica Acta* **1997**, 30, 75.
- [8] While there are examples of highly activated dienophiles reacting under strictly thermal conditions: a) P. A. Wender, R. M. Keenan, H. Y. Lee, *J. Am. Chem. Soc.* **1987**, 109, 4390; b) A. Hosomi, Y. Sakata, H. Sakurai, *Tetrahedron Lett.* **1985**, 26, 5175, simple aliphatic aldehydes typically require high pressure for noncatalyzed reactions: c) M. Chmielewski, J. Jurczak, *J. Carbohydr. Chem.* **1987**, 6, 1; d) J. Jurczak, A. Golebiowski, *Chem. Rev.* **1989**, 89, 149, and references therein.
- [9] a) G. R. Krow, R. Rodebaugh, R. Cramosin, W. Figures, H. Pannella, G. DeVicar, M. Grippi, *J. Am. Chem. Soc.* **1973**, 95, 5273; b) G. R. Krow, C. Pyun, C. Leitz, J. Marakowski, *J. Org. Chem.* **1974**, 39, 2449;

- c) G. R. Krow, K. J. Henz, S. W. Szczepanski, *J. Org. Chem.* **1985**, *50*, 1888.
- [10] L. S. Liebeskind, L. J. Lescosky, C. M. McSwain Jr., *J. Org. Chem.* **1989**, *54*, 1435; b) H. Dürr, H. Nickels, *J. Org. Chem.* **1980**, *45*, 973; c) O. Abou-Teim, M. C. Goodland, M. F. W. McOmie, *J. Chem. Soc. Perkin Trans. 1* **1983**, 2659; d) L. S. Liebeskind, M. S. South, *J. Org. Chem.* **1982**, *47*, 3815.
- [11] In a competition experiment between compounds **4** and **14**, 2-cyclohexen-1-one reacted preferentially with **14**.
- [12] The Diels–Alder reaction of **14** with methyl vinyl ketone gave a 4:1 ratio of regioisomers **26:27**, while that between 2-cyclohexen-1-one and **14** gave a 2:1 ratio of **28:29**. Poor regioselectivity of 4-methoxy-substituted *o*-quinone dimethides in Diels–Alder cycloadditions is preceded in reactions with methyl acrylate: Y. Ito, M. Nakatsuka, T. Saegusa, *J. Am. Chem. Soc.* **1982**, *104*, 7609. For information regarding the regiochemistry of **13**, see reference [3].
- [13] a) M. E. Jung, *J. Org. Chem.* **1976**, *41*, 1479; b) M. E. Jung, Y.-G. Pan, *J. Org. Chem.* **1977**, *42*, 3961; c) J. Muzart, *Synthesis* **1993**, 11.
- [14] a) K. Arai, T. Yoshimura, Y. Itatani, Y. Yamamoto, *Chem. Pharm. Bull.* **1983**, 925; b) A. Latvala, M. Oenuer, T. Goetzler, A. Linden, B. Kivcak, M. Hesse, *Phytochemistry* **1995**, *39*, 1229; c) P. Mingo, S. Zhang, L. S. Liebeskind, *J. Org. Chem.* **1999**, *64*, 2145, and references therein.



easily obtained by other experimental techniques. While there have been many PE spectroscopic studies of stable closed-shell molecules over the past 30 years, corresponding studies of open-shell radicals have been fewer.^[1–3] Both the methylthio ($\text{CH}_3\text{S}^\bullet$)^[4–14] and methoxy ($\text{CH}_3\text{O}^\bullet$)^[15–18] radicals are important intermediates in combustion, photochemical smog and atmospheric reactions. The competitive effects of the dynamic Jahn–Teller distortion and spin–orbit coupling and their influences on the ground states of both $\text{CH}_3\text{S}^\bullet$ and $\text{CH}_3\text{O}^\bullet$ are the subject of numerous experimental and theoretical studies.^[19–26]

Studies on these two radicals have been based on ab initio calculations to determine the energetics and geometry of the $\text{CH}_3\text{S}^\bullet$ radical in its ground and excited electronic states,^[27–29] but as yet there is no report on the different ionic states for $\text{CH}_3\text{S}^\bullet$ or of direct experimental evidence of the ionization energies of different ionic states for $\text{CH}_3\text{O}^\bullet$. Two separate photoionization mass spectrometry (PIMS) studies gave the first ionization energy of the $\text{CD}_3\text{O}^\bullet$ isotopomer ($(10.726 \pm 0.008) \text{ eV}$,^[23] $(10.74 \pm 0.02) \text{ eV}$ ^[30]). Slightly different calculated ionization energies of $\text{CH}_3\text{O}^\bullet$ have been reported by Curtiss et al.^[20] and Dyke et al.^[25] (10.78 and $(10.72 \pm 0.02) \text{ eV}$, respectively). Clearly, experimental measurements of the ionization energies for both radicals are necessary.

In our laboratory, we generate a well characterized source of radicals or atoms by in situ pyrolysis and microwave discharge of precursor molecules.^[31–35] In this communication, we would like to report the PES studies on the electronic structures of both methylthio and methoxy radicals. To our knowledge, this is the first experimental observation of different ionic states for both radicals.

The methylthio radical

The thermal, homolytic dissociation of suitable precursors is the most widely used method for generating radicals. Thermal decomposition of disulfides gives alkylthio radicals.^[36] Calculations on CH_3SSCH_3 indicate that the S–S bond is the weakest present and therefore pyrolysis of CH_3SSCH_3 offers a simple route to the $\text{CH}_3\text{S}^\bullet$ radical [Eq. (1)].



Figure 1a shows the in situ PE spectrum of the species generated by pyrolysis of the CH_3SSCH_3 molecule at 285°C and Figure 1b shows the lower ionization energy region ($E < 11.00 \text{ eV}$) in greater detail. The three bands at $E = 9.26$, 9.91 , and 10.32 eV are very sharp and correspond to the ionization of nonbonding electrons of the generated radical, because, in this case, the vertical ionization energy E_V is equivalent to the adiabatic ionization energy E_A . The first ionization energy of the $\text{CH}_3\text{S}^\bullet$ radical^[36] (9.26 eV) is in good agreement with both

First Experimental Observation on Different Ionic States of both Methylthio ($\text{CH}_3\text{S}^\bullet$) and Methoxy ($\text{CH}_3\text{O}^\bullet$) Radicals**

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When combined with ab initio or density-functional theory (DFT) calculations, HeI photoelectron spectroscopy (PES) richly provides information on orbital energies, ionic states, bonding, and vibrational fine structure data that cannot be

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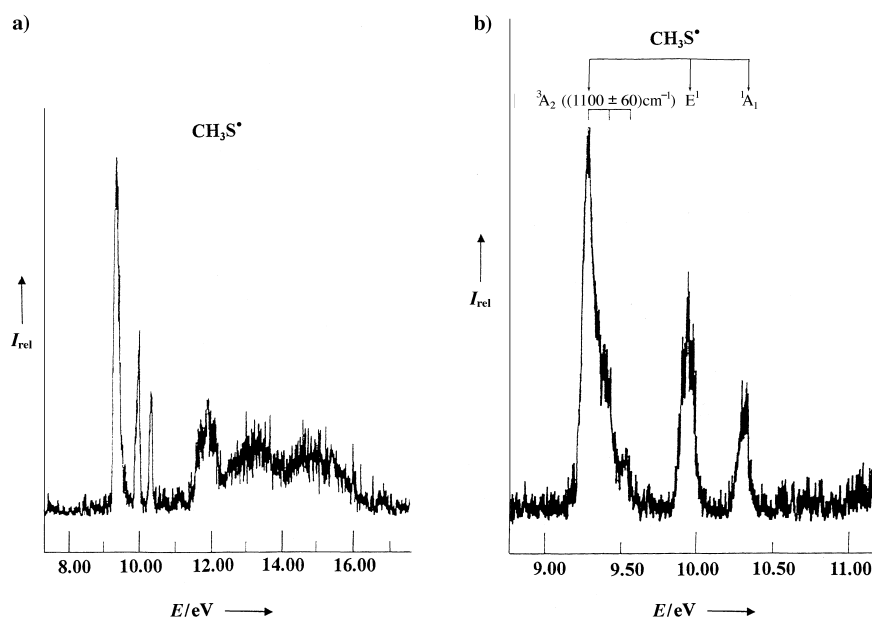


Figure 1. a) An in situ HeI-PE spectrum (given as relative intensities I_{rel}) of the product ($\text{CH}_3\text{S}^\bullet$) from the pyrolysis of CH_3SSCH_3 ; b) the expanded spectrum in the lower ionization energy region ($E < 11.00$ eV).

adiabatic ionization energy values obtained from two PIMS studies^[37, 38] and a G2 first ionization energy calculation (9.25 eV).^[39, 40] The vibrational fine structure spacing of this band also supports the assignment, as the spacing energy ($\tilde{\nu} = (1100 \pm 60)\text{cm}^{-1}$) is in good agreement with the presence of the $\nu_{\text{C-S}}$ stretching mode of the CH_3S^+ ion ($\tilde{\nu} = (1098 \pm 2)\text{cm}^{-1}$).^[14, 41]

Assignment of the sharp bands (Figure 1) to ionic states proceeds as follows: According to the C_{3v} symmetry of $\text{CH}_3\text{S}^\bullet$, as determined by a microwave study,^[8] and assuming a $\tilde{\chi}^2\text{E}$ ground state, as proposed by theoretical studies,^[39, 40] the valence shell molecular orbitals would be, in the order of increasing energy, $(5a_1)^2(2e)^4(6a_1)^2(7a_1)^2(3e)^3$. The HOMO ($3e$) is essentially derived from the nonbonding lone-pair orbital of the sulfur atom with a smaller contribution from C–S antibonding. The removal of an electron from the HOMO will generate a CH_3S^+ ion in the $^3\text{A}_2$, ^1E , and $^1\text{A}_1$ ionic states ($J = 1, 1/2, 0$, respectively) and the three PES bands corresponding to these three ionic states of $\text{CH}_3\text{S}^\bullet$ should be very sharp. The degeneracy of the ionized states is given by $2J + 1$, so that the ratio of the PES band areas of the ionic states should be 3:2:1 for $^3\text{A}_2: ^1\text{E}: ^1\text{A}_1$. Figure 1b reveals a band area ratio of 3.02:1.95:1.00 for the bands at $E = 9.26$, 9.91, and 10.32 eV, respectively, which is very close to the expected ratio. The measured PE spectrum has provided, for the first time, evidence of different ionic states of the $\text{CH}_3\text{S}^\bullet$ radical.

This assignment of the PES bands for $\text{CH}_3\text{S}^\bullet$ is also supported by the results of improved DFT calculations on the ground state of the $\text{CH}_3\text{S}^\bullet$ radical and of the ground and (several) ionic states of the CH_3S^+ ion. Such a calculation was used to assign PES bands for a number of other radicals.^[32, 34, 35] Table 1 includes the ionization energies E , as determined by the maxima of the bands in the PE spectra, and

the computed ionization energies E_v for different ionic states of $\text{CH}_3\text{S}^\bullet$. According to the Frank–Condon principle, E_v is obtained from the total energy difference between the ground states of the cation and the neutral radical in C_{3v} symmetry. According to Table 1 the low energy ionization energies from the PE spectrum for the $\text{CH}_3\text{S}^\bullet$ radical (9.26, 9.91, and 10.32 eV) are in good agreement with the computed ionization energies (9.266, 10.024, and 10.421 eV).

The higher energy PES bands for the methylthio radical at $E = 11.92$, 13.60, and 14.73 eV (Figure 1a) are very broad, which indicates that the corresponding ionized electrons occupied bonding orbitals or several orbitals of $\text{CH}_3\text{S}^\bullet$. The broad PES band at 11.92 eV is assigned to the ionization of the $7a_1$ orbital to consequently form both triplet ^3E and singlet ^1E ionic states. This

Table 1. PES ionization energies E , computed (DFT) ionization energies E_v , and relative intensities I_{rel} of signals observed from different ionic states for the methylthio radical.

E (PES) [eV] ^[a]	E_v (DFT) [eV]	Cationic state	Relative intensity	
			measured ^[b]	expected
9.26	9.266	$^3\text{A}_2$	3.02	3
9.91	10.024	^1E	1.95	2
10.32	10.421	$^1\text{A}_1$	1.00	1
11.92	11.724	^3E		
	12.156	^1E		
13.60	12.810	^3E		
	13.916	^1E		
14.73	15.102	$^3\text{A}_2$		

[a] Values are ± 0.02 eV and derived from the maxima of the PES bands.

[b] Corrected for analyzer sensitivity ± 0.02 .

assignment is supported on the basis of the computed ionization energies of 11.724 (^3E) and 12.156 eV (^1E) as the two states are very similar in energy and thereby appear overlapped. Similarly, the broad PES band at 13.60 eV is assigned to the ionization of the $6a_1$ orbital as the computed ionization energies of the formed triplet and singlet states 12.810 (^3E) and 13.916 eV (^1E) are again close and overlap. The very broad band at 14.73 eV is assigned to ionization of the less energetic valence orbitals of $\text{CH}_3\text{S}^\bullet$.

The methoxy radical

The incomplete combustion of methanol leads to the production of the methoxy radical, which, in the presence of nitrogen oxides, produces the mutagenic methyl nitrite $\text{CH}_3\text{ONO}^\bullet$.^[42] Methyl nitrite has also attracted considerable attention from atmospheric contamination studies since photodissociation processes cleave the molecule into NO and $\text{CH}_3\text{O}^\bullet$ species.^[43–46] That is, methyl nitrite can be used as a

source of methoxy radicals. We generate a $\text{CH}_3\text{O}^\bullet$ source by pyrolysis of CH_3ONO at 275°C and its PE spectrum was recorded in situ.

The dissociation of CH_3ONO to produce $\text{CH}_3\text{O}^\bullet$ and NO , should lead to contamination of the PE spectrum of $\text{CH}_3\text{O}^\bullet$ with bands due to the NO species. Figure 2a shows the PE

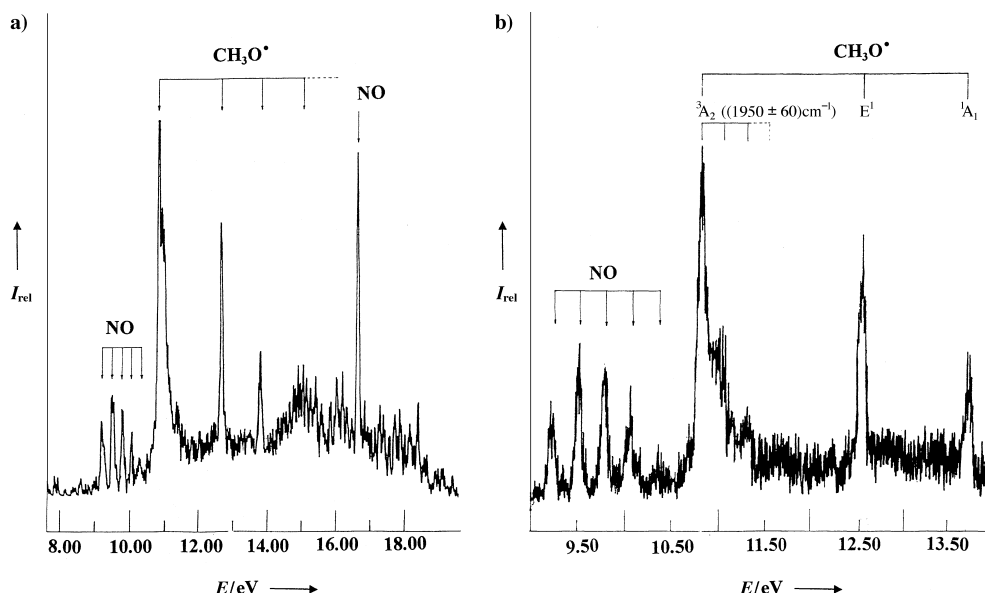


Figure 2. a) An in situ HeI-PE spectrum (given as relative intensities I_{rel}) of the products ($\text{CH}_3\text{O}^\bullet$ and NO) from the pyrolysis of CH_3ONO ; b) the expanded spectrum in the lower ionization energy region ($E < 14.00$ eV).

spectrum of products generated by pyrolysis of CH_3ONO and Figure 2b shows the lower ionization energy region ($E < 14.00$ eV) in greater detail. Five bands in the region $E < 10.70$ eV can be assigned to NO since the fine structure energy ($\tilde{\nu} = 2260 \text{ cm}^{-1}$) and the band at 9.54 eV with the greatest local intensity match the bands of NO alone. The two bands in the higher energy region ($E = 16.56$ and 18.32 eV) are also characteristic of NO species. The remaining bands in Figure 2 are therefore derived from the ionization of $\text{CH}_3\text{O}^\bullet$.

Assignment of the three sharp bands in Figure 2 for $\text{CH}_3\text{O}^\bullet$ follows the same process used for $\text{CH}_3\text{S}^\bullet$: For C_{3v} symmetry and a ${}^2\text{E}$ ground state, the molecular orbitals would be, in the order of increasing energy, $(3a_1)^2(4a_1)^2(1e)^4(5a_1)^2(2e)^3$. The HOMO ($2e$) of $\text{CH}_3\text{O}^\bullet$ has a dominant contribution from the lone-pair electrons of the oxygen atom with a small degree of C–O antibonding character.^[20] The removal of an electron from the HOMO will generate a CH_3O^+ ion in the ${}^3\text{A}_2$, ${}^1\text{E}$, and ${}^1\text{A}_1$ states. The sharp band at 10.78 eV matches the computed G2 adiabatic ionization energy (10.78 eV^[20]), and is in good agreement with the reported first ionization energy of the $\text{CD}_3\text{O}^\bullet$ isotopomer from two PIMS studies ($(10.726 \pm 0.008)^{[23]}$ and $(10.74 \pm 0.02) \text{ eV}^{[30]}$). This band is therefore attributed to the A_2 ionic state of CH_3O^+ and the vibrational fine structure spacing ($\tilde{\nu}(1950 \pm 60) \text{ cm}^{-1}$) is as expected for a $\nu_{\text{C-O}}$ stretching mode. The bands at 12.50 and 13.72 eV are designated as the ${}^1\text{E}$ and ${}^1\text{A}_1$ ionic states of CH_3O^+ , respectively, because of the sharpness of the bands and because the expected 3:2:1 band area ratio for ${}^3\text{A}_2: {}^1\text{E}: {}^1\text{A}_1$ is indeed observed (3.11:2.01:1.00 for the bands at 10.78, 12.50, and 13.72 eV).

This assignment is again supported by the improved DFT calculations performed in a manner similar to those for the methylthio radical. The calculation presumed a $\tilde{\chi}^2\text{E}$ ground state and the radical and its ionic states were restricted to C_{3v} symmetry. The total energy of the singlet state, described by a multideterminant wave function, was calculated using the

sum method of Ziegler et al.^[47] Table 2 shows the good agreement between the ionization energies measured ($E = 10.78$ (${}^3\text{A}_2$), 12.50 (${}^1\text{E}$), and 13.72 (${}^1\text{A}_1$) eV) and calculated ($E = 10.791$, 12.502 , and 13.728 eV). Also of interest is a vibrational fine structure at $\tilde{\nu} = (1950 \pm 60) \text{ cm}^{-1}$ present on the first band but absent on the other two. This phenomenon is currently under investigation.

Finally, the broad, higher energy PES band at 15.20 eV (Figure 2a) can be assigned to ionization of electrons of the $5a_1$ orbital leading to the ${}^3\text{E}$ and ${}^1\text{E}$ ionic states (calculated ionization energies are 14.994 (${}^3\text{E}$) and 15.381 eV (${}^1\text{E}$)).

Table 2. PES ionization energies E , computed (DFT) ionization energies E_v , and relative intensities I_{rel} of signals observed from different ionic states for the methoxy radical.

$E(\text{PES})$ [eV] ^[a]	$E_v(\text{DFT})$ [eV]	Cationic state	Relative intensity measured ^[b] expected	
10.78	10.791	${}^3\text{A}_2$	3.11	3
12.50	12.502	${}^1\text{E}$	2.01	2
13.72	13.728	${}^1\text{A}_1$	1.00	1
15.20	14.994	${}^3\text{E}$		
	15.381	${}^1\text{E}$		

[a] Values are ± 0.02 eV and derived from the maxima of the PES bands.

[b] Corrected for analyzer sensitivity ± 0.02 .

In summary, the PE spectra of both $\text{CH}_3\text{S}^\bullet$ and $\text{CH}_3\text{O}^\bullet$ radicals provide direct experimental evidence for the existence of different ionic states. The C_{3v} symmetry of both radicals could be deduced from their respective PE spectra. The PE spectrum is therefore able to provide both the orbital energy levels and also molecular symmetry.

Experimental Section

Experiments were performed on a double-chamber ultraviolet-photoelectron spectrometer (UPS), which was built specifically to detect transient species.^[48] The continuous $\text{CH}_3\text{S}^\bullet$ or $\text{CH}_3\text{O}^\bullet$ radical beams were produced in situ by pyrolysis of gas-phase CH_3SSCH_3 at $(285 \pm 0.5)^\circ\text{C}$ or CH_3ONO at $(275 \pm 0.5)^\circ\text{C}$, respectively, in a quartz tube. The heating system employed a double-heater inlet device. The purity of CH_3SSCH_3 was compared to a reference PE spectrum.^[49] The CH_3ONO was prepared by standard methods^[50, 51] and its purity was confirmed by mass spectrom-

etry and by a reference PE spectrum^[46]. The UPS delivered an operational resolution of about 30 meV for the $^2P_{3/2}$ band of Ar^+ . Experimental ionization energies were calibrated by the simultaneous addition of small amounts of Ar and CH_3I with the sample.

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- [1] H. Sitzmann, H. Bock, R. Boese, T. Dezember, Z. Havlas, W. Kaim, M. Moscherosch, L. Zanathy, *J. Am. Chem. Soc.* **1993**, *115*, 12003.
- [2] F. A. Honle, B. Beauchamp, *J. Am. Chem. Soc.* **1984**, *106*, 7336.
- [3] R. T. Boere, R. T. Oakley, R. W. Reed, P. C. Westwood, *J. Am. Chem. Soc.* **1989**, *111*, 1180.
- [4] A. B. Callear, J. Connor, D. R. Dickson, *Nature* **1969**, *221*, 1238.
- [5] P. C. Engelking, G. B. Ellison, W. C. Lineberger, *J. Chem. Phys.* **1978**, *69*, 1826.
- [6] S. Moran, G. B. Ellison, *J. Phys. Chem.* **1988**, *92*, 1794.
- [7] B. K. Janousek, J. I. Brauman, *J. Chem. Phys.* **1980**, *72*, 694.
- [8] Y. Endo, S. Satio, E. Hirota, *J. Chem. Phys.* **1986**, *85*, 1770.
- [9] Y. P. Lee, *Proc. SPIE Int. Soc. Opt. Eng.* **1993**, *44*, 1858.
- [10] K. Ohbayashi, H. Akimoto, I. Tanaka, *Chem. Phys. Lett.* **1977**, *52*, 47.
- [11] G. Black, L. E. Jusinski, *J. Chem. Soc. Faraday Trans.* **1986**, *82*, 2143.
- [12] G. Black, L. E. Jusinski, *J. Chem. Phys.* **1986**, *85*, 5379.
- [13] S. Y. Chiang, Y. P. Lee, *J. Chem. Phys.* **1991**, *95*, 66.
- [14] Y. Y. Lee, S. Y. Chiang, Y. P. Lee, *J. Chem. Phys.* **1990**, *93*, 4487.
- [15] K. Ito, T. Yano, Y. Sakai, *Proc. Int. Symp. Alcohol Fuels Technol.* **1984**, *2*, 2.
- [16] W. A. Glasson, *Environ. Sci. Technol.* **1975**, *9*, 1049.
- [17] A. Jonsson, M. B. Brite, *Environ. Sci. Technol.* **1982**, *16*, 106.
- [18] D. Kyokun, S. Terue, *Proc. Int. Symp. Alcohol Fuels Technol.* **1984**, *B3*, 2.
- [19] G. D. Bent, *J. Chem. Phys.* **1990**, *92*, 1547.
- [20] L. A. Curtiss, L. D. Kock, J. A. Pople, *J. Chem. Phys.* **1991**, *95*, 4040.
- [21] S. Kuo, Z. Zhang, R. B. Klemm, J. F. Liebman, L. J. Stief, F. L. Nesbitt, *J. Phys. Chem.* **1994**, *98*, 4026.
- [22] S. R. Lin, Y. P. Lee, J. B. Lee, *J. Chem. Phys.* **1988**, *88*, 171.
- [23] B. Ruscic, J. Berkowitz, *J. Chem. Phys.* **1991**, *95*, 4033.
- [24] D. L. Osborn, D. J. Leahy, E. M. Ross, D. M. Neumark, *Chem. Phys. Lett.* **1995**, *235*, 484.
- [25] J. Baker, J. M. Dyke, A. R. Ellis, A. Morris, *J. Electron Spectrosc. Relat. Phenom.* **1995**, *73*, 125.
- [26] Q. Cui, K. Morokuma, *Chem. Phys. Lett.* **1996**, *263*, 54.
- [27] R. Fournier, A. E. DePristo, *J. Chem. Phys.* **1992**, *96*, 1183.
- [28] C. W. Hsu, C. L. Liao, Z. X. Ma, P. J. H. Tjossem, C. Y. Ng, *J. Chem. Phys.* **1992**, *97*, 6283.
- [29] A. C. Curtiss, R. H. Nobes, J. A. Pople, L. Radom, *J. Chem. Phys.* **1994**, *101*, 7538.
- [30] S. C. Kuo, Z. Y. Zhang, R. B. Klemm, J. F. Liebman, L. J. Stief, F. L. Nesbitt, *J. Phys. Chem.* **1994**, *98*, 4026.
- [31] D. X. Wang, Y. Li, S. Li, H. Q. Zhao, *Chem. Phys. Lett.* **1994**, *222*, 167.
- [32] D. X. Wang, P. Jiang, X. M. Qian, G. Y. Hong, *J. Chem. Phys.* **1997**, *106*, 3003.
- [33] D. X. Wang, C. H. Li, X. M. Qian, S. D. Gablin, *J. Electron Spectrosc. Relat. Phenom.* **1998**, *97*, 59.
- [34] C. H. Qiao, G. Y. Hong, D. X. Wang, *J. Phys. Chem.* **1999**, *103*, 1972.
- [35] C. H. Qiao, M. F. Ge, D. X. Wang, *Chem. Phys. Lett.* **1999**, *305*, 359.
- [36] G. G. Odian, *Principles of Polymerization*, 3rd ed., Wiley, New York, **1991**, pp. 211–240.
- [37] B. Ruscic, J. Berkowitz, *J. Chem. Phys.* **1992**, *97*, 1818.
- [38] S. Nourbakhsh, K. Norwood, G. Z. He, C. Y. Ng, *J. Am. Chem. Soc.* **1991**, *113*, 6311.
- [39] L. A. Curtiss, R. H. Nobes, J. A. Pople, L. Radom, *J. Chem. Phys.* **1992**, *97*, 6766.
- [40] R. H. Nobes, L. Radom, *Chem. Phys. Lett.* **1992**, *189*, 554.
- [41] Y. C. Hsu, X. Liu, T. A. Miller, *J. Chem. Phys.* **1989**, *90*, 6582.
- [42] A. Jonsson, *Mutat. Res.* **1981**, *47*, 117.
- [43] C. S. Effenhauser, P. Felder, J. R. Huber, *J. Phys. Chem.* **1990**, *94*, 296.
- [44] E. Kades, M. Rosslein, U. Bruhlmann, J. R. Huber, *J. Phys. Chem.* **1993**, *97*, 989.

- [45] D. Schröder, D. Sülzle, O. Dutuit, T. Baer, H. Schwarz, *J. Am. Chem. Soc.* **1994**, *116*, 6395.
- [46] D. X. Wang, S. Li, Y. Li, S. J. Zheng, C. F. Ding, Y. Q. Gao, W. W. Chen, *J. Electron Spectrosc. Relat. Phenom.* **1996**, *82*, 19.
- [47] T. Ziegler, A. Rauk, E. J. Baerends, *Theor. Chim. Acta* **1977**, *43*, 261.
- [48] H. Zhao, D. X. Wang, G. Xu, *J. Anal. Instrum.* **1992**, *4*, 23; G. D. Willett, Ph.D. Thesis, La Trobe University (Australia), **1977**.
- [49] K. Kimura, S. Kalsumala, Y. Achiba, T. Yamagaki, S. Iwata in *Handbook of HeI Photoelectron Spectra of Fundamental Organic Molecules*, Japan Scientific Societies Press, Tokyo, **1981**, p. 228.
- [50] W. H. Hartung, F. Crossley in *Organic Synthesis Collective, Vol. 2* (Ed.: A. H. Blatt), Wiley, New York, **1948**, p. 364.
- [51] W. L. Semon, V. R. Damerell in *Organic Synthesis Collective, Vol. 2* (Ed.: A. H. Blatt), Wiley, New York, **1948**, p. 204.

The Kinetic Order of an Interfacial Diels–Alder Reaction Depends on the Environment of the Immobilized Dienophile**

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Substituent effects are used widely in physical organic chemistry to elucidate the relationships between the structures, reactivities, and properties of molecules. This mechanistic tool finds routine use in many areas, including the determination of reaction mechanisms,^[1] the development of catalysts,^[2] and the design of selective inhibitors of proteins.^[3] We have a program to extend the principles of physical organic chemistry to the reactions of molecules confined at the solid–liquid interface in order to elucidate the factors that uniquely affect these reactions. Here we show one such effect wherein the reaction of an immobilized molecule is influenced by a substituent effect arising from the surrounding micro-environment. We use the Diels–Alder reaction of soluble cyclopentadiene with quinone immobilized to a self-assembled monolayer (SAM) on gold as a model system.

This work uses monolayers prepared from an alkanethiol terminated in a hydroquinone group and a second alkanethiol terminated in either a methyl or hydroxy group (Figure 1). The reversible two-electron electrochemical oxidation of this hydroquinone to the quinone is well known.^[4] In previous work we showed that this quinone undergoes a Diels–Alder reaction with cyclopentadiene (Cp) dissolved in the electrolyte to give the cycloaddition adduct.^[5] Since this adduct is not

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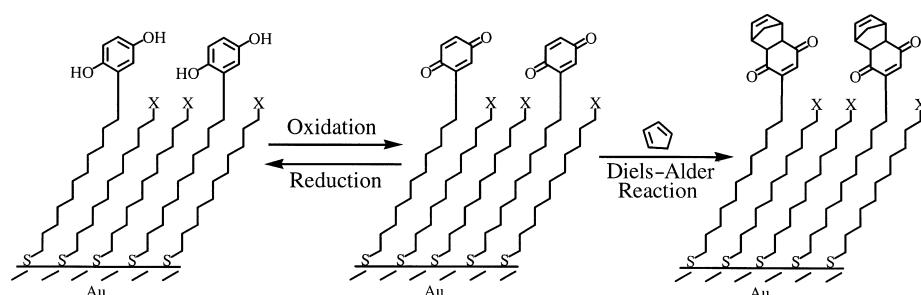


Figure 1. Reversible electrochemical oxidation of a monolayer that presents hydroquinone groups together with either methyl ($X = \text{CH}_3$) or hydroxy ($X = \text{OH}$) groups affords a monolayer that presents the quinone group. The resulting quinone reacts with cyclopentadiene to give the Diels–Alder adduct.

redox active, the rate of quinone loss, and hence the rate constant for the reaction, can be measured using cyclic voltammetry. Figure 2a shows a set of consecutive cyclic voltammograms for a monolayer that presents quinone groups ($\chi_Q = 0.10$) and hydroxy groups in 1:1 tetrahydrofuran:water containing sodium phosphate (2 mM), sodium chloride (75 mM), and cyclopentadiene (15.2 mM).^[6] Under these

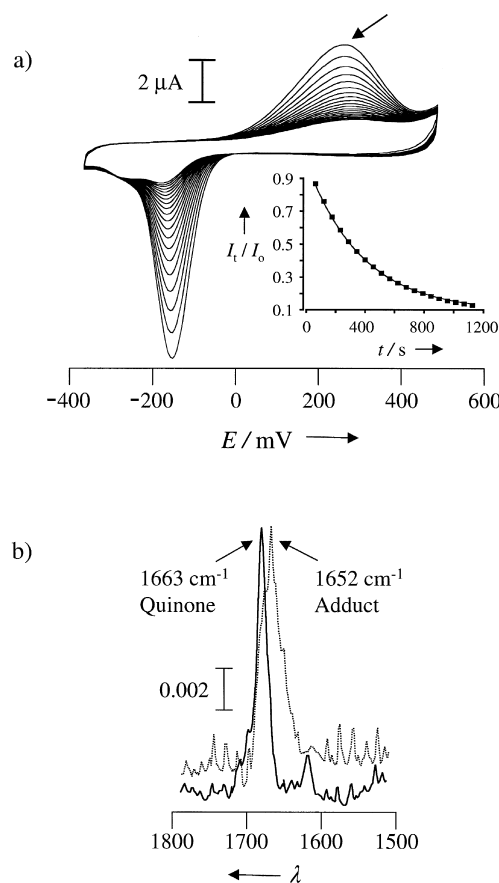


Figure 2. a) Consecutive cyclic voltammograms of a monolayer presenting hydroquinone groups ($\chi_Q = 0.10$) and hydroxy groups at a scan rate of 25 mV s^{-1} versus the Ag/AgCl reference electrode. The arrow denotes the first scan. Inset: A plot of the peak current showing the reduction in the amount of immobilized quinone versus time. See text for further information. b) Grazing-angle infrared spectra of the carbonyl stretching region of a monolayer presenting quinone groups prior (solid line) and after complete reaction with cyclopentadiene (dashed line). The two spectra are offset slightly for clarity and have intensities given by the scale bar.

conditions the hydroquinone group undergoes oxidation at 220 mV (versus Ag/AgCl) and the resultant quinone group is reduced at -150 mV .^[7] The waves for both oxidation and reduction decrease at the same rate over consecutive voltammograms. Several observations confirm that the decrease in the amount of quinone is due to the Diels–Alder reaction occurring. First, in the absence of cyclopentadiene the voltammograms showed no change over 100 cycles. Second,

the use of the structurally related molecules cyclopentane and cyclopentene resulted in no change in the voltammograms over as many cycles. Third, a comparison of grazing angle IR spectra of the monolayers before and after reaction are consistent with the expected product of the Diels–Alder reaction (Figure 2b). Monolayers presenting only quinone groups showed an absorption band at 1663 cm^{-1} in the IR spectra, which corresponds to a carbonyl stretch of the quinone molecules.^[8] After this monolayer was exhaustively treated with cyclopentadiene a new band appeared at 1652 cm^{-1} , which corresponds to the carbonyl stretch of the Diels–Alder adduct. The shift of the carbonyl stretch by approximately 10 cm^{-1} has been reported for the reaction of quinone and cyclopentadiene in solution.^[9] Fourth, ellipsometry showed that the thickness of the monolayer increased by the expected amount following reaction with 1-hexylcyclopentadiene.^[10] Finally, this reaction has been used to immobilize ligands for the biospecific recognition of proteins.^[5]

To determine the rate constant for the Diels–Alder reaction we analyzed the loss in peak current for the reduction of quinone over consecutive cyclic voltammograms (inset Figure 2a).^[11] Since the concentration of cyclopentadiene was much greater than that of immobilized quinone, the loss in peak current could be fit to an exponential decay [Eq. (1)] to obtain a pseudo first-order rate constant k' .

$$I_t = I_i + (I_0 - I_i)\exp^{-k't} \quad (1)$$

I_t is the peak current at time t , I_0 is the initial peak current, and I_i is the residual nonfaradaic current. The excellent fit of the experimental data with this equation indicates that the reactivity of the quinone groups is homogeneous and independent of the extent of the reaction. We repeated this experiment using different concentrations of cyclopentadiene, and in all cases found the reaction proceeded with homogeneous first-order kinetics and to completion for monolayers with quinone ($\chi_Q = 0.10$) and hydroxy groups. The pseudo first-order rate constants increased linearly with the concentration of cyclopentadiene ($0.76\text{--}58 \text{ mM}$). The slope of the best-fit line to these data provided a second-order rate constant of $k_{\text{DA}} = 0.26 \text{ M}^{-1} \text{ s}^{-1}$, after correction for the fraction of time that quinone was present (Figure 3a). This rate constant was independent of the density of quinone over the range $\chi_Q = 0.1\text{--}0.5$ and shows that the reactivity of the immobilized quinone was not affected by lateral interactions with neighboring quinones molecules on the monolayer.^[12]

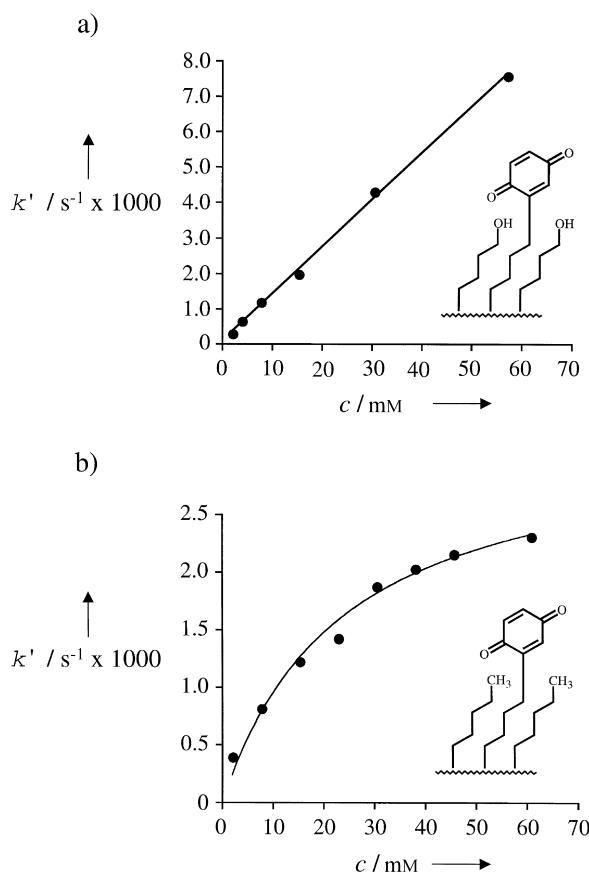


Figure 3. A comparison of the kinetic behavior for the reaction of cyclopentadiene with monolayers presenting quinone groups mixed with hydroxy groups (a) or with methyl groups (b). See the text for an explanation of the best fit curve in (b).

We next repeated these experiments for monolayers that presented quinone groups surrounded by methyl groups ($\chi_Q = 0.10$). All experimental conditions were identical to those described above and we again found, for all concentrations of cyclopentadiene, that the reaction proceeded with homogeneous first-order kinetics and to completion. A comparison of the first-order rate constants with the concentration of cyclopentadiene showed that they were not linearly related (Figure 3b). Instead, the first-order rate constant reached a limiting value with higher concentrations of cyclopentadiene. Since this kinetic behavior is inconsistent with a bimolecular reaction, we reasoned that the diene might first adsorb onto the monolayer and subsequently react with an immobilized quinone moiety.^[13] In this scheme the reaction of quinone with adsorbed diene is effectively intramolecular, and hence should proceed with apparent first-order kinetics. The overall rate is then the product of the first-order rate constant (k_{rxn}), the surface concentration of quinone (Γ_Q), and the fraction of available quinone sites on the monolayer to which cyclopentadiene is adsorbed [Eq. (2), θ is the fractional coverage and varies between 0 and 1].

$$\text{rate} = k_{\text{rxn}} \theta \Gamma_Q \quad (2)$$

We used a Langmuir isotherm to relate θ to the concentration of cyclopentadiene, and the association constant for

the adsorption of diene onto the monolayer (K_{ads}). This relation makes the assumption that adsorption is reversible, at equilibrium, and that the energy of association of a molecule at a given site is independent of the adsorption of a second molecule at a neighboring site. The data relating the first-order rate constant (k') and the concentration of diene were fit to Equation (3) with k_{rxn} and K_{ads} as adjustable parameters. The fit was excellent and gave values for K_{ads} and k_{rxn} of 41.0 M^{-1} and 0.0032 s^{-1} , respectively (Figure 3b).

$$k' = \frac{k_{\text{rxn}} K_{\text{ads}} [\text{Cp}]}{1 + K_{\text{ads}} [\text{Cp}]} \quad (3)$$

This work provides a dramatic example of the extent to which a change in the microenvironment of an immobilized group can affect the kinetic course of a reaction of that group with a soluble reactant. The second-order rate constant observed when quinone was surrounded with hydroxy groups is consistent with a bimolecular reaction of these two groups in a manner analogous to that for the same reaction in solution. The kinetic pathway changes completely on changing to an environment of methyl groups. We believe that the data are most consistent with an equilibrium adsorption of diene onto the monolayer followed by a first-order reaction of the two molecules. The velocity of the second-order reaction on monolayers presenting hydroxy groups is faster than the first-order reaction for monolayers presenting methyl groups. Even so, the second-order bimolecular reaction does not compete with the slower first-order process on monolayers that present methyl groups. This second-order pathway apparently is not inhibited by association of cyclopentadiene with the SAM, since low concentrations of cyclopentadiene (which give small θ , and hence leave many quinone groups uninhibited) display only the first-order pathway. The partitioning of the reaction between these two pathways therefore depends not only on the equilibrium adsorption constant (K_{ads}) of the diene with the monolayer, but also on the structure and solvation of the interface. We are currently exploring the effects that several other parameters have on this partitioning, including composition of the solvent, structure of the diene, identity of the functional groups surrounding the quinone, and the conformational flexibility of the tether connecting the quinone to the monolayer.

The use of SAMs and electrochemistry were essential to the studies described here. SAMs are structurally well-defined surfaces that permit wide flexibility in tailoring the environments of reactive groups and in controlling the densities of these groups. The use of cyclic voltammetry—and the related choice of a reactant that displays a reversible redox couple—permitted measurement of the rate of reaction with a resolution on the order of tens of seconds. The combination of techniques described here—SAMs and electrochemistry—provides an effective model system for physical organic studies of interfacial reactions^[14] and will be important to elucidating other factors that are unique to reactions at surfaces.

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- [1] a) M. D. Su, S. Y. Chu, *J. Phys. Chem. A* **1998**, *102*, 10159; b) C. J. Bradaric, W. J. Leigh, *Organometallics* **1998**, *17*, 645.
- [2] a) M. Palacki, N. S. Finney, P. J. Pospisil, M. L. Guler, T. Ishida, E. N. Jacobsen, *J. Am. Chem. Soc.* **1998**, *120*, 948; b) D. W. Nelson, A. Gypser, P. T. Ho, H. C. Kolb, T. Kondo, H. C. Kwong, D. V. McGrath, A. E. Rubin, P. O. Norrby, K. D. Gable, K. B. Sharpless, *J. Am. Chem. Soc.* **1997**, *119*, 1840.
- [3] a) J. B. Aggen, J. M. Humphrey, C.-M. Gauss, H.-B. Huang, A.-C. Nairn, A. R. Chamberlin, *Bioorg. Med. Chem.* **1999**, *7*, 543; b) A. C. Bishop, C. Y. Kung, K. Shah, L. Witucki, K. M. Shokat, Y. Liu, *J. Am. Chem. Soc.* **1999**, *121*, 627.
- [4] a) J. J. Hickman, D. Ofer, P. E. Laibinis, G. M. Whitesides, M. S. Wrighton, *Science* **1991**, *252*, 688; b) Y. Sato, M. Fujita, F. Mizutani, K. Uosaki, *J. Electroanal. Chem.* **1996**, *409*, 145.
- [5] M. N. Yousaf, M. Mrksich, *J. Am. Chem. Soc.* **1999**, *121*, 4286.
- [6] χ_0 is the fraction of alkanethiolates in the monolayer that present quinone groups, and was determined by integrating the waves in the cyclic voltammograms.
- [7] Cyclic voltammetry was performed with a Bioanalytical Systems CV-50W potentiostat using a cell with the gold/SAM as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl as the reference electrode.
- [8] S. Ye, A. Yashiro, Y. Sato, K. Uosaki, *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 3813.
- [9] a) P. Yates, M. I. Ardaro, L. F. Feiser, *J. Am. Chem. Soc.* **1955**, *78*, 650; b) A. P. Marchand, R. W. Allen, *J. Org. Chem.* **1974**, *39*, 1596.
- [10] Ellipsometric measurements were made with a Gaertner model L116S Research Type manual thin-film ellipsometer operating at 632.8 nm (He-Ne laser) and an angle of incidence of 70°. A value of 1.45 was used for the average refractive index of the SAM. The SAM presenting quinone groups had an average thickness of 21.4 ± 0.7 Å. After reaction with 1-hexyl-cyclopentadiene the SAM had a thickness of 34.4 ± 0.8 Å. Both values are consistent with the proposed structures.
- [11] For an example of previous work that used cyclic voltammetry to measure the rate of an interfacial reaction, see M. Maskus, H. Abruna, *Langmuir* **1996**, *12*, 4455.
- [12] A monolayer presenting only quinone groups gave heterogeneous kinetic behavior in that the reactivity of the quinone groups was dependent on the extent of reaction. This contrasting behavior gives evidence that the mixed monolayers used in this work were not phase-separated.
- [13] This model is analogous, though not identical, to the formalism of Langmuir–Hinshelwood. For a review, see W. H. Weinberg, *Acc. Chem. Res.* **1996**, *29*, 479.
- [14] For examples of the characterization of interfacial reactions, see a) K. L. Sun, R. C. Thomas, R. M. Crooks, A. J. Ricco, *J. Am. Chem. Soc.* **1991**, *113*, 8550; b) V. Chechik, C. J. M. Stirling, *Langmuir* **1998**, *14*, 99.

Highly Efficient Phosphapalladacyclic Catalysts for the Hydroarylation of Norbornene

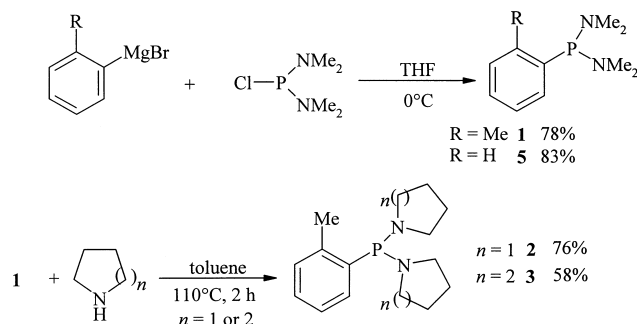
Jean Michel Brunel,* Andreas Heumann, and Gérard Buono

In the last decade, organopalladium-catalyzed C–C bond formation has become one of the most important tools in the synthesis of organic molecules.^[1] In particular, the Heck reaction is an important method for the preparation of aryl-functionalized alkenes in synthetic chemistry and the pharmaceutical industry.^[2] Attempts to achieve industrial-scale application of this reaction proved difficult due to the need for relatively large amounts of palladium catalysts and/or expensive aryl iodides. The former problem can be overcome by more efficiently recycling the catalyst, and the latter by using catalysts of higher activity or by activating the cheaper aryl bromides and chlorides. Herrmann, Beller et al.^[3] and Milstein et al.^[4] described recently the synthesis of phosphapalladacycles and their use as efficient catalysts for the Heck reaction that give turnover numbers (TON)^[5] one to three orders of magnitude higher than those reported before (TON > 100 000). Cyclopalladated complexes, formed in situ from mixtures of Pd^{II} salts and tri-*o*-tolylphosphane were investigated.^[6] More recently, it was found that fine tuning of the phosphorus ligand sphere could improve these results, and chelating diphosphanes containing P–N bonds gave higher turnover values (TON > 200 000).^[7] In recent years, we have studied and developed the potential of oxazaphospholidine and diazaphospholidine ligands in various catalytic reactions.^[8–10] Systems in which the phosphorus atom is substituted by two nitrogen and one oxygen atom or one aromatic carbon atom proved to be excellent mediators for, inter alia, highly selective allylic substitutions,^[8] copper-catalyzed Diels–Alder reactions,^[9] and asymmetric cyclopropanations.^[10] Here we report the synthesis of a new class of bis(alkylamino)-*p*-tolylphosphane ligands and their high efficiency in the palladium-catalyzed hydroarylation of norbornene and norbornadiene; they surpass all previously known catalysts as regards stability, lifetime, and turnover frequency (TOF).^[5]

The synthesis of bis(dimethylamino)-*o*-tolylphosphane (**1**) was achieved in 78 % yield from *o*-tolylmagnesium bromide and chloro-bis(dimethylamino)phosphane in THF at 0 °C (Scheme 1). Ligands **2** and **3** were synthesized from **1** by exchange reaction in refluxing toluene with two equivalents of the corresponding amine in 76 and 58 % yield, respectively (Table 1).^[11]

Treating palladium(II) acetate with **1–3** in refluxing toluene gave the expected palladacyclic complexes **4a–c** in high yields (90–95 %) as thermally stable yellow solids insensitive to air and moisture (Scheme 2). The cyclometallated structure

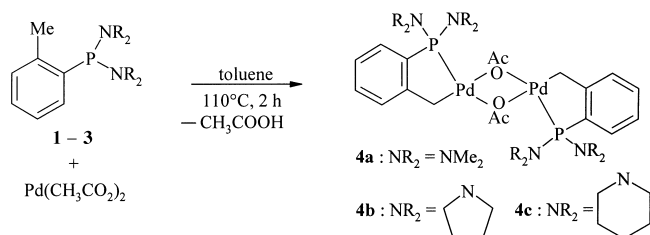
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Scheme 1. Synthesis of **1–3** and **5**.

Table 1. Characteristic data of compounds **1–3**, **4a–c**, **5** in CDCl₃.

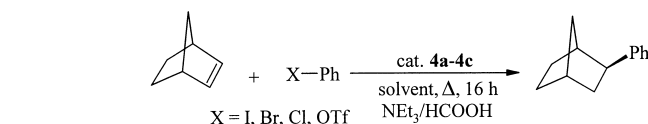
1 : Purification by distillation afforded 1 in 78% yield as an oil, b.p. 80 °C (10 Pa); ¹ H NMR (200 MHz): δ = 2.31 (s, 3H), 2.69 (d, <i>J</i> = 35.5 Hz, 6H), 2.72 (d, <i>J</i> = 35.5 Hz, 6H), 7.17–7.46 (m, 4H); ¹³ C NMR (50 MHz): δ = 20.1 (d, <i>J</i> = 26.0 Hz), 41.0 (s), 41.3 (s), 121.5 (s), 125.1 (s), 127.6 (s), 130.4 (s), 138.5 (s), 140.0 (d, <i>J</i> = 43.6 Hz); ³¹ P NMR (40.54 MHz): δ = 96.6
2 : Purification by distillation afforded 2 in 76% yield as an oil, b.p. 95 °C (3 Pa); ¹ H NMR (200 MHz): δ = 1.76 (m, 8H), 2.41 (s, 3H), 3.01 (m, 8H), 6.63–7.48 (m, 4H); ¹³ C NMR (50 MHz): δ = 26.4 (d, <i>J</i> = 4.8 Hz), 36.4 (d, <i>J</i> = 14.0 Hz), 49.9 (d, <i>J</i> = 13.5 Hz), 125.3 (s), 127.7 (s), 128.7 (s), 129.8 (d, <i>J</i> = 5.0 Hz), 130.5 (s), 143.3 (d, <i>J</i> = 42.0 Hz); ³¹ P NMR (40.54 MHz, CDCl ₃): δ = 89.1
3 : Purification by distillation afforded 3 in 58% yield as an oil, b.p. 130 °C (2 Pa); ¹ H NMR (200 MHz): δ = 1.49 (m, 12H), 2.46 (s, 3H), 3.0 (m, 8H), 7.0–7.42 (m, 4H); ¹³ C NMR (50 MHz): δ = 25.6 (d, <i>J</i> = 4.5 Hz), 41.2 (d, <i>J</i> = 35.0 Hz), 46.2 (s), 50.4 (d, <i>J</i> = 15.0 Hz), 125.1 (s), 128.6 (s), 127.7 (s), 129.2 (s), 130.7 (s), 141.2 (d, <i>J</i> = 41.0 Hz); ³¹ P NMR (40.54 MHz): δ = 91.2
4a : ¹ H NMR (200 MHz): δ = 2.40 (dd, <i>J</i> = 14.0, <i>J</i> _{PH} = 1.8 Hz, 2H), 2.55 (d, <i>J</i> = 35.5 Hz, 12H), 2.69 (d, <i>J</i> = 35.5 Hz, 12H), 2.82 (dd, <i>J</i> = 14.1, <i>J</i> _{PH} = 1.8 Hz, 2H), 3.01 (s, 6H), 7.15–7.56 (m, 8H); ¹³ C NMR (50 MHz): δ = 24.7 (d, <i>J</i> = 3.1 Hz), 30.8 (s), 41.2 (s), 41.8 (s), 121.5 (s), 125.1 (s), 127.6 (s), 131.4 (s), 138.5 (s), 148.0 (d, <i>J</i> = 31.3 Hz), 178.5 (s); ³¹ P NMR (40.54 MHz): δ = 89.2; elemental analysis calcd for C ₂₆ H ₄₂ N ₄ O ₄ P ₂ Pd ₂ (749.41): C 41.67, H 5.65, N 7.48, P 8.27, Pd 28.4; found C 41.8, H 5.9, N 7.45, P 8.35, Pd 28.12
5 : Purification by distillation afforded 5 in 83% yield as an oil, b.p. 60 °C (1 Pa); ¹ H NMR (200 MHz): δ = 2.68 (d, <i>J</i> = 33.2 Hz, 6H), 2.71 (d, <i>J</i> = 33.2 Hz, 6H), 7.18–7.49 (m, 5H); ¹³ C NMR (50 MHz): δ = 41.0 (s), 41.5 (s), 122.5 (s), 124.1 (s), 128.6 (s), 131.4 (s), 139.5 (s), 140.2 (d, <i>J</i> = 44.6 Hz); ³¹ P NMR (40.54 MHz): δ = 100.7



Scheme 2. Synthesis of **4a–c**.

as the result of simple C–H activation of the *ortho*-methyl group of the arylphosphane component was clearly established by NMR spectroscopy and elemental analysis (Table 1). The formulation of **4a–c** as μ -acetato dimeric structures was made by analogy to known palladium chemistry.^[12]

We chose the arylation of norbornene as our test reaction and the reductive (NEt₃/formic acid) conditions described by Larock et al.^[13] and Brunner et al.^[14] (Scheme 3). Besides the



Scheme 3. Catalytic hydroarylation of norbornene (OTf = CF₃SO₃).

simplicity and easy handling of this reaction, another reason was that the formation of chiral centers would allow enantioselective catalysis, and reaction with heteroaryl groups should lead to interesting analogues of epibatidine.^[15] We initially investigated the reaction of iodobenzene with norbornene under various experimental conditions. Carbon–carbon coupling in the presence of NEt₃/formic acid as hydrogen source and catalytic amounts of **4a–4c**, generated in situ, led to the corresponding *exo*-phenylnorbornane in good to quantitative yield (Table 2). No other isomer was formed nor did disubstitution occur.^[16]

Increasing the reaction temperature from 25 to 110 °C led to the first major improvement in the reaction yield (Table 2, entries 1 and 2; 0 and 72% yield, respectively). The acetate-bridged palladacycles **4a–c** become catalytically active at about 100 °C (TON of 144). Moreover, a dramatic solvent effect controls the outcome of the reaction (entries 3 and 4). Although polar solvents such as 2-(dimethylamino)ethanol (DMAE) or DMF led to excellent results (entries 6 and 8), DMSO appeared to be a much better solvent since extremely low catalyst concentrations (ca. 5×10^{-7} mol %) are fully sufficient (entry 13). Exceptional TONs of up to 196×10^6 , yields of 98%, and TOFs of up to 12×10^6 were obtained with this amount of catalyst. To our knowledge, these are the highest TOFs reported to date for palladium catalysis. Furthermore, regardless of whether **4a**, **4b**, or **4c** was used in the reaction, high TONs (14×10^6 to 158×10^8) were obtained (entries 13–16). The use of Herrmann's catalyst or triphenylphosphane as ligand was less effective, and a TOF of up to 8×10^3 was obtained (entries 21–23).^[17] Phenyl bromide and triflate were also successfully used and allowed the desired product to be obtained with a TOF of up to 8×10^3 (entries 19 and 20). The use of chlorobenzene instead of iodobenzene led to a marked drop in TON for the formation of phenylnorbornane (entry 17). When the catalyst concentration was decreased, no conversion occurred. We also studied the influence of the hydrogen source, and a significant effect of the nature of the reducing agent was found (Table 3, entries 1–3). Furthermore, the use of different aryl iodides led in all cases to excellent yields and high TOFs (entries 4–6). Replacement of norbornene with norbornadiene led to the formation of the expected phenylnorbornene in 98% yield with a TOF of up to 13×10^6 .

The mechanism of this reaction in the presence of palladacycles **4a–c** and the exact nature of the palladium species involved in this highly efficient catalytic process are not fully established. Nevertheless, it is clear that the presence of a methyl group on the aromatic ring bonded to the phosphorus atom is necessary for high reactivity. The use of the parent bis(dimethylamino)phenylphosphane **5** did not lead to any conversion when less than 0.5 mol % of palladium catalyst was used.^[18]

Table 2. Catalytic hydroarylation of norbornene with palladacycles **4a–4c** (Scheme 3).

Entry	ArX	Catalyst (mol % Pd)	Solvent	T [°C]	Conversion [%] ^[a]	Yield [%] ^[b]	TON ^[c]
1	PhI	4a (0.5)	toluene	25	0	–	–
2	PhI	4a (0.5)	toluene	110	80	72	1.4 × 10 ²
3	PhI	4a (5 × 10 ^{−4})	toluene	110	5	–	–
4	PhI	4a (0.5)	CH ₃ CN	80	12	–	–
5	PhI	4a (0.5)	DMF	120	100	99	1.9 × 10 ²
6	PhI	4a (5 × 10 ^{−7})	DMF	120	75	62	124 × 10 ⁶
7	PhI	4a (0.5)	DMAE	120	100	99	1.9 × 10 ²
8	PhI	4a (5 × 10 ^{−7})	DMAE	120	50	42	80 × 10 ⁶
9	PhI	4a (0.5)	DMSO	120	100	99	1.9 × 10 ²
10	PhI	4a (5 × 10 ^{−2})	DMSO	120	100	99	1.9 × 10 ²
11	PhI	4a (5 × 10 ^{−4})	DMSO	120	100	99	198 × 10 ³
12	PhI	4a (5 × 10 ^{−6})	DMSO	120	100	98	19 × 10 ⁶
13	PhI	4a (5 × 10 ^{−7})	DMSO	120	100	98	196 × 10 ⁶
14	PhI	4a (5 × 10 ^{−9})	DMSO	120	100	79	158 × 10 ⁸
15	PhI	4b (5 × 10 ^{−6})	DMSO	120	100	85	17 × 10 ⁶
16	PhI	4c (5 × 10 ^{−6})	DMSO	120	100	71	14 × 10 ⁶
17	PhCl	4a (0.5)	DMSO	120	100	99	1.9 × 10 ²
18	PhCl	4a (5 × 10 ^{−3})	DMSO	120	5	–	–
19	PhBr	4a (5 × 10 ^{−4})	DMSO	120	100	98	196 × 10 ³
20	PhOTf	4a (5 × 10 ^{−4})	DMSO	120	100	99	198 × 10 ³
21 ^[d]	PhI	5 × 10 ^{−4}	DMSO	120	100	63	126 × 10 ³
22 ^[d]	PhI	5 × 10 ^{−6}	DMSO	120	5	–	–
23 ^[e]	PhI	5 × 10 ^{−6}	DMSO	120	5	–	–

[a] Conversion determined by gas chromatography. [b] Yield of isolated product. [c] Moles product per mole catalyst. [d] With Herrmann's catalyst, prepared from Pd(OAc)₂ and tri-*o*-tolylphosphane. [e] With catalyst prepared from Pd(OAc)₂ and triphenylphosphane.

Table 3. Catalytic hydroarylation of norbornene (and norbornadiene) with various aryl iodides in the presence of different hydrogen sources.

Entry	ArX	Catalyst (mol % Pd)	Hydrogen source	Conversion [%] ^[a]	Yield [%] ^[b]	TON ^[c]
1	PhI	4a (5 × 10 ^{−7})	NET ₃ /HCOOH	100	98	196 × 10 ⁶
2	PhI	4a (5 × 10 ^{−7})	HCOONH ₄	100	97	194 × 10 ⁶
3	PhI	4a (5 × 10 ^{−7})	PHMS	0	–	–
4	1-iodonaphthalene	4a (5 × 10 ^{−7})	NET ₃ /HCOOH	100	95	190 × 10 ⁶
5	2-iodoanisole	4a (5 × 10 ^{−7})	NET ₃ /HCOOH	100	91	182 × 10 ⁶
6	4-iodoanisole	4a (5 × 10 ^{−7})	NET ₃ /HCOOH	100	96	192 × 10 ⁶
7 ^[d]	Ph-I	4a (5 × 10 ^{−7})	NET ₃ /HCOOH	100	98	196 × 10 ⁶

[a]–[c] See footnotes to Table 2. [d] Norbornadiene was used instead of norbornene.

In conclusion, we have described the synthesis of several new phosphapalladacycles and their use as catalysts in the hydroarylation of norbornene and norbornadiene. For the moment only aryl iodides are sufficiently reactive to allow TONs greater than 10¹⁰ to be attained. Further studies on the mechanism and the exact nature of the active species are in progress.

Experimental Section

General procedure for the hydroarylation of norbornene (Table 2, entry 13): the appropriate catalyst concentration was obtained by successive dilution of an initial catalyst solution prepared by mixing Pd(OAc)₂ (2.5 mg, 11 × 10^{−6} mol) and ligand **1** (4.8 mg, 23 × 10^{−6} mol) in 5 mL of DMSO under argon at 60 °C for 10 min (the mixture turned black after complete formation of the catalyst). The catalyst solution (5 × 10^{−7} mol %) was introduced into 10 mL of DMSO under argon. To the stirred solution were added 459 mg of iodobenzene (2.3 × 10^{−3} mol), 700 mg of norbornene (7.4 × 10^{−3} mol), 760 mg of NET₃ (7.5 × 10^{−3}), and 280 mg of formic acid (6 × 10^{−3} mol). The mixture was heated at 120 °C for 16 h. After cooling, 15 mL of water was added, and the mixture was extracted with pentane (3 × 30 mL). The combined organic phase was dried over MgSO₄ and filtered, and the solvents were removed under reduced pressure. The product was purified by distillation to afford phenylnorbornene in 98 % yield as a viscous oil, b.p. 124 °C (0.2 mbar); ¹H NMR (CDCl₃, 200 MHz): δ = 1.25–1.92 (m, 8 H), 2.44 (s, 2 H), 2.83 (q, *J* = 8.0 Hz, 1 H), 7.20–7.41 (m,

5 H); ¹³C NMR (CDCl₃, 50 MHz): δ = 29.0 (s), 30.7 (s), 36.1 (s), 36.9 (s), 39.2 (s), 43.0 (s), 47.4 (s), 125.4 (s), 127.1 (s, 2C), 128.3 (s, 2C), 142.7 (s).

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- [1] a) A. de Meijere, F. E. Meyer, *Angew. Chem.* **1994**, *106*, 2473; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2379; b) W. Cabri, I. Candiani, *Acc. Chem. Res.* **1995**, *28*, 2; c) K. C. Nicolaou, E. J. Sorensen, *Classics in Total Synthesis*, VCH, Weinheim, **1996**; d) G. Poli, G. Giambastiani, A. Heumann, *Tetrahedron* **1999**, in press.
- [2] a) “Catalytic Carbon–Carbon Coupling by Palladium Complexes: Heck Reactions”: W. A. Herrmann in *Applied Homogeneous Catalysis with Metal Complexes* (Ed.: B. Cornils), VCH, Weinheim, **1996**; b) V. V. Grushin, H. Alper, *Chem. Rev.* **1994**, *94*, 1047; c) R. F. Heck, *Palladium Reagents in Organic Synthesis*, Academic Press, London, **1985**; d) J. Tsuji, *Palladium Reagents and Catalysts, Innovation in Organic Synthesis*, Wiley, Chichester, **1995**.
- [3] a) M. Beller, T. H. Riermeier, S. Haber, H. J. Kleiner, W. A. Herrmann, *Chem. Ber.* **1996**, *129*, 1259; b) W. A. Herrmann, C. Broßmer, C. P. Reisinger, T. H. Riermeier, K. Öfele, M. Beller, *Chem. Eur. J.* **1997**, *3*, 1357; c) M. Beller, H. Fischer, W. A. Herrmann, K. Öfele, C. Broßmer, *Angew. Chem.* **1995**, *107*, 1992; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1848; d) W. A. Herrmann, V. P. W. Böhm, *J. Organomet. Chem.* **1999**, *572*, 141; e) W. A. Herrmann, C. Broßmer, K. Öfele, K.-P. Reisinger, T. Priermeier, M. Beller, H. Fischer, *Angew. Chem.* **1995**, *107*, 1989; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1844; see also: f) G. Poli, C. Scolastico, *Chemtracts: Org. Chem.* **1999**, *12*, 643.

- [4] a) Y. Ben-David, M. Portnoy, M. Gozin, D. Milstein, *Organometallics* **1992**, *11*, 1995; b) M. Portnoy, Y. Ben-David, I. Rouso, D. Milstein, *Organometallics* **1994**, *13*, 3465; c) M. Portnoy, Y. Ben-David, D. Milstein, *Organometallics* **1993**, *12*, 4734; d) M. Ohff, A. Ohff, M. E. van der Boom, D. Milstein, *J. Am. Chem. Soc.* **1997**, *119*, 11 687.
- [5] TON = mole product per mole Pd; TOF = mole product per mole Pd per h.
- [6] Highly efficient palladium carbene complexes are also known: W. A. Herrmann, M. Elison, J. Fischer, C. Köcher, G. R. Artus, *Angew. Chem.* **1995**, *107*, 2602; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2371.
- [7] B. L. Shaw, S. D. Perera, *Chem. Commun.* **1998**, 1863.
- [8] a) T. Constantieux, J. M. Brunel, A. Labande, G. Buono, *Synlett* **1998**, 49; b) G. Muchow, J. M. Brunel, M. Maffei, O. Pardigon, G. Buono, *Tetrahedron* **1998**, *54*, 10435; c) J. M. Brunel, T. Constantieux, G. Buono, *J. Org. Chem.* **1999**, *64*, 8940.
- [9] J. M. Brunel, B. Del Campo, G. Buono, *Tetrahedron Lett.* **1998**, *39*, 9663.
- [10] J. M. Brunel, O. Legrand, S. Reymond, G. Buono, *J. Am. Chem. Soc.* **1999**, *121*, 5807.
- [11] Although **1** and **2** are air- and moisture-stable, **3** should be stored under nitrogen atmosphere to avoid decomposition.
- [12] All attempts to obtain crystals suitable for an X-ray structure analysis failed until now.
- [13] R. C. Larock, P. L. Johnson, *J. Chem. Soc. Chem. Commun.* **1989**, 1368.
- [14] H. Brunner, K. Kramler, *Synthesis* **1991**, 1121.
- [15] a) A. Kasyan, C. Wagner, M. E. Maier, *Tetrahedron* **1998**, *54*, 8047; b) J. C. Namyslo, D. E. Kaufmann, *Synlett* **1999**, 114.
- [16] K. Fugami, S. Hagiwara, H. Oda, M. Kosugi, *Synlett* **1998**, 477.
- [17] It is noteworthy that in the presence of a smaller amount of Hermann's catalyst no reaction occurs (Table 2, entry 22).
- [18] The use of 0.5 mol % of Pd(OAc)₂/5 led to phenylnorbornane in 83 % yield (TON = 166), but no conversion occurred at lower concentrations.

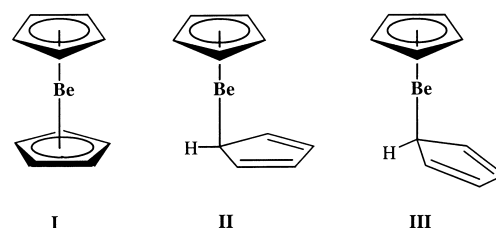
Synthesis and X-ray Structures of [Be(C₅Me₄H)₂] and [Be(C₅Me₅)₂]*

María del Mar Conejo, Rafael Fernández, Enrique Gutiérrez-Puebla, Ángeles Monge,* Caridad Ruiz, and Ernesto Carmona*

Dedicated to Professor José Elguero

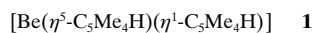
Beryllocene, [Be(C₅H₅)₂], the lightest member of the alkaline earth metallocenes^[1] and of the general bis(metallocene) series of compounds [MCp₂'], has aroused intense

structural debate since its preparation by Fischer and Hofmann in 1959.^[2] Structures **I**–**III** are some of the proposed models. Its unexpected dipolar nature^[3] is in disagreement

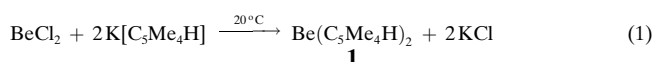


with the symmetrical ferrocene-like structure **I**. The most recent X-ray study,^[4a] confirmed the slip-sandwich η^5/η^1 structure **II** with disordered Be atoms at -145°C . This geometry is also in accord with electron diffraction data^[5] and with molecular dynamics calculations,^[6] and it has been rationalized by Beattie and co-workers, along with the structures displayed by other main-group metal cyclopentadienyls.^[7] Very recent density-functional theory (DFT) calculations on structure **I** and **III** concluded that the σ - π structure **III** is lower in energy.^[8]

Despite this long controversy and the existence of many half-sandwich Be compounds, including [Be(η^5 -C₅Me₅)Cl],^[9] no other beryllocenes have been reported to date, with the exception of [Be(η^5 -C₅Me₅)(η^1 -C₅H₅)], which has been characterized only in solution.^[10] Related dihydro-1*H*-azaboryl species have also been investigated.^[11] Here we present our initial results on the synthesis and solid-state structures of the beryllocenes **1** and **2**.



Compound **1** can be synthesized by the simple room-temperature reaction of BeCl₂ and K[C₅Me₄H] in diethyl ether [Eq. (1)]. Following crystallization from petroleum ether, **1** can be isolated in about 70 % yield in the form of white crystals, soluble in hexane, benzene, and other nonpolar organic solvents. Both solid samples and solutions of **1** exhibit high thermal stability, but the compound is very reactive towards H₂O and O₂ and decomposes immediately in the presence of air. Similar to [Be(C₅H₅)₂],^[12] compound **1** is highly fluxional in solution; the two rings are equivalent down to -90°C according to the ¹H and ¹³C NMR spectra (C₇D₈; see Experimental Section).



Single crystals of **1** suitable for X-ray structure analysis were investigated at -160°C and were found to be orthorhombic.^[13] Figure 1a shows the molecular structure and Figure 1b a schematic representation of the Be- η^1 -C₅Me₄H moiety. In contrast to the structure of [Be(C₅H₅)₂], in which the Be atom is disordered between two crystallographically equivalent sites, no positional or thermal disorder appears

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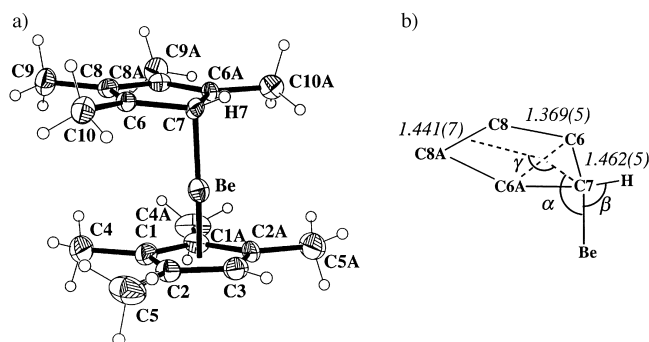
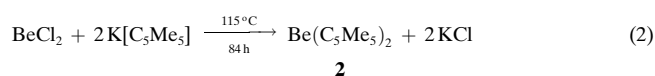


Figure 1. a) Molecular structure of compound **1**. b) Schematic representation of the Be- η^1 -C₅Me₄H moiety of **1**; $\alpha = 100.5(5)$, $\beta = 106(2)$, $\gamma = 173.7(4)^\circ$.

to exist in **1**. The Be atom is coordinated to one η^5 -C₅Me₄H ring and to one η^1 -C₅Me₄H ring. The distance between Be and the centroid of the former (1.471(7) Å) and its carbon atoms (av. 1.90(7) Å) compare well with those found in [Be(C₅H₅)₂].^[4] In the Be- η^1 -C₅Me₄H moiety the Be-C7 separation of 1.769(8) Å is in the middle of the range of Be-C bonds (1.70–1.85 Å^[14]). This ring is not planar. The plane defined by carbon atoms C6, C6A, and C7 forms an angle of 11.7(3)° with the η^5 -C₅Me₄H ring, but the best mean plane is almost parallel to the η^5 ring (6.6(1)°). The hydrogen atom H7 deviates by 0.46 Å from the plane of C7 and the adjacent carbons and points away from Be. Altogether these data indicate a geometry around C7 closer to distorted tetrahedral than in [Be(C₅H₅)₂].^[4a, 7] In the latter, the σ -bonded carbon is nearly coplanar with the neighboring carbons and the attached hydrogen, hence its geometry has been described as trigonal pyramidal.^[4a, 7]

The facile formation of **1** encouraged us to search for a suitable synthesis of the C₅Me₅ analogue, [Be(C₅Me₅)₂], even though the Be center of this complex is thought to be sterically overcrowded.^[1] Solid-angle estimations predict the putative [Be(η^5 -C₅H₅)₂] species to be a severely congested molecule.^[15] Despite this, we have found that BeCl₂ and K[C₅Me₅] react slowly (3.5 days) in a mixture of toluene and diethyl ether at 115 °C to give the desired compound **2** at about 50 % yield [Eq. (2)]. Similar to compound **1**, [Be(C₅Me₅)₂] **2** is soluble in nonpolar organic solvents and decomposes instantly in the presence of O₂ or H₂O. Its ¹H NMR spectrum merely shows a singlet at $\delta = 1.83$, whereas in the ¹³C{¹H} spectrum the ring carbons resonate at $\delta = 110.5$ (20 °C; $\delta = 109.7$ at –90 °C). Since in other Be and Mg compounds containing η^5 -C₅Me₅ ligands (for example, [Be(η^5 -C₅Me₅)Cl],^[9, 10] [Be(η^5 -C₅Me₅)(η^1 -C₅H₅)],^[10] [Mg(η^5 -C₅Me₅)₂],^[16] among others^[14a, 16]) the corresponding signal appears invariably in the very narrow range of $\delta = 110$ –108, **2** can be represented as [Be(η^5 -C₅Me₅)₂]. This proposal has been confirmed by X-ray studies at –160 °C.



Compound **2** forms monoclinic crystals,^[13] with four formula units per unit cell (Figure 2). The molecular structure represented in Figure 3 shows that the beryllium atom is

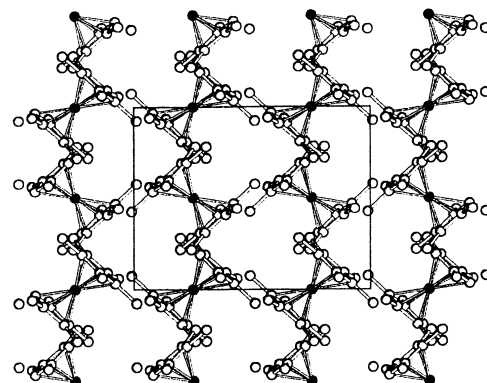


Figure 2. View of the cell of **2** along [100].

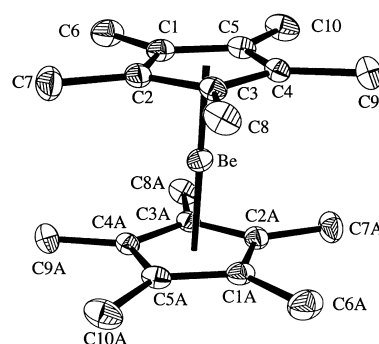


Figure 3. Molecular structure of **2**.

bonded to two planar and parallel C₅Me₅ ligands (the average deviation from the mean plane is only 0.01 Å), which are separated by 3.310(1) Å (the interlayer separation in graphite is 3.35 Å).^[17] The Be-Cp' centroid separation (1.655(1) Å) and Be-C_{ring} distances are comparable to those found in the related [Mg(η^5 -C₅Me₅)₂]^[18] and [Al(η^5 -C₅Me₅)₂]^[19] species after allowance is made for the differences in the radii of the metals (see below). The five Be-C distances to the rings in **2** are not identical since they range from 1.969(1) (Be-C2) to 2.114(1) Å (Be-C5). Consequently, the Be-Cp' centroid vectors are not exactly perpendicular to the rings but form an angle of 87.1(5)°. It is difficult to decide whether these facts are meaningful in terms of Be-C₅Me₅ bonding (the similarity between this structure and the η^3/η^3 model^[6] suggested for the transition state of the ring inversion mechanism in [Be(C₅H₅)₂] is evident) or are determined by crystal packing effects. Considering the long Be-C_{ring} bonds, we favor the second possibility. A difference of only 11 kJ mol⁻¹ has been calculated to exist among several model structures for beryllocene^[6] and a compression by 0.02 Å of the metal–ring distances from equilibrium in a compound like [Mg(η^5 -C₅H₅)₂] has been estimated to require^[20a] only 0.7 kJ mol⁻¹.

The present study on beryllocenes confirms the tendency of the small beryllium atom to adopt an η^5/η^1 slip-sandwich structure in [BeCp'₂] compounds. Moreover, it has been unexpectedly revealed for the C₅Me₅ ligand that the η^5/η^5 structure becomes more favorable in the solid state. It thus appears that if one of the Cp' rings forms a sufficiently strong σ Be-CH_{ring} bond, it prevails over the somewhat weaker

Be- η^5 -Cp' binding and the η^5/η^1 structure is adopted. If the input to the binding energy that arises from the Be-C σ -bond becomes weaker, as expected for Be- η^1 -C₅Me₅, the contribution from the delocalization of the ring π -system could prevail,^[7] with the result that the [Be(η^5 -C₅Me₅)₂] structure is preferred. Crystal packing effects may also enforce the symmetric sandwich structure, whereas the alternative slip-sandwich formulation, [Be(η^5 -C₅Me₅)(η^1 -C₅Me₅)], could be destabilized by van der Waals repulsions between the Me groups of the η^5 -C₅Me₅ ring and the Me group on the carbon atom of the η^1 -C₅Me₅ ring σ -bonded to beryllium. DFT calculations and gas-phase electron diffraction studies on compound **2**, currently in progress, are expected to clarify some of the above aspects and to provide further relevant information on the bonding and structural properties of **2**. Naturally, bonding in **2** is weaker than in the transition metal metallocene analogues, as attested by the very long Be-C_{ring} separations (recall that Be-C bonds range from 1.70 to 1.85 Å^[14]). It appears, however, comparable to that in [Mg(η^5 -C₅Me₅)₂]^[18, 20] and [Al(η^5 -C₅Me₅)₂]^[19]. In the three species, the M-C_{ring} separations amount to 2.05 (**2**, average value), 2.34 (Mg), and 2.16 Å (Al), whilst the effective ionic radii of these metals in six-fold coordination are 0.45 (calculated), 0.72, and 0.535 Å, respectively.^[21]

Experimental Section

Caution! Beryllium compounds are very toxic by inhalation and in contact with the skin, are irritating to the respiratory system, and present the danger of very serious irreversible effects.

1: BeCl₂ (0.824 g, 10 mmol) and K[C₅Me₅H] (3.2 g, 20 mmol) were suspended in diethyl ether (200 mL) and stirred overnight at room temperature. Removal of the solvent in vacuo and extraction with petroleum ether afforded crystals of **1** after cooling to -30 °C. Yield 70%; m.p. 83 °C; ¹H NMR (500 MHz, [D₆]benzene, 25 °C): δ = 1.80 (s, 12H; Cp'CH₃), 1.83 (s, 12H; Cp'CH₃), 4.39 (s, 2H; Cp'CH); ¹H NMR (400 MHz, [D₈]toluene, -90 °C): δ = 1.75 (s, 12H; Cp'CH₃), 1.78 (s, 12H; Cp'CH₃), 4.14 (s, 2H; Cp'CH); ¹³C{¹H} NMR (125 MHz, [D₆]benzene, 25 °C): δ = 10.1 (s, CH₃), 12.4 (s, CH₃), 82.8 (m, CH), 117.3 (s, CCH₃), 117.7 (s, CCH₃); ¹³C{¹H} NMR (100 MHz, [D₈]toluene, -90 °C): δ = 10.7 (s, CH₃), 13.0 (s, CH₃), 79.8 (s, CH), 118.0 (s, CCH₃), 119.1 (s, CCH₃); IR (Nujol): $\tilde{\nu}$ = 3065 (m), 2728 (w), 1244 (m), 1040 (m), 798 (s) cm⁻¹; elemental analysis calcd. for C₁₈H₂₆Be: C 86.0, H 10.4; found C 85.1, H 10.6.

2: The synthesis is similar to that of compound **1**. BeCl₂ (0.482 g, 6 mmol) and K[C₅Me₅] (2.088 g, 12 mmol) are stirred for 84 h in a 1:1 mixture of toluene:diethyl ether (80 mL) while the temperature of the heating bath is maintained at 115 °C. The solvent is then removed under vacuum and the residue extracted with petroleum ether. Cooling to -30 °C overnight gives crystals of **2** in ca. 50% yield; m.p. 237 °C; ¹H NMR (400 MHz, [D₆]benzene, 25 °C): δ = 1.83 (s, 30H, Cp'CH₃); ¹³C{¹H} NMR (100 MHz, [D₆]benzene, 25 °C): δ = 10.4 (s, CH₃), 110.5 (s, CCH₃); IR (Nujol): $\tilde{\nu}$ = 2723 (w), 1030 (m), 715 (s) cm⁻¹; elemental analysis calcd. for C₂₀H₃₀Be: C 85.9, H 10.8; found: C 85.7, H 10.8.

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- Schmidbaur in *Gmelin Handbook of Inorganic Chemistry*, 8th ed., Organoberyllium Compounds, Part 1, Springer, Berlin, 1987.
- [2] E. O. Fischer, H. P. Hofmann, *Chem. Ber.* **1959**, 92, 482.
 - [3] S. J. Pratten, M. K. Cooper, M. J. Aroney, S. W. Filipczuk, *J. Chem. Soc. Dalton Trans.* **1985**, 1761.
 - [4] a) K. W. Nugent, J. K. Beattie, T. W. Hambley, M. R. Snow, *Aust. J. Chem.* **1984**, 37, 1601; b) C. H. Wong, T. Y. Lee, K. J. Chao, S. Lee, *Acta Crystallogr. Sect. B* **1972**, 28, 1662; c) C. H. Wong, T. Y. Lee, T. J. Lee, T. W. Chang, C. S. Liu, *Inorg. Nucl. Chem. Lett.* **1973**, 9, 667.
 - [5] A. Almenningen, A. Haaland, J. Luszyk, *J. Organomet. Chem.* **1979**, 170, 271.
 - [6] P. Margl, K. Schwarz, P. E. Blöchl, *J. Chem. Phys.* **1995**, 103, 683.
 - [7] J. K. Beattie, K. W. Nugent, *Inorg. Chim. Acta* **1992**, 198–200, 309.
 - [8] L. W. Mire, S. D. Wheeler, E. Wagenseller, D. S. Marynick, *Inorg. Chem.* **1998**, 37, 3099.
 - [9] C. J. Burns, R. A. Andersen, *J. Organomet. Chem.* **1987**, 325, 31.
 - [10] S. J. Pratten, M. K. Cooper, M. J. Aroney, *J. Organomet. Chem.* **1990**, 381, 147.
 - [11] G. Schmid, O. Boltsch, R. Boese, *Organometallics* **1987**, 6, 435.
 - [12] K. W. Nugent, J. K. Beattie, L. D. Field, *J. Phys. Chem.* **1989**, 93, 5371.
 - [13] Crystal data for C₁₈H₂₆Be (**1**) and C₂₀H₃₀Be (**2**). The compounds crystallize in the *Pnma* and *C2/c* space groups, respectively, with cell parameters: *a* = 12.918(3), *b* = 15.735(3), *c* = 7.621(2) Å (**1**) and *a* = 14.9127(5), *b* = 11.9919(4), *c* = 9.4979(3) Å, β = 94.839(1)° for (**2**). The crystals, with dimensions 0.10 × 0.20 × 0.20 mm³ (**1**) and 0.35 × 0.30 × 0.30 mm³ (**2**), were mounted on a Bruker-Siemens Smart CCD diffractometer equipped with a low-temperature device and a normal focus, 2.4 kW sealed tube X-ray source (Molybdenum radiation, λ = 0.71067 Å) operating at 50 kV and 20 mA. Data were collected at -160 °C using ω scans ($3 < \theta < 21^\circ$) for **1** (whose crystals exhibited poor quality so there were no reflections over 21°) and $2 < \theta < 31^\circ$ for **2**. The total number of reflections measured was 2661 and 8398 of which 777 and 2450 were considered independent, respectively. The structure was solved by direct methods (G. M. Sheldrick, SHELX-92, Program for Crystal Structure Determination, University of Cambridge, 1992). Hydrogen atoms were located in difference Fourier maps. Refinements were by full-matrix least-squares analysis with anisotropic thermal parameters for all non-hydrogen atoms and isotropic for hydrogen atoms in both cases. Final *R* values are *R*1 = 0.054 and 0.053, respectively. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-137573 and -137574. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
 - [14] a) C. J. Burns, R. A. Andersen, *J. Am. Chem. Soc.* **1987**, 109, 5853; b) W. A. Herrmann, O. Runte, G. Artus, *J. Organomet. Chem.* **1995**, 501, C1; c) M. Niemeyer, P. P. Power, *Inorg. Chem.* **1997**, 36, 4688; d) R. Han, G. Parkin, *Inorg. Chem.* **1993**, 32, 4968.
 - [15] E. B. Lobkovsky, *J. Organomet. Chem.* **1984**, 277, 53.
 - [16] M. M. Conejo, R. Fernández, E. Carmona, unpublished results.
 - [17] F. A. Cotton, G. Wilkinson, C. A. Murillo, M. Bochmann, *Advanced Inorganic Chemistry*, 6th ed., Wiley, New York, 1999.
 - [18] R. A. Andersen, R. Blom, J. M. Boncella, C. J. Burns, H. V. Volden, *Acta Chem. Scand.* **1987**, A41, 24.
 - [19] C. Dohmeier, H. Schnöckel, C. Robl, U. Schneider, R. Ahlrichs, *Angew. Chem.* **1993**, 105, 1714; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 1655.
 - [20] a) K. Faegri, Jr., J. Almlöf, H. P. Lüthi, *J. Organomet. Chem.* **1983**, 249, 303; b) A. J. Bridgeman, *J. Chem. Soc. Dalton Trans.* **1997**, 2887.
 - [21] R. D. Shannon, *Acta Crystallogr. Sect. A* **1976**, 32, 751.

- [1] a) P. Jutzi, N. Burford, *Chem. Rev.* **1999**, 99, 969; b) P. Jutzi, N. Burford in *Metallocenes*, Vol. 1 (Eds.: A. Togni, R. L. Halterman), Wiley-VCH, 1998, chap. 2; c) N. A. Bell in *Comprehensive Organometallic Chemistry II*, Vol. 1 (Eds.: G. Wilkinson, F. G. A. Stone, W. Abel), Pergamon, Oxford, 1995, chap. 2; d) M. L. Hays, T. P. Hanusa, *Adv. Organomet. Chem.* **1996**, 40, 117; e) D. Stalke, *Angew. Chem.* **1994**, 106, 2256; *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 2168; f) H.

Adrenaline Release by Chromaffin Cells: Constrained Swelling of the Vesicle Matrix Leads to Full Fusion**

Christian Amatore,* Yann Bouret, Eric R. Travis, and R. Mark Wightman

Release of neurotransmitters by secretory vesicles is a fundamental neurobiological process but some of its physicochemical basis remains a mystery. In particular, the reason is still unknown why some vesicles proceed to a stage involving full inclusion of their former membrane into that of the cell (full fusion), whereas others remain poised at the initial stage of the process, where release occurs through a nanometer-sized pore (fusion pore).

In this paper, we investigate quantitatively this problem by examining the kinetic information contained in the experimental dynamics of the vesicle membrane during the full-fusion stage. We take advantage of our recent work, in which we established quantitatively that the dynamics of full fusion can be determined with unprecedented precision through deconvolution of chronoamperometric currents during individual exocytotic secretion events measured relative to release of adrenaline by chromaffin cells.^[1] This treatment demonstrated that full fusion secretion events are regulated by the interplay between the swelling of the core of the vesicle and the dynamics of the membrane unfolding although the efflux of catecholamine cations (adrenaline, in this work) remains limited kinetically by its slow diffusion inside the exposed matrix. The present work affords a quantitative description of the physicochemical forces regulating the transition between fusion-pore and full-fusion states. During the pore-release phase, the internal vesicle matrix is confined by its membrane, so it cannot expand significantly. This restrained swelling of the confined matrix drastically weakens the stability of the fusion pore by increasing the membrane tension. When the surface tension forces overcome its edge resistance, the fusion pore becomes unstable and expands rapidly, thereby initiating full fusion. This concept implies that very small vesicles should never reach the transition point because they cannot develop a sufficient membrane surface tension due to an insufficient internal swelling pressure, whereas large vesicles should always proceed to the full-fusion stage and their matrix is thereby fully exposed to the extracellular fluid at the end of the process.

In chromaffin cell vesicles, catecholamine cations are tightly associated with a negatively charged matrix composed of chromogranins and ATP. Because of electroneutrality, catecholamine cations can only escape upon exchange with hydrated monovalent cations from the extracellular solution.^[2] As reported for synthetic hydrogels,^[3, 4] this exchange disrupts hydrogen bonds and creates weak dipole–dipole repulsion in the matrix structure and thereby imparts upon it a tendency to swell.^[5–7] For example, the radius of mast cell granules is reported to expand by a factor of about 1.35 upon full release.^[6, 7] Swelling increases ionic diffusivity in the matrix, which allows further penetration of solvated ions and eventually leads to a cascade of released catecholamine cations. However, the matrix swelling is initially impeded by the tight cellular membrane and the mechanism of catecholamine cation release is necessarily more complex.

Exocytosis of catecholamines by chromaffin cells can be measured with a carbon-fiber ultramicroelectrode placed adjacent to the cell.^[8–11] This ensures the electrooxidation (two electrons per molecule^[12]) of secreted molecules that diffuse from the cell to the electrode. The released catecholamine flux $\frac{dN(t)}{dt}$, where $N(t)$ is the number of catecholamine molecules released at time t , is converted into a current $I(t)$ according to Faraday's law: $I(t) = 2F \frac{dN(t)}{dt}$, where F is the Faraday constant. The variation of the current with time (Figure 1) is thus a direct kinetic measurement of the catecholamine efflux. Simultaneous amperometric and

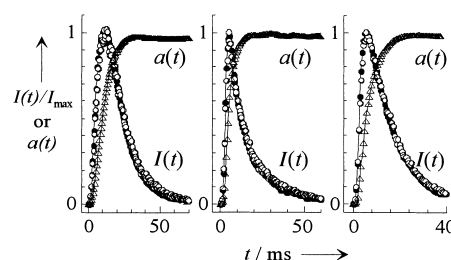


Figure 1. Normalized chronoamperometric spikes $I(t)/I_{\max}$, measured as described previously,^[26, 27] and the variations of $a(t)$ ^[1] for three representative individual exocytotic events observed at chromaffin cells stimulated to release adrenaline upon the addition of Ba^{2+} .^[26, 27] $I(t)$: Current at time t ; $I_{\max}$: Maximum current of each event; $a(t)$: Fraction of the vesicle surface area exposed to the extracellular fluid at some time t after the beginning of full fusion (see Figure 2). Experimental (open circles) and theoretical (solid circles) currents based on the extracted $a(t)$ functions (open triangles).^[1]

patch–clamp investigations demonstrated that release starts through the formation of a fusion pore that connects the inside of the vesicle to the extracellular fluid through the two contacting membranes.^[13–15] Any event prior to the formation of the initial fusion-pore aperture is not considered here, since our method gives only access to the rate of efflux of catecholamine cations. For most chromaffin vesicles, this pore-release phase proceeds to the full fusion of vesicle and cell membrane.^[13–15]

A steady flux during the pore lifetime is observed by chronoamperometry as a small constant current, termed I_{foot} (about 5 pA, merged with the initial current rise in Figure 1).

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Transition to the full-fusion stage results in a rapid current increase that decays within a few milliseconds.^[8–11, 13, 14] As established previously,^[11] such a sharp spike is inconsistent with the matrix swelling rate alone controlling the kinetics,^[3] in contradiction to previous claims.^[7] Rather, the current spike is consistent with convoluted kinetics involving the dynamics of the vesicle membrane and the rate of diffusion of catecholamine out of the matrix.^[1] Application of this model to a large number of chronoamperometric spikes allowed its experimental validation,^[1] as well as measurement of catecholamine concentration and diffusion inside the swollen matrix.^[16] Extraction of the diffusive kinetic components thereby afforded the vesicle-membrane dynamics with unprecedented precision.^[1, 16]

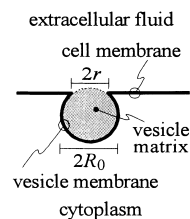


Figure 2. Schematic representation of the vesicle and cell membranes during full fusion, with a definition of the vesicle radius R_0 and the expanding pore radius r .

The surface area function $a(t)$ extracted from the current trace^[1] (Figure 1) represents the fractional area of the spherical vesicle matrix of radius R_0 (Figure 2) exposed to the extracellular fluid at time t after the beginning of the full-fusion event. In agreement with patch-clamp capacitance measurements,^[13, 14] $a(t)$ has an approximately sigmoidal shape (see triangles in Figure 1). However, the present precision demonstrates that $a(t)$ exhibits a profound asymmetry between short- and long-term behaviors. During the initial period, $a(t)$ increases explosively, yet later it increases at a much smoother

pace. We have suggested that this arises because different physicochemical factors control the vesicle membrane dynamics.^[1] We demonstrate herein that this intuitive claim is quantitatively supported.

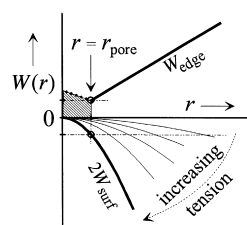


Figure 3. Schematic representation of the edge potential energy $W_{\text{edge}} = 2\pi\rho_0 r$ and the surface-tension potential energy $W_{\text{surf}} = -\pi\sigma r^2$ as a function of the pore radius r . The component W_{surf} is represented for different surface-tension energy coefficients (σ) to illustrate the effect of an increase in this parameter due to the increasing internal pressure in the vesicle. The shadowed box symbolizes the edge energy of the initial fusion-pore structure that opposes its closure (see text).

The full-fusion event can be described as a pore-enlargement process.^[16] In a membrane, a circular pore of radius r has a potential energy $W(r)$, which is the sum of two components,^[17] $W(r) = W_{\text{surf}} + W_{\text{edge}}$ (see Figure 3). The term $W_{\text{surf}} = -\pi\sigma r^2$ represents the surface-tension work which tends to enlarge the pore radius (the surface-tension energy coefficient σ , for a differential pressure ΔP between the membrane surfaces, is given by $\sigma = \frac{1}{2}\Delta P R_0$) and the term $W_{\text{edge}} = 2\pi\rho_0 r$ represents the potential energy of the pore edge that opposes its enlargement (ρ_0 is the edge energy coefficient). The value of W_{edge} during the pore-release phase, represented by the shaded box in Figure 3, is determined by biological processes that are still not fully characterized. In any case, during this initial phase, the overall energy has a minimum at $r = r_{\text{pore}} =$

~ 1 nm, since initial fusion pores are known to have this radius.^[11, 13, 14, 18] Since these pores are reported to flicker ($0 \leq r \leq r_{\text{pore}}$) in phase with thermal fluctuations,^[13–15, 18] the value of $W_{\text{edge}}(r_{\text{pore}})$ must be comparable to $k_B T$ (k_B is Boltzmann's constant).

During the pore-release stage, $W_{\text{edge}}(r_{\text{pore}}) > -W_{\text{surf}}(r_{\text{pore}})$ (Figure 3). However, during this stage, a steady-state flux of catecholamine cations exchanges with hydrated cations from the extracellular fluid. This results in a continuous modification of the matrix near the fusion pore and an increasing drive for these altered matrix zones to swell.^[5–7] While the vesicle is constricted by its membrane, it cannot swell significantly and thereby ΔP and the surface-tension coefficient σ increase.^[19] The tightening of the vesicle membrane proceeds up to the moment when the initial fusion pore becomes metastable,^[17] a situation requiring $dW(r)/dr = 0$, that is, $\Delta P_{\text{rupt}} = 2\sigma_{\text{rupt}}/R_0 = 2\rho_0/r_{\text{pore}}R_0$ (ΔP_{rupt} and σ_{rupt} are the pressure and surface-tension coefficient at the moment of pore rupture). Any further alteration of the matrix provokes the pore rupture. Provided that the membrane tension does not relax significantly, during the rupture phase the pore must enlarge irreversibly since $W_{\text{surf}}(r)$ varies with r^2 but $W_{\text{edge}}(r)$ varies only with r (see Figure 3).^[17] It is beyond the scope of this work to propose a precise relationship between σ and r during the pore expansion stage. However, it seems realistic to consider that σ should not vary significantly while the membrane tightens the matrix, that is, the boundary between the cell and vesicle membranes has not yet reached the equatorial plane of the vesicle (Figure 4). Conversely,

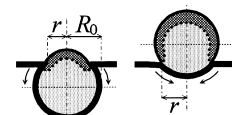


Figure 4. Schematic representation of the different topologies before and after the equatorial plane of the vesicle has been passed during the full-fusion process. The symbols are identical to those used in Figure 2, with the unaltered zones of the matrix shown lightly shaded and the swollen zones darker.

when more than half of the matrix is already exposed, it may freely expand towards the extracellular fluid and the membrane tension should relax significantly, and thus $\sigma \leq \sigma_{\text{rupt}}$. This intuitive view^[1] suggests that the pore edge energy can be neglected ($W(r) \approx -\pi\sigma r^2$, where $\sigma \approx \sigma_{\text{rupt}}$) during the first half of the full-fusion process but it dominates ($W(r) \approx +2\pi\rho_0 r$)^[20] during the second half.^[16] This imposes an accelerated expansion up to the matrix equatorial plane. Afterwards, the pore closes irreversibly, being tantamount to resorbing the remaining vesicle cupola on which the vesicle rested (Figure 4). The two phases occur at different speeds because of the different physicochemical factors involved. This view^[1] explains qualitatively the asymmetric shape of the experimentally measured $a(t)$ function.

To proceed quantitatively, we need to investigate the dynamics of the full-fusion process. We suppose that $W(r)$ is converted into viscous dissipation, Equation (1), where η_s is

$$4\pi\eta_s r(dr/dt) = -W(r) \quad (1)$$

the surface viscosity.^[21] We will consider the two halves of the full-fusion process separately since they obey different driving

forces. Equation (1) predicts that during the first half, the radius should expand exponentially at $r = r_{\text{pore}} \exp(\sigma t / 4\eta_s)$, which is clearly inconsistent with our experimental data: Figure 5a shows that the pore radius^[22] expands at an initial rate comparable to the average rate over the entire time interval, rather than being comparatively negligible if an exponential growth occurred, as is usual in pore-nucleation processes.^[21]

This establishes that surface-tension potential energy cannot be solely responsible for the rapid expansion of the initial pore. Our results are consistent with the additional dissipation of a constant energy E_0 during this process.^[23] Let γ be the value of E_0 relative to $4\pi\sigma R_0^2$, the entire surface-tension potential energy immediately after the rupture; Equation (1) thereby affords Equation (2). Integration of Equation (2)

$$2(r/R_0) \frac{d(r/R_0)}{dt} = (\sigma/2\eta_s) [(r/R_0)^2 + 4\gamma] \quad (2)$$

gives $(r/R_0) = 2\gamma^{1/2} [\exp((\sigma t)/(2\eta_s)) - 1]^{1/2}$. Figure 5b shows that this prediction agrees extremely well with the experimental measurements (Figure 5a). This is further confirmed by the remarkably good correlation shown in Figure 5c, which includes all of the 232 events which could be treated at this stage of the study. This treatment showed that $\gamma \approx 0.005$ and thus the mean value of E_0 is a minute amount of the entire surface-tension energy at the beginning of full fusion. This is, nevertheless, a few hundred $k_B T$,^[23] which explains its considerable role on the initial pore expansion rate.

When the matrix is already more than half exposed, the main driving force stems from the pore edge, $W(r) = +2\pi\rho_0 r$. Still supposing the viscous dissipation mode, Equation (1) transforms to Equation (3). This predicts that the pore radius

$$\frac{d(r/R_0)}{dt} = -\frac{\rho_0}{2R_0\eta_s} \quad (3)$$

decreases linearly with time ($r \propto -(\rho_0/2\eta_s)t$), as recently observed for transient pore closure,^[21] albeit in a very different context. This prediction agrees strikingly with the experimental variations observed for all the 596 experimental spikes treated in this study (Figure 6). The statistical treatment of these data revealed that the mean value of $2R_0\eta_s/\rho_0$ is about 7 ms. This corresponds to an average closure rate of $20 \mu\text{m s}^{-1}$, which seems realistic for a weakly stressed membrane.^[21]

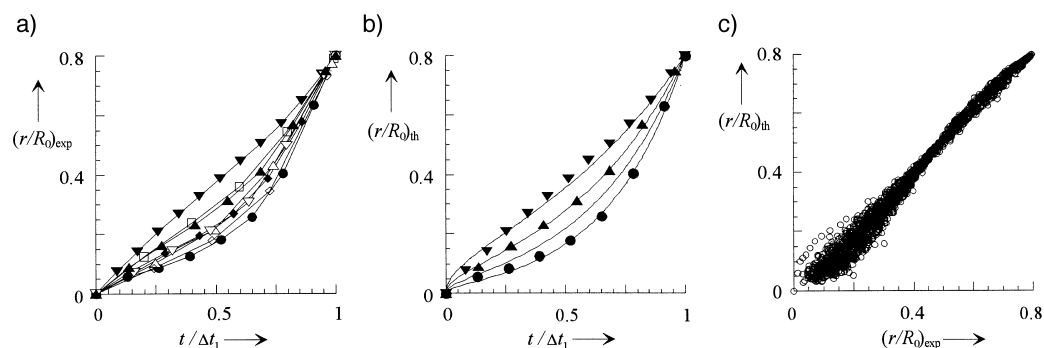


Figure 5. Variations of $r(t)/R_0$,^[22] the full-fusion pore radius relative to that of the vesicle during the first half of the full-fusion process (see the left panel of Figure 4). a) Experimental variations for eight representative events; Δt_1 is the time for which $r(\Delta t_1) = 0.8 R_0$. b) Theoretical predictions by Equation (2) for (from top to bottom) $\lg \gamma = -2, -2.5, -3, \text{ and } -3.5$. Three representative events shown in (a) are overlaid (with the same symbols) to illustrate the remarkable agreement. c) Correlation between theoretical predictions for $r(t)/R_0$ [Eq. (2)] and experimental measurements for all of the 232 events which could be treated here (correlation coefficient 0.993).

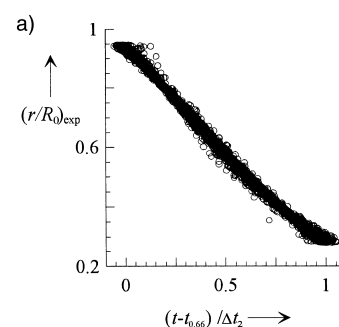


Figure 6. Correlation between theoretical predictions [Eq. (3)] and experimental variations of $r(t)/R_0$ during the second half of the full-fusion process (see the right panel of Figure 4; all of the 596 events are treated here; correlation coefficient 0.998); Δt_2 is the time required for $a(t)$ to increase from 0.66 to 0.98; $t_{0.66}$ is the time at which $a(t) = 0.66$.

The above two physicochemical and kinetic analyses and their remarkable quantitative agreement with the experimental measurements demonstrate that the dynamics of the full-fusion process is controlled by two different mechanisms, depending on the system topology. The initial events, when the matrix is still constricted by the vesicle membrane, are dictated by the surface-tension energy due to the pressure created by the restricted swelling of the vesicle. Thus, in agreement with previous conjecture by Neher on secretion without full fusion,^[15] this model indicates that small vesicles cannot achieve full fusion because they cannot develop a sufficient pressure for their membrane surface tension to overcome their pore edge resistance. Conversely, larger vesicles should always proceed irreversibly to full fusion unless the pore closes by another mechanism before its rupture point is reached (namely, as in “kiss and run” events^[14, 15]).

For the chromaffin cells investigated here, the mean initial fusion-pore duration is $t_{\text{rupt}} = (2.2 \pm 0.2) \text{ ms}$.^[1, 16] Since I_{foot} is about 5 pA, this establishes, by Faraday’s law, that to reach the rupture point, about 20000 catecholamine molecules among the 3–5 million that the matrix contains must be released.^[11, 16] Since the mean value of R_0 is about 150 nm,^[24] the depleted volume due to catecholamine efflux at t_{rupt} corresponds approximately to that of a sphere of about 25 nm radius. Assuming that the energetics of the present

vesicles can be extrapolated to other catecholaminergic vesicles,^[25] vesicles containing less than about 20000 molecules and with radii less than about 25 nm (such as small synaptic vesicles) should never reach full fusion and thereby be released only through their fusion pores. Conversely, larger ones (such as large, dense core vesicles) should always fully fuse unless another mechanism closes the pore before about 20000 molecules have been released. It is interesting that these values correspond precisely to the molecule content (~ 10000) and size (20–30 nm) of many catecholaminergic neuronal vesicles. In this perspective, these vesicles would represent the optimum balance between a nonfusional release and a maximum load of neurotransmitter.

Experimental Section

All experiments and procedures were identical to those previously described, either for the stimulation of chromaffin cells and monitoring of chronoamperometric traces,^[26, 27] or for the deconvolution of the chronoamperometric data.^[1, 16] In this study we treated 596 representative events. All events were used in the kinetic treatment of the second half of the full-fusion stage. However, to perform a significant kinetic treatment of the initial half of full fusion, several of these events could not be used, either because insufficient data points (< 5) were available in the time range of interest or because residual foot features altered the spike rise. This reduced the series to 232 meaningful events, but the data presented here are consistent with all the discarded events.

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- [1] C. Amatore, Y. Bouret, L. Midrier, *Chem. Eur. J.* **1999**, *5*, 2151–2162.
- [2] F. Helfferich, M. S. Plesset, *J. Chem. Phys.* **1958**, *28*, 418–424.
- [3] T. Tanaka, D. J. Fillmore, *J. Chem. Phys.* **1979**, *70*, 1214–1218.
- [4] J. L. Barrat, J. F. Joanny in *Advances in Chemical Physics*, Vol. XCIV (Eds.: I. Prigogine, S. Rice), Wiley, New York, **1966**, pp. 27–33.
- [5] J. Zimmerberg, M. Curran, F. S. Cohen, M. Brodwick, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 1585–1589.
- [6] P. E. Marszalek, B. Farrell, P. Verdugo, J. M. Fernandez, *Biophys. J.* **1997**, *73*, 1160–1168.
- [7] P. E. Marszalek, B. Farrell, P. Verdugo, J. M. Fernandez, *Biophys. J.* **1997**, *73*, 1169–1183.
- [8] R. M. Wightman, J. A. Jankowski, R. T. Kennedy, K. T. Kawagoe, T. J. Schroeder, D. J. Leszczyszyn, E. J. Diliberto, Jr., J. A. Near, O. H. Viveros, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10754–10758.
- [9] T. J. Schroeder, J. A. Jankowski, K. T. Kawagoe, R. M. Wightman, C. Lefrou, C. Amatore, *Anal. Chem.* **1992**, *64*, 3077–3083.
- [10] R. M. Wightman, T. J. Schroeder, J. M. Finnegan, E. L. Ciolkowski, K. Pihel, *Biophys. J.* **1995**, *68*, 383–390.
- [11] T. J. Schroeder, R. Borges, J. M. Finnegan, K. Pihel, C. Amatore, R. M. Wightman, *Biophys. J.* **1996**, *70*, 1061–1068.
- [12] E. L. Ciolkowski, K. M. Maness, P. S. Cahill, R. M. Wightman, D. H. Evans, B. Fosset, C. Amatore, *Anal. Chem.* **1994**, *66*, 3611–3617.
- [13] G. Alvarez de Toledo, R. Fernandez-Chacon, J. M. Fernandez, *Nature* **1993**, *363*, 554–558.
- [14] A. Albillos, G. Dernick, H. Horstmann, W. Almers, G. Alvarez de Toledo, M. Lindau, *Nature* **1997**, *389*, 509–512.
- [15] E. Neher, *Nature* **1993**, *363*, 497–498.
- [16] C. Amatore, Y. Bouret, E. R. Travis, R. M. Wightman, *Biochimie*, submitted.
- [17] C. Taupin, M. Dvolaitzky, C. Sauteret, *Biochemistry* **1975**, *14*, 4771–4775.
- [18] A. F. Oberhauser, J. M. Fernandez, *Biophys. J.* **1993**, *65*, 1844–1852.
- [19] C. Nanavati, J. M. Fernandez, *Science* **1993**, *259*, 963–965.
- [20] For simplicity, we still use the same notation ρ_0 for the edge-energy coefficient of the pore, although the pore considered during the full-fusion stage is necessarily bilipidic and may differ from the initial fusion pore. However, since the value of $W_{\text{edge}}(r_{\text{pore}})$ has been estimated above to be in the range of $k_B T$, that is, close to that of a

bilipidic pore of identical size (1 nm), in practical terms the two coefficients are similar even if the two pore structures may differ.

- [21] O. Sandre, L. Moreaux, F. Brochard-Wyart, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10591–10596.
- [22] The pore radius $r(t)$ is readily obtained from the experimental relative surface area $a(t)$ by $r(t)/R_0 = [1 - [1 - 2a(t)]^{1/2}]^{1/2}$.
- [23] The use of a simple pore model in our treatment neglects the fact that the connection of the cell and vesicle membranes creates a “bottle neck” invagination. Therefore, a negative additional energy (termed the Gaussian curvature energy; see, for example: U. Seifert, R. Lipowsky in *Structure and Dynamics of Membranes* (Eds.: R. Lipowsky, E. Sackmann), Elsevier, Amsterdam, **1995**, pp. 409–411, must be added to $W(r)$. The modulus of Gaussian curvature energies is predicted to be about $300 k_B T$, which compares excellently with the mean value of $E_0 \approx 120 k_B T$ obtained here. Note that considering that the edge energy of the initial fusion pore is $2.5 k_B T$, as is implicitly assumed here, would afford exactly $E_0 = 300 k_B T$.
- [24] R. E. Coupland, *Nature* **1968**, *217*, 384–388.
- [25] This extrapolation is less provocative than it may seem at first glance. Indeed, the present theory shows that the number of altered sites N_{rupt} required to provoke the full-fusion process depends only on 1) the initial pore radius r_{pore} , 2) the initial pore edge energy W_{edge} , and 3) the energy ΔE associated with one cation exchange per matrix site. Irrespective of the vesicle, W_{edge} has to be necessarily in the range of a few $k_B T$ to allow flickering, r_{pore} needs to be in the nanometer range to agree with any reasonable fusion pore architecture (either an ion channel protein or a lipidic structure), and ΔE is also expected to be a close invariant whenever the catecholamine monocations are replaced by monovalent metal cations. This ion exchange alters the compacted vesicle matrix by suppressing stabilizing hydrogen bonds and by creating weak dipole–dipole repulsions, so ΔE ought to be always in the range of a few $k_B T$ per matrix site. Thus, N_{rupt} should be closely (that is, within less than an order of magnitude) invariant for all catecholamine-releasing vesicles.
- [26] D. J. Leszczyszyn, J. A. Jankowski, O. H. Viveros, E. J. Diliberto, Jr., J. A. Near, R. M. Wightman, *J. Biol. Chem.* **1990**, *265*, 14736–14737.
- [27] J. A. Jankowski, J. M. Finnegan, R. M. Wightman, *J. Neurochem.* **1994**, *63*, 1739–1747.

Giant Artificial Ion Channels Formed by Self-Assembled, Cationic Rigid-Rod β -Barrels**

Bodo Baumeister, Naomi Sakai, and Stefan Matile*

Dedicated to Professor Koji Nakanishi
on the occasion of his 75th birthday

The interior of toroidal biomacromolecules is a privileged site for molecular recognition, translocation, and transformation.^[1–4] Giant ion channels (or nanopores) are a particularly

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appealing class of toroidal biomacromolecules because of the additional spatial compartmentalization provided by the bilayer membrane, which gives vectorial accessibility to their confined interior and allows rapid stochastic detection of intratoroidal processes.^[5–7] This unique feature has stimulated inspired expansions of intratoroidal chemistry beyond biological function and has culminated so far in stochastic recognition of organic molecules^[5] and oligonucleotides.^[6] The conceivable intriguing perspectives for future stochastic intratoroidal chemistry make the construction of synthetic nanopores highly attractive. It seemed, however, questionable whether or not large intratoroidal space of variable chemical nature could be established using small synthetic molecules^[8–17] instead of biomacromolecules.^[5–7] Here, we report direct experimental evidence for the first synthetic toroidal supramolecule capable of providing a) large, b) transmembrane, and c) functionalized intratoroidal space of d) remarkable stability.

The design of intratoroidal “nanospace” was based on electrostatic repulsion at the internal surface of rigid-rod β -barrels (Figure 1). These very recently devised synthetic

internal destructive interactions introduced to maintain central space, overcompensating constructive interactions were needed at the hydrophobic exterior. In rigid-rod β -barrel **1⁶** this situation is created by repulsive, partially protonated Lys-residues^[21–24] at the inner and attractive hydrophobic Leu residues at the outer surface of the barrel.

We thus synthesized Leu-Lys-Leu-rod **1** using our previously established, general method for the preparation of peptide-rods.^[1] The CD spectrum of **1** in uniformly-sized EYPC-SUVs was indicative of the formation of rigid-rod β -barrels.^[1, 17, 25] A similar CD spectrum obtained in CHCl_3 indicated the presence of a similar supramolecular organization of **1**, but poor solubility prevented further structural studies by less sensitive methods.^[17, 1] In clear contrast to the previously described self-assembly of Leu-rod **2** into ionophoric “minimalist” β -barrels **2²** no evidence for β -barrel **1⁶**

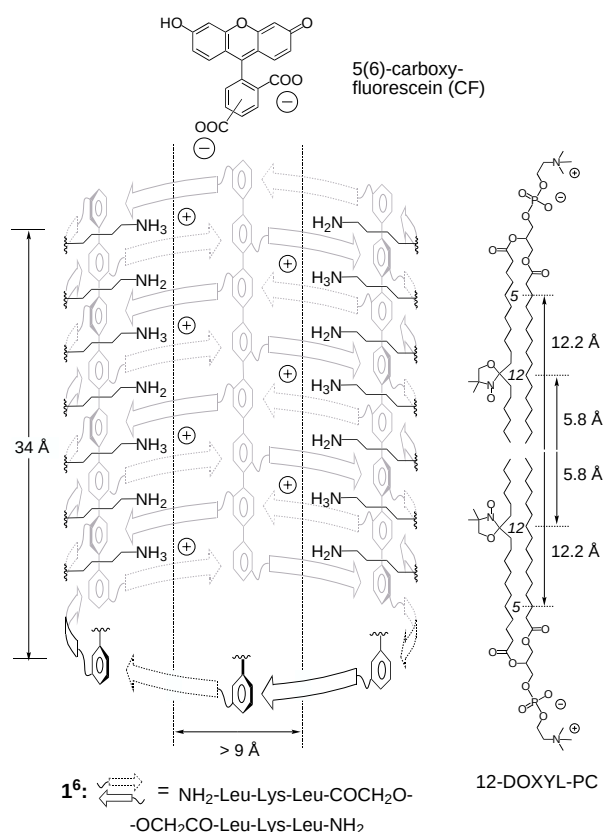
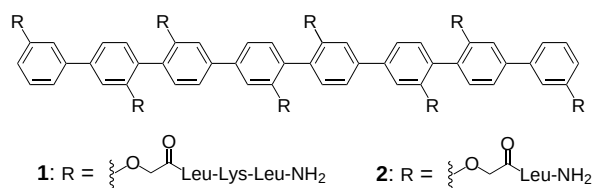


Figure 1. Schematic structure of rigid-rod β -barrel **1⁶** formed by Leu-Lys-Leu-rods **1** in EYPC bilayers and the probes used to determine the transmembrane barrel orientation, barrel size, and internal diameter.^[27] The distribution of intratoroidal amines and ammonium cations in **1⁶** is unknown and depicted arbitrarily to illustrate the concept.

nanobarrels^[1, 17] are composed of preorganizing rigid-rod scaffolds^[18–20] that are connected through short, antiparallel β -sheets^[16, 21–24] formed by interdigitating lateral peptide strands. To assure supramolecular organization, despite the

was found in other solvents and by electrospray ionization mass spectrometry (ESI-MS).^[17] This observation is also in excellent agreement with the operation of destructive internal electrostatic interactions.

The interaction of rigid-rod β -barrel **1⁶** with hydrophobically matching EYPC bilayers was studied by fluorescence depth quenching; this method provides the most precise information on binding to and orientation in bilayer membranes at relevant concentrations of around $1\ \mu\text{M}$ or less.^[26–30] For orientational studies using depth quenching, the emission intensities of fluorophore **1** were measured in EYPC-SUVs containing either 8.7 % of 12-DOXYL-PC or 5-DOXYL-PC. These labeled membranes thus carry quencher Q_1 and Q_2 at defined distances d_1 (5.8 Å) and d_2 (12.2 Å) from the center of the EYPC bilayer, respectively (Figure 1).^[26, 27] Since collisional quenching is proportional to the average distance between the fluorophore and the quencher,^[26, 27] a reduced quenching efficiency of Q_1 relative to Q_2 ($Q_1 < Q_2$) indicates the location of the fluorophore near the membrane surface.^[28, 29] On the other hand, $Q_1 > Q_2$ is consistent with a central location,^[28] while $Q_1 \sim Q_2$ directly reveals a transmembrane orientation in the unique case of rigid-rod fluorophores.^[29, 30] The latter situation ($Q_1 \sim Q_2$, Figure 2a) was found for **1** (namely, rigid-rod β -barrel **1⁶**, Figure 1). The changes of oligo(*p*-phenylene) emission as a function of relative concentrations and time further revealed that about sixty lipids per rod **1** are needed to assure complete incorporation (Figure 2c) and that this process is finished in less than a minute (Figure 2b).^[29]

These findings provide a concise description of the interaction of polyamine **1** with lipid bilayers and may further contribute to clarifying the question as to whether or not polyamines in general are capable of spanning bilayer membranes or rather act by supramolecular disorganization

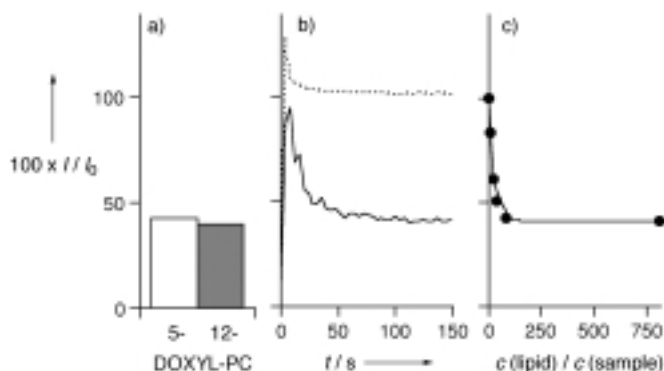


Figure 2. Fluorescence quenching of the emission intensity of oligo(*p*-phenylene) **1** in spin-labeled EYPC-SUVs ($\lambda_{\text{ex}} = 328$ nm, $\lambda_{\text{em}} = 390$ nm, 10 mM potassium phosphate buffer, 100 mM KCl, pH 6.4). a) Emission intensities (I/I_0) of **1** (100 nM) with 0.25 mM of EYPC-SUVs containing 8.7% 5- and 12-DOXYL-PC (I) relative to identical measurements with unlabeled EYPC-SUVs (I_0). b) Time course of the emission intensity of **1** (100 nM) after addition to 0.25 mM of unlabeled (---) and 12-DOXYL-PC-labeled EYPC-SUVs (—). c) Dependence of the quenching of **1** by 12-DOXYL-PC as a function of lipid/rod ratio at constant lipid concentration.^[29]

at the membrane/water interface.^[8, 30, 31] Namely, the above direct orientational evidence proved that the permanent location of polyamines within the hydrophobic core of bilayer membranes is possible at biologically relevant concentrations.

Insights into the chemical nature of the intratoroidal space of barrel **1**⁶ were obtained using EYPC-SUVs loaded with self-quenching 5(6)-carboxyfluorescein (CF).^[29] Expanded β -barrels **1**⁶ (but not contracted β -barrels **2**²)^[17] were capable of mediating rapid CF-efflux at nanomolar concentrations as judged from the increase in the CF-emission intensity as a function of time (Figure 3). Facile efflux of anionic fluoro-

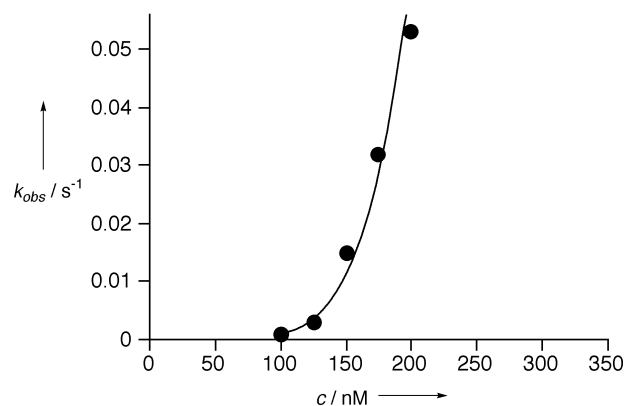


Figure 3. Observed rate constants of CF-efflux as a function of the concentration of oligo(*p*-phenylene) **1** (EYPC-SUVs, 10 mM HEPES, 50 mM CF_{in}, 10 mM NaCl_{in}, 107 mM NaCl_{out}, pH 7.4). The solid line represents the best fit curve ($n = 6.1 \pm 0.7$). Efflux rates were obtained from observed changes in fluorescence intensity ($I_t - I_0$)/($I_\infty - I_0$) of EYPC-SUV-entrapped CF as a function of time ($\lambda_{\text{em}} = 514$ nm, $\lambda_{\text{ex}} = 492$ nm).

phores of more than 9 Å minimal diameter implied the formation of at least hexameric barrels ($d \sim 20$ Å; Cerius2/Insight II Discover)^[1] with a cationic interior.

Kinetic information on transmembrane ion flux directly relates to the suprastructure of the mediating nanopore.

Namely, the dependence of the CF-efflux rates on pore concentration is given by Equation (1), where K_D is the dissociation constant of supramolecule **1**^{*n*} composed of *n* monomers **1**, k_0 the rate constant for CF-efflux in the absence of **1**, and k_{int} an intrinsic rate constant.^[8]

$$k_{\text{obs}} = k_0 + k_{\text{int}}[\text{monomer}]^n/K_D \quad (1)$$

Application of Equation (1) to CF-efflux mediated by rigid-rod β -barrel **1**⁶ revealed a sixth-order dependence of k_{obs} on the concentration of peptide-rod **1** (Figure 3). This corroborated the self-assembly of **1** into active hexamers, namely, the rigid-rod β -barrel **1**⁶ (Figure 1).

The presence of rigid-rod β -barrel **1**⁶ in black lipid membranes (EYPC-BLMs) caused the fluctuating appearance of single channel currents of 178 pA at –50 mV applied voltage (Figure 4).^[32] Their unusually high magnitude is

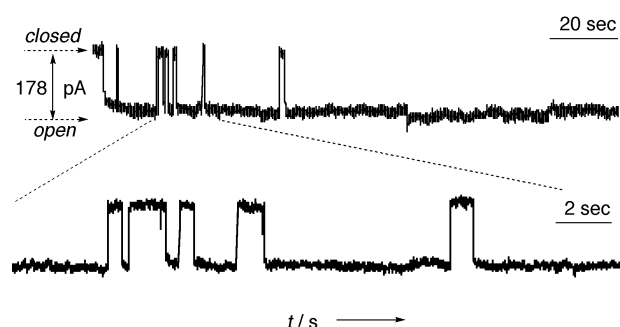


Figure 4. Conductance of EYPC-BLMs at –50 mV as a function of time in the presence of oligo(*p*-phenylene) **1**.

consistent with a minimal diameter of nanopore **1**⁶ that is in the range of the paradigmatic natural β -barrel formed by α -hemolysin, a self-assembled polypeptide containing 293 amino acids.^[5–7] The “infinite” lifetime of these high-conductance, supramolecular synthetic nanopores exceeded those of the best low-conductance, unimolecular artificial ion channels by far.^[14] Together with the absence of transient “bursts” frequently seen with artificial ion channels,^[12, 13, 15, 33] this result revealed the remarkable stability of the roughly ohmic nanopore **1**⁶.

In summary, a combination of the unique advantages of self-assembled, rigid-rod oligo(*p*-phenylene)s with tunable electrostatic repulsion was used to design artificial nanopores of remarkable stability and intratoroidal amine/ammonium functional groups. Direct evidence for the stoichiometry, transmembrane orientation, large internal diameter, and long lifetime of rigid-rod β -barrels **1**⁶ was obtained under comparable experimental conditions. These superb properties strongly suggest that future ion-channel engineering within functionalized intratoroidal space will not be restricted to biomacromolecules.

Experimental Section

Leu-Lys-Leu-rod **1** was obtained from tripeptide H-Leu-Lys(Boc)-Leu-NH₂ (Boc = *tert*-butoxycarbonyl) that was made in four steps (overall 23%) from commercial amino acid derivatives (Bachem, Calbiochem-Novabiochem) as described for other sequences.^[1] H-Leu-Lys(Boc)-Leu-

NH₂ was coupled with benzotriazole-1-yl-oxy-tris(pyrrolidinophosphonium)hexafluorophosphate/*N,N*-diisopropylethylamine to the octiphenylene scaffold (55%) and deprotected with trifluoroacetic acid to give **1** (quant.) following previously described protocols without significant changes.^[1] Selected data: H-Leu-Lys(Boc)-Leu-NH₂: [α]_D²⁰ = −29.5 (*c* = 1.00 in MeOH); m.p. 179.5–180.1 °C; elemental analysis calcd for C₂₅H₄₅N₅O₅: C 58.57, H 9.62, N 14.85; found: C 58.47, H 9.65, N 14.71. Leu-Lys(Boc)-Leu-rod (**1**-Boc): ESI-MS (MeOH): *m/z* (%): 1633 (100) [*M*+3Na]³⁺, 2438 (85) [*M*+2Na]²⁺. Leu-Lys-Leu-rod (**1**): CD (EYPC-SUVs, pH 6.4): λ_{max} [nm] ($\Delta\epsilon_{\text{max}}$ [M^{−1}cm^{−1}]) = 333 (+6.4), 307 (−5.1), 252 (−3.7), 237 (+32.6), 215 (−56.0); ESI-MS (MeOH): *m/z* (%): 1008 (100) [*M*+4H]⁴⁺, 1344 (13) [*M*+3H]³⁺. Before use, spectroscopically pure **1** was repurified by RP-HPLC (YMC pack ODS-A, 10 × 250 mm coupled with a Jasco PU-980 pump and a Jasco UV-970 UV/Vis detector, acetonitrile: methanol:trifluoroacetic acid = 49.5:49.5:1, *R*_t = 4.10 min) until constant activity was confirmed (dye efflux).

Fluorescence depth quenching (Figure 2) and dye efflux (Figure 3) were performed as described.^[29] To measure the conductances, a bilayer of EYPC was formed by painting a solution of EYPC (Northern Lipids Inc.) in *n*-decane (33 mg mL^{−1}) containing 0–0.5 mol% of **1** on an orifice (*d* = 150 μm) in a polystyrene cup separating two chambers of a bilayer apparatus (BCH-13, Warner Instrument Corp.). These chambers contained 1 mL saline buffer (5 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 2 M NaCl, pH 7.4), a magnetic stirring bar, and a glass KCl (1 M) agar bridge connection to Ag/AgCl electrodes. Oligophenylenes **1** (0.8 mmol in MeOH, 0–30 μL) were added to the stirred *cis* compartment. Formation of EYPC-BLM in the presence of **1**, and addition of **1** to final EYPC-BLM gave comparable results. Currents were recorded at different holding potentials (*trans* at *I* = 0) in a home-made Faraday cage with a bilayer clamp amplifier (BC-525c, Warner Instrument Corp.), low-pass filtered with an 8-pole Bessel filter at 1 kHz (LPF-8, Warner Instrument Corp.), converted (Digipack 1200-2, Axon Instruments), and sampled at 2 kHz by computer. Data were analyzed with pClamp 8.0 software (Axon Instruments).

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- [1] B. Baumeister, S. Matile, *Chem. Eur. J.* **2000**, *6*, 1739, and references therein.
- [2] G. Beste, F. S. Schmidt, T. Stibora, A. Skerra, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1898.
- [3] C. F. Barbas III, A. Heine, G. Zhong, T. Hoffmann, S. Gramatikova, R. Björnstedt, B. List, J. Anderson, E. A. Stura, I. A. Wilson, R. A. Lerner, *Science* **1997**, *278*, 2085.
- [4] P. C. Babbitt, J. A. Gerlt, *J. Biol. Chem.* **1997**, *272*, 30591.
- [5] L.-Q. Gu, O. Braha, S. Conlan, S. Cheley, H. Bayley, *Nature* **1999**, *398*, 686.
- [6] M. Akeson, D. Branton, J. J. Kasianowicz, E. Brandin, D. W. Dreame, *Biophys. J.* **1999**, *77*, 3227.
- [7] P.-G. de Gennes, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 7262.
- [8] S. Otto, M. Osifchin, S. L. Regen, *J. Am. Chem. Soc.* **1999**, *121*, 7276; S. Otto, M. Osifchin, S. L. Regen, *J. Am. Chem. Soc.* **1999**, *121*, 10440.
- [9] G. R. Dieckmann, J. D. Lear, Q. Zhong, M. L. Klein, W. F. DeGrado, K. A. Sharp, *Biophys. J.* **1999**, *76*, 618.
- [10] Z. Qi, M. Sokabe, K. Donowaki, H. Ishida, *Biophys. J.* **1999**, *76*, 631.
- [11] M. G. Fritz, P. Walde, D. Seebach, *Macromolecules* **1999**, *32*, 574.
- [12] J. de Mendoza, F. Cuevas, P. Prados, E. S. Meadows, G. W. Gokel, *Angew. Chem.* **1998**, *110*, 1650; *Angew. Chem. Int. Ed.* **1998**, *37*, 1534.
- [13] T. M. Fyles, D. Loock, X. Zhou, *J. Am. Chem. Soc.* **1998**, *120*, 2997.
- [14] E. Abel, E. M. Maguire, O. Murillo, I. Suzuki, S. L. DeWall, G. W. Gokel, *Chem. Commun.* **1997**, 1145.
- [15] N. Voyer, L. Potvin, E. Rousseau, *J. Chem. Soc. Perkin Trans. 2* **1997**, 1469.
- [16] J. D. Hartgerink, T. D. Clark, M. R. Ghadiri, *Chem. Eur. J.* **1998**, *4*, 1367.
- [17] N. Sakai, N. Majumdar, S. Matile, *J. Am. Chem. Soc.* **1999**, *121*, 4294.
- [18] P. F. H. Schwab, M. D. Levin, J. Michl, *Chem. Rev.* **1999**, *99*, 1863.
- [19] A. J. Berresheim, M. Müller, K. Müllen, *Chem. Rev.* **1999**, *99*, 1747.

- [20] R. E. Martin, F. Diederich, *Angew. Chem.* **1999**, *111*, 1440; *Angew. Chem. Int. Ed.* **1999**, *38*, 1351.
- [21] E. K. Koepf, M. H. Petrassi, M. Sudol, J. W. Kelly, *Protein Sci.* **1999**, *8*, 841.
- [22] F. A. Syud, J. F. Espinosa, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 11577.
- [23] M. S. Searle, S. R. Griffiths-Jones, H. Skinner-Smith, *J. Am. Chem. Soc.* **1999**, *121*, 11615.
- [24] T. Kortemme, J. Ramirez-Alvarado, L. Serrano, *Science* **1998**, *281*, 253.
- [25] EYPC-SUVs: small unilamellar vesicles composed of egg yolk phosphatidylcholine; 5-DOXYL-PC: 1-palmitoyl-2-stearoyl(5-DOXYL)-*sn*-glycero-3-phosphocholine; 12-DOXYL-PC: 1-palmitoyl-2-stearoyl(12-DOXYL)-*sn*-glycero-3-phosphocholine (DOXYL = 4,4-dimethyl-3-oxazolinyl).
[26] J. Ren, S. Lew, Z. Wang, E. London, *Biochemistry* **1997**, *36*, 10213.
[27] A. S. Ladokhin, *Methods Enzymol.* **1997**, *278*, 462.
[28] C. Ni, S. Matile, *Chem. Commun.* **1998**, 755.
[29] L. A. Weiss, N. Sakai, B. Ghebremariam, C. Ni, S. Matile, *J. Am. Chem. Soc.* **1997**, *119*, 12142.
[30] N. Sakai, S. Matile, *Chem. Eur. J.* **2000**, *6*, 1731.
[31] N. Sakai, S. Matile, *Tetrahedron Lett.* **1997**, *38*, 2613.
[32] B. Hille, *Ionic Channels of Excitable Membrane*, 2nd ed., Sinauer, Sunderland, MA, **1992**.
[33] N. Sakai, C. Ni, S. M. Bezrukov, S. Matile, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2743.

A Novel Catalytic System for the Mannich-Type Reaction of Silyl Enolates: Stereoselective Synthesis of β -Aminoketones**

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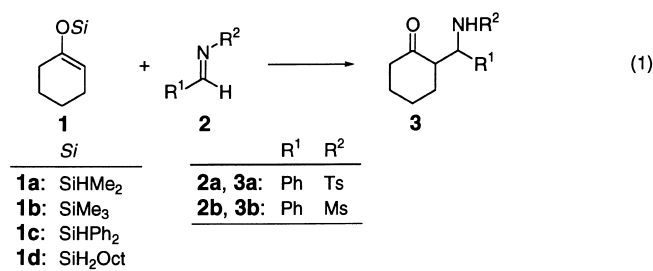
The Lewis acid promoted addition of ester silyl enolates to imines and iminium salts enables the stereoselective construction of β -aminoesters and β -lactams.^[1] However, it is not widely known that the Mannich-type reaction with ketone silyl enolates reveals high stereoselectivity in terms of the relative configuration between the newly formed chiral centers.^[2, 3] Here, we describe that dimethylsilyl (DMS) enolates react with *N*-sulfonylimines in the presence of H₂O and a catalytic amount of a base to afford β -aminoketones with high levels of diastereoselectivity.

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

Treatment of imine **2a** with DMS enolate **1a**, H₂O, and *i*Pr₂NH (**2a**:**1a**:H₂O:amine = 1:1.2:0.6:0.024) in DMF at room temperature for 1 h gave **3a** (*anti*:*syn* = 94:6) in 95 % yield [Eq. (1)].^[4] The yield dropped to 9–16 % without H₂O and/or



*i*Pr₂NH. Other amines and aqueous NaOH also worked as efficient catalysts for this process (base, yield, *anti*:*syn*: pyridine, 82 %, 93:7; Et₃N, 91 %, 83:17; 2M NaOH (aq), 91 %, 68:32). The H₂O-*i*Pr₂NH-mediated reaction proceeded even at –78 °C to achieve higher *anti* selectivity (d.r. = 99:1). Trimethylsilyl enolate **1b** was insensitive to **2a** even in the presence of H₂O/*i*Pr₂NH. In contrast, diphenylsilyl and octylsilyl enolates, **1c** and **1d**, smoothly added to **2a** at room temperature (enolate, yield, *anti*:*syn*: **1c**, 98 %, 95:5; **1d**, 91 %, 91:9). *N*-Mesylimine **2b** (mesyl = methanesulfonyl) as well underwent the base-catalyzed Mannich-type reaction with **1a** to give **3b** in 92 % yield (*anti*:*syn* = 88:12); however, other imines (R¹ = Ph, R² = Ph, Bn, NHTs, OH, SPh, and SPh) gave no adduct. The high reactivity of *N*-sulfonylimines to **1a** arises from their high electrophilicity as a result of the presence of the sulfonyl group.

The use of aromatic and α,β -unsaturated imines led to high yield and selectivity (Table 1), while aliphatic imines **2i, j** (R¹ = *c*Hex, *t*Bu, R² = Ts) did not react with **1a** at all. Acyclic

Table 1. Base-catalyzed reaction of **1a** with imines **2**.

Entry	Imine R ¹ (R ² = Ts)	T [°C]	t [h]	β -Aminoketone 3 Yield [%]	<i>anti</i> : <i>syn</i> ^[a]
1	2a Ph	–78	18	3a 91	99:1
2	2c <i>p</i> -MeC ₆ H ₄	–50	9	3c 88	99:1
3	2d <i>p</i> -MeOC ₆ H ₄	–50	18	3d 95	99:1
4	2e <i>p</i> -NCC ₆ H ₄	–50	18	3e 90	95:5
5	2f <i>p</i> -O ₂ NC ₆ H ₄	–50	18	3f 90	97:3
6	2g 2-furyl	–78	24	3g 88	98:2
7	2h (<i>E</i>)-PhCH = CH	–78	9	3h 82	96:4

[a] Determined by HPLC analysis.

silyl enolates **4a–e** and ketene silyl thioacetal **4f** also underwent reaction with **2a** (Table 2). In all cases, *anti*-selectivity was observed irrespective of the geometry of the enolates. The low yield in entry 2 (Table 2) appeared to be a result of the low reactivity of **4b**, which has no substituent at the position of electrophilic attack,^[5] as well as it undergoing hydrolysis. Indeed, the use of D₂O instead of H₂O to reduce the rate of hydrolysis raised the yield to 58 %.

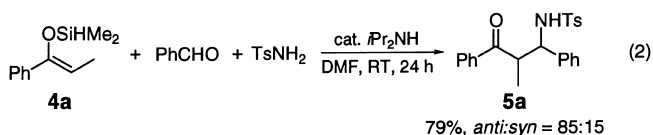
A competitive reaction of **1a** with **2a** versus PhCHO (**1a**:**2a**:PhCHO = 1.2:1:1) proved that **1a** activated by H₂O and *i*Pr₂NH has high reactivity to **2a** (**3a**, 96 %; aldol adduct,

Table 2. Base-catalyzed reaction of enolates **4** with **2a**.

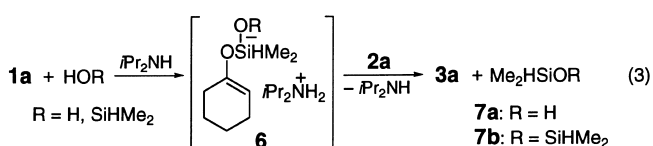
Entry	Silyl enolate 4 R ³ R ⁴ E:Z	T [°C]	t [h]	β -Aminoketone 5 Yield [%]	<i>anti</i> : <i>syn</i> ^[a]
1	4a Ph Me <2:98	–78	24	5a	98 99:1
2	4b Ph H –	–78	67	5b	27 –
3	4c Et Me 67:33	–78	24	5c	79 92:8
4	4c Et Me 20:80	–78	24	5c	97 90:10
5 ^[b]	4d <i>i</i> Pr Me 44:56	–50	24	5d	84 90:10
6 ^[b]	4e <i>t</i> Bu Me <2:98	–50	48	5e	88 93:7
7 ^[b]	4f <i>t</i> BuS Me 8:92	–78	18	5f	98 95:5

[a] Determined by HPLC or ¹H NMR (entries 5 and 7) analysis. [b] Increased amounts of reagents were used: **2a**:**4**:H₂O:*i*Pr₂NH = 1:2:1:0.04.

<1 %).^[6] From this observation, it occurred to us that a three-component coupling reaction among aldehydes, TsNH₂, and DMS enolates could be realized with only a catalytic amount of *i*Pr₂NH since the condensation between aldehydes and TsNH₂ into imines yields H₂O.^[7] As expected, the *i*Pr₂NH-catalyzed reaction of PhCHO, TsNH₂, and **4a** (PhCHO:TsNH₂:**4a**:amine = 1:1:2:0.02) gave β -aminoketone **5a** in good yield with no aldol adduct [Eq. (2)].



The H₂O-*i*Pr₂NH-catalyzed reaction of **1a** with **2a** in [D₇]DMF followed by ¹H NMR analysis revealed that **3a** and (Me₂HSi)₂O (**7b**) were formed in an approximately 2:1 molar ratio before aqueous work up. This observation suggests the possibility of the following catalytic cycle [Eq. (3)]: 1) **1a** reacts with H₂O and *i*Pr₂NH to form



ammonium silicate **6** as an intermediate, 2) the addition of **6** to **2a** provides **3a** along with *i*Pr₂NH and silanol **7a**, and 3) **7a** also works as an activator of **1a** to form disiloxane **7b**. The intermediate arising from **1a** might be a naked enolate bearing a diisopropylammonium ion as a counterion. However, the low reactivity to aldehydes and the change in diastereoselectivity with **1a**, **1c**, and **1d** support the presence of **6** rather than a naked enolate.^[8] The observed difference in reactivity between **1a** and **1b** is attributable to the ease of oxygen coordination in the formation of **6**.^[4] The origin for the *anti*-selective addition of DMS enolates is unclear, but it can be rationalized by an antiperiplanar transition-state model disposing tosyl and R⁴ groups on the opposite sides, which seems to be energetically favorable in view of nonbonding

interaction and dipole–dipole repulsion between C=O and C=N bonds.^[9]

In conclusion, we have found a unique catalytic system in which DMS enolates activated by *i*Pr₂NH and H₂O exhibit high reactivity to *N*-tosylimines. To the best of our knowledge, the base-catalyzed Mannich-type reaction of metal enolates is unprecedented. The present reaction provides an efficient method for the stereoselective synthesis of β -aminocarbonyl compounds, which are versatile building blocks for the synthesis of numerous, biologically significant compounds.

Experimental Section

Typical procedure (entry 1 in Table 1): **1a** (94 mg, 0.60 mmol) and *i*Pr₂NH (1.6 μ L, 12 μ mol) were added to a solution of **2a** (130 mg, 0.50 mmol) and H₂O (5.4 mL, 0.30 mmol) in DMF (1.0 mL) at -78°C . After 18 h, the resultant mixture was quenched with 2 M aqueous HCl (10 mL), then neutralized with saturated aqueous NaHCO₃. The aqueous phase was extracted with ethyl acetate and the combined organic phases dried over Na₂SO₄ before being evaporated. Purification of the residue by column chromatography on silica gel (hexane:ethyl acetate, 4:1) afforded **3a** (166 mg, 91 %). Further details can be found in the Supporting Information.

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- [1] a) M. Arend, R. Westermann, N. Risch, *Angew. Chem.* **1998**, *110*, 1096–1122; *Angew. Chem. Int. Ed.* **1998**, *37*, 1044–1070; b) E. F. Kleinman in *Comprehensive Organic Synthesis*, Vol. 2 (Eds.: B. M. Trost, I. Fleming), Pergamon, Oxford, **1991**, pp. 893–951.
- [2] a) E. G. Nolen, A. Allocco, M. Broody, A. Zuppa, *Tetrahedron Lett.* **1991**, *32*, 73–74; b) A. Zarghi, M. R. Naimi-Jamal, S. A. Webb, S. Balalaie, M. R. Saidi, J. Ipaktschi, *Eur. J. Org. Chem.* **1998**, 197–200.
- [3] M. Arend, N. Risch, *Angew. Chem.* **1995**, *107*, 2861–2862; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2639–2640.
- [4] For our previous report on the aldol reaction of DMS enolates, see K. Miura, H. Sato, K. Tamaki, H. Ito, A. Hosomi, *Tetrahedron Lett.* **1998**, *39*, 2585–2588.
- [5] J. Burfeindt, M. Patz, M. Müller, H. Mayr, *J. Am. Chem. Soc.* **1998**, *120*, 3629–3634.
- [6] Imino-selective addition of enolates: S. Kobayashi, S. Nagayama, *J. Am. Chem. Soc.* **1997**, *119*, 10049–10053.
- [7] This type of three-component coupling reaction has been achieved using an acid catalyst: a) S. Kobayashi, T. Busujima, S. Nagayama, *Synlett* **1999**, 545–546; b) T. Akiyama, J. Takaya, H. Kagoshima, *Synlett* **1999**, 1045–1048.
- [8] Naked enolate anions generated from silyl enolates by the action of a fluoride ion exhibit high reactivity to benzaldehyde: a) E. Nakamura, M. Shimizu, I. Kuwajima, J. Sakata, K. Yokoyama, R. Noyori, *J. Org. Chem.* **1983**, *48*, 932–945; b) R. Noyori, I. Nishida, J. Sakata, *J. Am. Chem. Soc.* **1983**, *105*, 1598–1608.
- [9] C. Gennari in *Comprehensive Organic Synthesis*, Vol. 2 (Eds.: B. M. Trost, I. Fleming), Pergamon, Oxford, **1991**, pp. 629–660.

An Unprecedented Hexapotassium-Hexamagnesium 24-Membered Macrocyclic Amide: A Polymetallic Cationic Host to Six Monodeprotonated Arene Anions**

Philip C. Andrews, Alan R. Kennedy, Robert E. Mulvey,* Colin L. Raston, Brett A. Roberts, and René B. Rowlings

In memory of Ron Snaith[†]

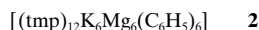
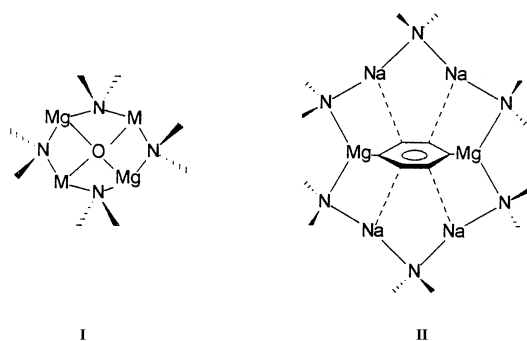
We are currently developing a new concept in macrocyclic host–guest chemistry of amide-supported heterodimetallic cationic rings which formally act as hosts to anionic guests.^[1–3] This idea emerged from our investigations into the effects of mixing an alkali-metal amide with a magnesium bis(amide). While some of the reaction mixtures studied follow a straightforward path leading to simple heterodimetallic compositions,^[4, 5] others take an unexpected turn to behave as powerful oxygen scavengers or as regioselective dideprotonating agents to yield two distinct categories of macrocyclic complex. One type of complex is the eight-membered [(MNMgN)₂]²⁺ (M = Li, Na, K) ring systems with oxo O^{2–} or peroxo (O₂)^{2–} cores;^[1] this first category has the generalized structure **I** (shown with the oxo core). We refer to this type as “inverse crown ether” complexes, on account of their inverse relationship to conventional crown ether complexes. The single potassium^[2] example **1** is strictly a poly(inverse crown ether) complex since its (KNMgN)₂ ring is not discrete (unlike the (LiNMgN)₂ or (NaNMgN)₂ analogues), but is linked into chains through intermolecular K $\cdots$ CH₃(SiMe₂) agostic interactions.^[6] The second category have the generalized structure **II**, which are twelve-membered [(NaNMgNNaN)₂]²⁺ ring systems with dianionic [C₆H₃(CH₃)₂]^{2–} or (C₆H₄)^{2–} cores derived from toluene or benzene, respectively.^[2] We reveal herein the latest and most remarkable category of macrocyclic complexes yet discovered in this special family of heterodimetallic compounds. Expecting structures akin to that of **II** on replacing sodium by a larger alkali metal, our synthesis and crystallographic characterization of the new potassium 2,2,6,6-tetramethylpiperidide (tmp) complexes **2** and **3** have, in contrast, uncovered an unprecedented twenty-four membered [(KNMgN)₆]⁶⁺ ring system, which acts as a polymetallic host to six *singly* deprotonated arene anions.

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[†] Ron Snaith was a close personal friend of R.E.M.; this dedication is in recognition of his inspired teaching and his many outstanding contributions to s-block chemistry.



In the first stage of the preparation of **2** and **3**, three equivalents of the parent amine tmpH are added to a 1:1 stoichiometric mixture of *n*BuK^[7] and Bu₂Mg. This generates an in situ mixture of K(tmp)/Mg(tmp)₂.^[8] This mixture smoothly converts benzene or toluene (when added in a large excess) into the new macrocyclic complexes **2** and **3**, respectively. The isolated solids of **2** and **3** each contain variable amounts of arene solvent as molecules of crystallization which complicate yield calculations; however, using the formulas obtained for the crystals studied by X-ray diffraction (**2**·5.07 C₆H₆ and **3**·2.15 C₆H₅CH₃), yields of 72.2% and 80.6%, respectively, are estimated. The substantially superior yields to that obtained previously for **II** (at best 27%) provide **2** and **3** in sufficient quantity to allow their reaction chemistry to be studied in detail.

In gross geometrical terms, **2** and **3** are essentially isostructural. For brevity, and because of disorder of the toluene groups within the latter structure, only details of the former^[9] are reported here. Figure 1 shows the molecular structure of **2**.

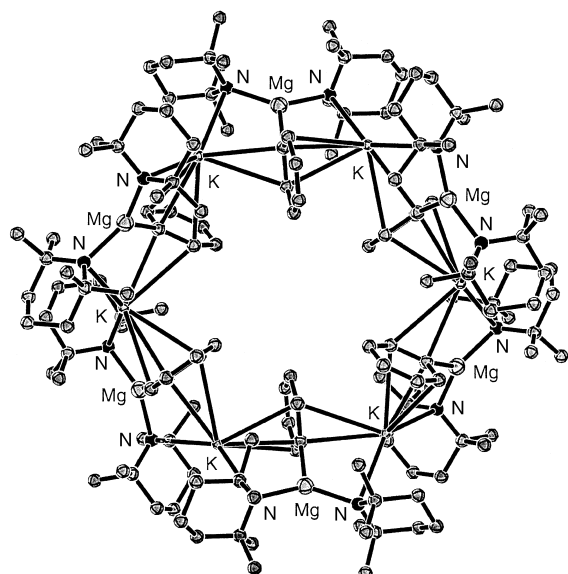


Figure 1. Molecular structure of **2** without hydrogen atoms and solvent molecules of crystallization. The (KNMgN)₆ ring is clearly labeled.

Its 24-membered ring comprises alternating metal and nitrogen atoms in KNMgN sections, onto each a phenyl group is secured through a combination of an *ipso* C–Mg σ bond and C–K π interactions. This large ring is severely puckered (Figure 2): Although the six K⁺ ions and six *ipso* C atoms

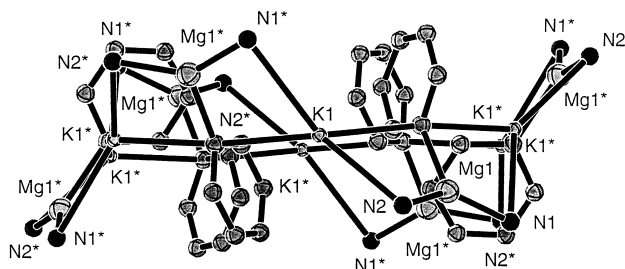


Figure 2. Alternative view of **2** emphasising the severe puckering of the twenty-four membered (KNMgN)₆ ring. Selected bond lengths [Å] and angles [°]: K1–N1* 3.191(4), K1–N2 3.090(4), Mg1–N1 2.016(4), Mg1–N2 2.022(4); N1*–K1–N2 141.5(1), C19–K1–C19* 124.9(2), N1*–K1–C19 123.3(2), N2–K1–C19 69.5(1), N1*–K1–C19* 67.7(1), N2–K1–C19* 139.3(2), N1–Mg–N2 136.7(2), N1–Mg–C19 110.3(2), N2–Mg–C19 113.0(2), where * is *y*, –*x* + *y*, –*z*.

approximate to a plane (RMS deviation 0.1379), the Mg and N atoms straddle this plane. Amide bridging within a large Mg ring system has previously been observed in the homonuclear dodecamer [(EtMgN(H)Dipp)₁₂] (Dipp = 2,6-diisopropylphenol),^[10] but it bears little resemblance otherwise to the structure of **2**. Mean K–N and Mg–N bond lengths in **2** are longer (3.141 Å) and slightly shorter (2.019 Å), respectively, than their counterparts in **1** (2.819 and 2.044 Å). Mimicking the arrangement found in the monoarene structure **II**, all the metal atoms in **2** project inwards towards the ring core—resulting in exocyclic bond angles of less than 180°, specifically 141.49(12)° at K and 136.72(18)° at Mg—while the nitrogen atoms project outwards, resulting in acute endocyclic bond angles (mean 89.32°). The interatomic distances involved suggest that the hapticity of the phenyl rings with respect to the K atoms is $\mu\text{-}\eta^3\text{:}\eta^2$, that is, three C atoms (one *ipso*, two *ortho*) on one face and two C atoms (one *ipso*, one *ortho*) on the opposing face engage in π interactions with the K atom projecting over that face at distances of 3.009(5), 3.246(5), 3.210(5), 3.083(5), and 3.354(5) Å, respectively (all other K⋯C(Ph) distances exceed 3.6 Å). The distorted trigonal planar geometry at the Mg atom is completed by the aforementioned C–Mg bond, the length of which (2.196(6) Å) is equivalent to that (mean) found in **II**. It may be significant that here, as in **II**, the Mg atom occupies the same in-plane site on the arene ring as that formerly occupied by the lost proton. One might logically assume from this observation that, in the absence of any major structural reorganization, the arene deprotonation takes place at the less reactive Mg center rather than at the more reactive K center. Unfortunately, however, as yet we have not been successful in isolating the intermediate that performs this deprotonation. Interestingly, a mirror is found in the situation for the toluene-based dianions of **II**, in which the deprotonation of the toluene molecules in **3** occurs selectively on the ring (either at *meta* or *para* positions), to leave the more acidic methyl

substituent intact. Conventional homometallic bases would conversely be expected to attack the latter group to generate resonance-stabilized benzyl carbanions.^[11] Presumably operating through a special ring templating effect,^[12] the active base(s) involved in the formation of **2** and **3** is a product of the heterodimetallic synergism taking place within the reaction mixture. In this respect, a parallel can be drawn with the chemistry of LiR·KOR' superbases,^[13] the mixed-metal reagents frequently utilized by synthetic organic chemists. More than thirty years since the first such application of superbases,^[14] their mechanistic intricacies still remain to be fully unravelled, although it is established that the participation of the two distinct metal atoms is essential to the enhanced performance of superbases over that of analogous homometallic bases. Logic dictates that the mixed-metal templating ring involved in the formation of **2** and **3** must be larger and have more breadth than its counterpart in **II** given that only one end of the arene molecule experiences deprotonation, implying that the "other end" lies distant from a metal center.

Experimental Section

2 or **3**: Preparations were carried out in Schlenk tubes under a protective atmosphere of dry, oxygen-free argon. Freshly prepared but unrefined *n*BuK (15.1 or 12.3 mmol) was suspended in hexane (30 mL) and subjected to ultrasound for 5 minutes until a fine brown suspension formed. To this was added an equimolar amount of dibutylmagnesium (15.1 or 12.3 mmol, in heptane) resulting in the formation of a congealed brown/cream mass. Three molar equivalents of 2,2,6,6-tetramethylpiperidine (45.3 or 36.9 mmol) were then added. A slightly exothermic reaction ensued as most of the solid dissolved in the solvent mixture. Fine particulates were removed by filtration through Celite and the orange-brown filtrate was concentrated in vacuo to half of its original volume. At this stage 15 mL of the arene (**2**: benzene; **3**: toluene) was introduced and the solution was heated to 60°C and surrounded by a Dewar water bath. The solution cooled slowly over several hours to deposit rodlike orange/brown (**2**) or yellow (**3**) crystals. Yields **2**: 72.2%; **3**: 80.5%. Satisfactory C,H,N elemental analyses were obtained for both compounds. ¹H NMR (300 MHz, [D₈]THF, 25°C, TMS): **2**: δ = 7.94 (m, 2H, *o*-C₆H₅), 7.31 (s, free C₆H₆), 6.87 (m, 2H, *m*-C₆H₅), 6.72 (m, 1H, *p*-C₆H₅), 1.68 (m, 2H, *α*-H), 1.25 (m, 4H, *β*-H), 1.06 (s, 12H, *γ*-H); **3**: δ = 7.83–7.65 (m, 2H, bound C₆H₄CH₃), 7.18–7.10 (m, free C₆H₅CH₃), 6.75–6.63 (m, 1H, bound C₆H₄CH₃), 6.08 (m, 1H, bound C₆H₄CH₃), 2.30 (s, free C₆H₅CH₃), 2.16 (s, 3H, bound C₆H₄CH₃), 1.65 (m, 2H, *α*-H), 1.25 (m, 4H, *β*-H) and 1.09 (s, 12H, *γ*-H); ¹³C NMR (75 MHz, [D₈]THF, 25°C, TMS): **2**: δ = 181.57 (*ipso*-C₆H₅), 142.28 (*o*-C₆H₅), 128.84 (free C₆H₆), 125.10 (*m*-C₆H₅), 122.61 (*p*-C₆H₅), 52.90 (tmp *γ*-C), 42.47 (tmp *α*-C), 36.13 (tmp *β*-C), 21.62 (tmp Me); **3**: δ = 158.64 (bound C₆H₄CH₃), 143.56 (bound C₆H₄CH₃), 142.13 (bound C₆H₄CH₃), 137.94 (free C₆H₅CH₃), 129.48 (free C₆H₅CH₃), 128.71 (free C₆H₅CH₃), 127.61 (bound C₆H₄CH₃), 125.70 (free C₆H₅CH₃), 123.10 (bound C₆H₄CH₃), 112.63 (bound C₆H₄CH₃), 52.75 (tmp *γ*-C), 42.44 (tmp *α*-C), 36.11 (tmp *β*-C), 24.64 (free C₆H₅CH₃), 21.14 (tmp Me), 19.98 (bound C₆H₄CH₃).

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- [1] A. R. Kennedy, R. E. Mulvey, R. B. Rowlings, *Angew. Chem.* **1998**, *110*, 3321; *Angew. Chem. Int. Ed.* **1998**, *37*, 3180.
- [2] D. R. Armstrong, A. R. Kennedy, R. E. Mulvey, R. B. Rowlings, *Angew. Chem.* **1999**, *111*, 231; *Angew. Chem. Int. Ed.* **1999**, *38*, 131.
- [3] A. R. Kennedy, R. E. Mulvey, C. L. Raston, B. A. Roberts, R. B. Rowlings, *Chem. Commun.* **1999**, 353.
- [4] W. Clegg, K. W. Henderson, R. E. Mulvey, P. A. O'Neil, *J. Chem. Soc. Chem. Commun.* **1994**, 769.
- [5] A. R. Kennedy, R. E. Mulvey, R. B. Rowlings, *J. Am. Chem. Soc.* **1998**, *120*, 7816.
- [6] K. W. Klinkhammer, *Chem. Eur. J.* **1997**, *3*, 1418.

- [7] Traces of lithium can be present. See L. Lochmann, J. Pospisil, D. Lim, *Tetrahedron Lett.* **1966**, 256.
- [8] P. E. Eaton, C.-H. Lee, Y. Xiong, *J. Am. Chem. Soc.* **1989**, *111*, 8016.
- [9] Crystal structure data for **2**: A colourless rod of dimensions 0.4 × 0.2 × 0.1 mm, trigonal, space group *R* $\bar{3}$ (No. 148), *a* = *b* = 37.2805(9), *c* = 11.8346(9) Å, *V* = 14244(1) Å³, *T* = 123 K, *Z* = 3, ρ_{calcd} = 1.022 Mg m⁻³, 2 θ_{max} = 46°, MoK α (λ = 0.71073 Å). The structure was solved, and refined on *F*², using programs of the Shelx family to convergence at *R*1 = 0.0862 (for 3240 reflections with *I* > 2 σ (*I*)), *wR*2 = 0.2653, and *S* = 1.062 for 287 parameters and 4384 unique reflections. Max. residual electron density 0.685 e Å⁻³. Hydrogen atoms were placed in calculated positions and the benzene solvent of crystallization was treated as rigid C₆H₆ groups. Compound **3** is isomorphous with **2** (*a* = *b* = 37.4442(6), *c* = 11.9489(6) Å). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140112 and -140113. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [10] M. M. Olmstead, W. J. Grigsby, D. R. Chacon, T. Hascall, P. P. Power, *Inorg. Chim. Acta* **1996**, *251*, 273.
- [11] D. Hoffmann, W. Bauer, F. Hampel, N. J. R. von E. Hommes, P. van R. Schleyer, P. Otto, U. Pieper, D. Stalke, D. S. Wright, R. Snaith, *J. Am. Chem. Soc.* **1994**, *116*, 528.
- [12] R. W. Saalfrank, I. Bernt, E. Uller, F. Hampel, *Angew. Chem.* **1997**, *109*, 2596; *Angew. Chem. Int. Ed.* **1997**, *36*, 2482.
- [13] A. Mordini, *Advances in Carbanion Chemistry*, Vol. 1 (Ed.: V. Snieckus), JAI Press, London, **1992**, p. 1.
- [14] M. Schlosser, *J. Organomet. Chem.* **1967**, *8*, 9.

Low-Temperature Hydrogenolysis of Alkanes Catalyzed by a Silica-Supported Tantalum Hydride Complex, and Evidence for a Mechanistic Switch from Group IV to Group V Metal Surface Hydride Complexes**

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We report herein a low-temperature hydrogenolysis of alkanes on a silica-supported tantalum hydride and the striking difference in reactivity compared to similar reactions

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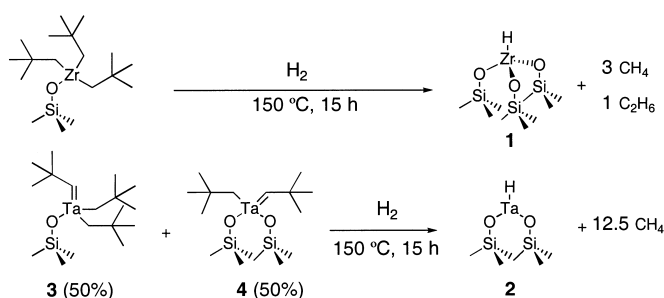
of silica-supported Group IV hydrides, for example in the hydrogenolysis of ethane.^[1]

Our group has shown that the silica-supported Group IV metal hydrides **1**, $(\equiv\text{SiO})_3\text{M}-\text{H}$ ($\text{M} = \text{Ti}$,^[1c], Zr ,^[1a] or Hf)^[1b], readily catalyze the hydrogenolysis of simple alkanes, such as propane, butanes, and pentanes (but with the exception of ethane), into mixtures of methane and ethane at low temperatures (25 to 150 °C). It even transforms polyethylene into lower molecular weight alkanes (all the way from diesels to mixtures of methane and ethane).^[2] These catalytic reactions are readily explained by a mechanism which involves a β -alkyl transfer^[3] as the key step for carbon–carbon bond cleavage. Interestingly, this elementary step corresponds to the microscopic reverse of insertion of an olefin into a metal–carbon bond, the carbon–carbon bond formation step (propagation) involved in Ziegler–Natta polymerization.^[4]

The surface complex **1** is usually obtained by selective hydrogenolysis of $(\equiv\text{SiO})\text{M}(\text{Np})_3$ with molecular H_2 at 150 °C (Np = neopentyl). During this hydrogenolysis, there is formation of a 3:1 mixture of methane and ethane, a product distribution which is in agreement with the β -alkyl transfer mechanism (Scheme 1).^[1a]

In sharp contrast to the behavior of group IV metal hydrides, the hydrogenolysis of the tantalum surface complexes **3** and/or **4** leads to the formation of methane as the only final product, thus showing a dramatic difference of reactivity (Scheme 1). This hydrogenolysis also yields a tantalum hydride complex $(\equiv\text{SiO})_2\text{Ta}-\text{H}$, **2**, which is a remarkable catalyst for the metathesis of paraffins.^[5, 6] We have been puzzled by the striking differences of behavior between **1** and **2** for the stoichiometric formation of the surface hydrides. This led us to further investigate the catalytic activity of **2** for the hydrogenolysis of various alkanes, including ethane.

In a glass batch reactor, a mixture of hydrogen and acyclic alkanes ($\text{C}_n\text{H}_{2n+2}$; $n > 1$) in the presence of **2** are typically converted into their lower homologues, that is, $\text{C}_{n-1}\text{H}_{2n}$ to CH_4 , and only CH_4 is detected at full conversion (Figure 1). The product distribution is typical of successive cleavage of the carbon–carbon bonds, for example, the successive formation and disappearance of propane and ethane in the hydrogenolysis of 2-methylpropane. The product selectivity is constant at conversions of 50–80% (Table 1), depending on the starting material; these constant selectivities are consistent with the observation of primary products, and can therefore provide potential mechanistic information regarding the carbon–carbon bond cleavage step. Moreover, the observed selectivities at low conversions are quite informative. For example, a 50:50



Scheme 1. The metals are attached to the silica surface by siloxy groups shown as $\equiv\text{SiO}$.

mixture of ethane and methane could be expected at low conversion in the hydrogenolysis of propane, yet a 43:57 mixture is obtained at various temperatures, showing that ethane resulting from the C–C bond cleavage of propane is partially cleaved before being desorbed (Table 1, entry 2).

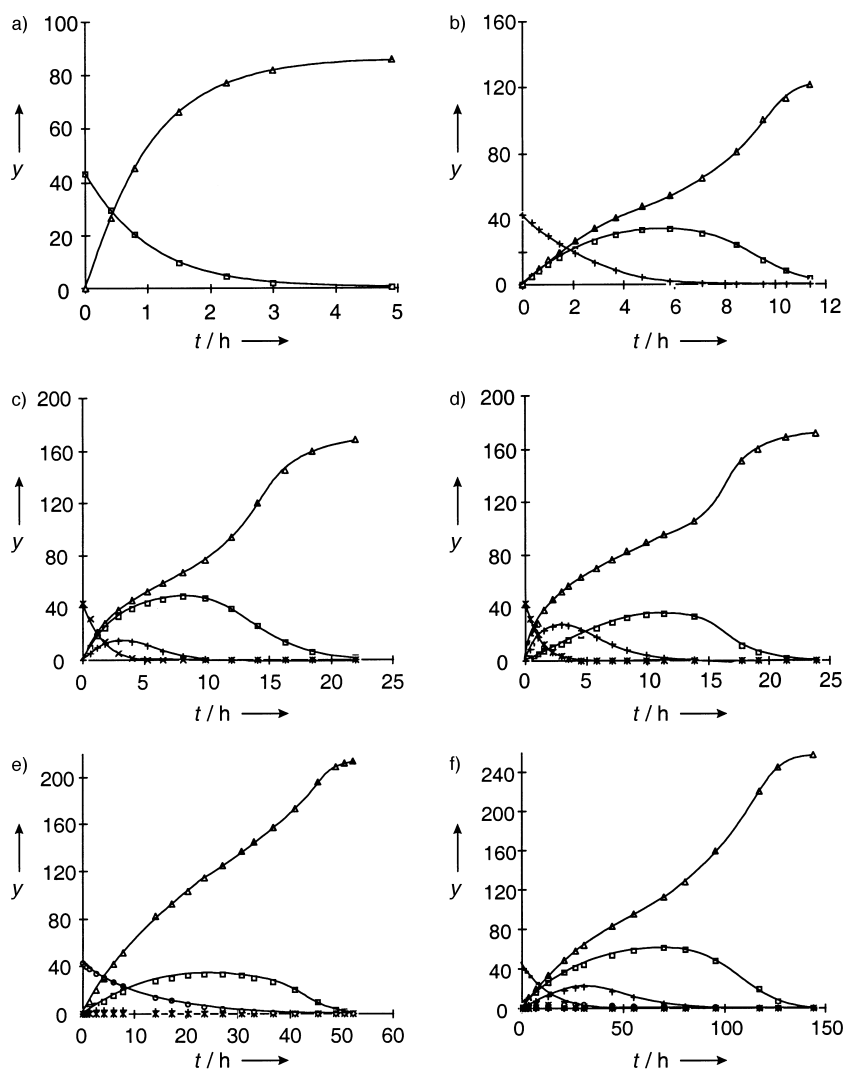


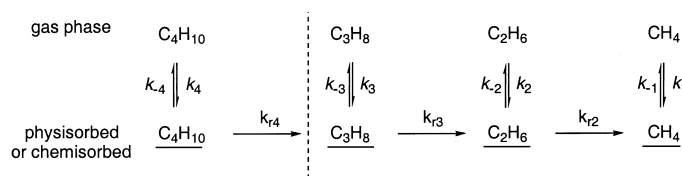
Figure 1. Hydrogenolysis of alkanes over a silica-supported tantalum hydride surface complex **2**. The y axis shows the equivalents of products formed relative to one equivalent of **2**. The x axis shows time in hours. a) Ethane hydrogenolysis; b) propane hydrogenolysis; c) butane hydrogenolysis; d) 2-methylpropane hydrogenolysis; e) neopentane hydrogenolysis; f) 2,2-dimethylbutane hydrogenolysis. Δ = Methane, $\square$ = ethane, $+$ = propane, $\times$ = butane, $*$ = 2-methylpropane, $\circ$ = neopentane, $\bullet$ = 2,2-dimethylbutane.

Table 1. Initial rate constants (*k*) and selectivities at low conversions for the hydrogenolysis of alkanes over (≡SiO)₂Ta–H at 160 °C.^[a]

Entry	alkane	<i>k</i> [h ^{−1}]	pentanes	butanes	propane	ethane	methane
1	ethane	1.0	–	–	–	–	100
2	propane	0.36	–	–	–	43 (50)	57 (50)
3	2-methylpropane	0.74	–	1.6 ^[b]	35.2 (50)	9.2 (0)	53.9 (50)
4	butane	0.65	–	1.8 ^[b]	19.5 (33)	36.3 (33)	42.4 (33)
5	Np–H	0.08	–	– ^[c]	– ^[c]	– ^[c]	– ^[c]
6	<i>n</i> -C ₅ H ₁₂	0.33	2.2 ^[d]	7.1 ^[e] (25)	20.0 (25)	37.5 (25)	33.0 (25)
7	Np–Me	0.07	8.5 ^[f,g] (10)	7.5 ^[f,h] (10)	11.7 (0)	29.8 (10)	40.8 (40)

[a] The numbers in parenthesis correspond to the expected selectivities if the first carbon–carbon bond cleavage were statistical. [b] This corresponds to the selectivity in the other isomer. [c] Selectivities are not constant even at low conversion since neopentane reacts slower than the products formed. [d] Selectivity in 2-methylbutane. [e] Selectivity in butane; the selectivity in 2-methylpropane is 0.2 %. [f] The selectivities decrease slowly with conversion. [g] Selectivity in neopentane; the selectivities in 2-methylbutane and pentane are 1.0 % (30), and 0 % (0), respectively. [h] Selectivity in 2-methylpentane only, the selectivity in butane is 0.7 % (0).

The same observation applies to higher alkanes. In fact, it is even more dramatic in the case of 2-methylpropane in which the selectivity in propane is only 35% compared with an expected 50 % (Table 1, entry 3). This shows that the process does not involve purely successive reactions and, also, that there is a greater probability for a higher alkane to react further rather than desorb (Scheme 2, physisorption and/or chemisorption).



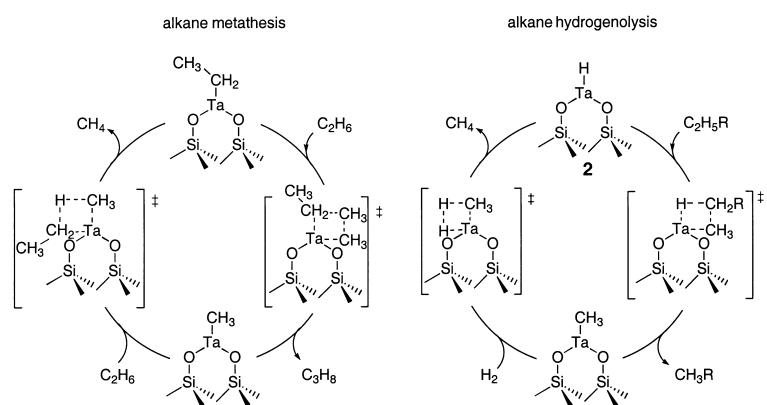
Scheme 2.

Three molecules deserve a special interest. Firstly, ethane is catalytically cleaved by **2** into methane (Table 1, entry 1), which obviously cannot be explained by a β -alkyl transfer mechanism since the corresponding ethyl–tantalum surface complex, (≡SiO)₂Ta–CH₂CH₃, does not have an alkyl group in the β -position. This shows that a one-carbon transfer process is possible with this catalyst, in contrast to what has been observed for Group IV metals (see above). However, it is difficult to discriminate between one- or two-atom transfer processes (like β -alkyl transfer) for simple linear or branched alkanes such as butane or 2-methylpropane. This led us to investigate the hydrogenolysis of 2,2-dimethylbutane (Np–Me, Table 1, entry 7). Under identical conditions, this molecule gave neopentane as the major product of higher alkanes (> C₃). In this case, neopentane cannot be produced by a β -alkyl transfer, which clearly shows that another process for carbon–carbon bond cleavage takes place even for higher alkanes. Finally, in the case of neopentane (Np–H, Table 1, entry 5), there is no build-up of intermediary products (like 2-methylpropane or propane), thus showing its slower reactivity relative to the lighter alkanes formed during its hydrogenolysis. This is in contrast to what has been observed for other alkanes including pentane (see above) and also for its hydrogenolysis in the presence of **1**.^[1a, 7] This is probably due to steric

reasons and also suggests that the actual carbon–carbon bond cleavage step might also be different from a β -alkyl transfer.

In the light of the specific abilities of tantalum hydride to also catalyze alkane metathesis,^[6] which transforms an alkane into its higher and lower homologues, we suggest that the mechanism for carbon–carbon bond cleavage could be closely related to that of alkane metathesis. The key step of the proposed mechanism involves the formation and cleavage of a carbon–carbon bond by interaction of an alkane molecule with a tantalum–carbon bond by σ -bond metathesis (Scheme 3). In the case of the hydrogenolysis of ethane (R = H), the interaction of the Ta–H bond directly with a carbon–carbon bond, via a four-centered transition state, leads to methane and a Ta–Me surface species; the latter, which can readily give methane through a related σ -bond metathesis mechanism under H₂, is regenerated into the catalyst, **2**.^[8] The same mechanism can be also extended to higher alkanes in the case of tantalum (see above, for example, neopentane and 2,2-dimethylbutane). This is dramatically different from group IV metals in which β -alkyl transfer is the only carbon–carbon bond cleavage process.

In conclusion, the tantalum hydride **2** catalyzes the low-temperature hydrogenolysis of alkanes, including that of ethane, in sharp contrast with Group IV metal hydrides. This surface complex also catalyzes alkane metathesis, while surface complexes like **1** do not. We are currently further investigating these two reactions.



Scheme 3. The tantalum is attached to the silica surface by siloxy groups shown as ≡SiO.

Experimental Section

The reactions were carried out with the same batch of catalyst in the absence of oxygen and water under the following conditions: $(\text{SiO})_2\text{Ta-H}$ (prepared by impregnation, 18.8×10^{-6} mol of Ta, 1 equiv), alkane (40 Torr, 43 equiv), and H_2 (630 Torr, 710 equiv) were heated with an oil bath to the desired temperature ($\pm 1^\circ\text{C}$) in a glass batch reactor (376 mL). During the reaction, aliquots were expended, brought to atmospheric pressure, and analyzed by gas chromatography (HP 5890 apparatus, $\text{Al}_2\text{O}_3/\text{KCl}$ on fused silica column, $50\text{m} \times 0.32\text{mm}$). Alkanes and H_2 were dried over freshly regenerated molecular sieves (3Å) and deoxo traps before addition.

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- [1] For the low-temperature hydrogenolysis of alkanes on group IV silica supported complexes, see: a) $(\text{SiO})_2\text{Zr-H}$: C. Lecuyer, F. Quignard, A. Choplin, D. Olivier, J.-M. Basset, *Angew. Chem.* **1991**, *103*, 1692; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1660; J. Corker, F. Lefebvre, C. Lecuyer, V. Dufaud, F. Quignard, A. Choplin, J. Evans, J.-M. Basset, *Science* **1996**, *271*, 966; b) $(\text{SiO})_2\text{Hf-H}$: L. D'Ornelas, S. Reyes, F. Quignard, A. Choplin, J.-M. Basset, *Chem. Lett.* **1993**, 1931; c) $(\text{SiO})_2\text{Ti-H}$: C. Rozier, G. P. Niccolai, J.-M. Basset, *J. Am. Chem. Soc.* **1997**, *119*, 848.
- [2] V. Dufaud, J.-M. Basset, *Angew. Chem.* **1998**, *110*, 848; *Angew. Chem. Int. Ed.* **1998**, *37*, 806; V. Dufaud, C. Marangelli, J.-M. Basset, unpublished results.
- [3] For some examples of β -alkyl transfer, see: P. Watson, C. Roe, *J. Am. Chem. Soc.* **1982**, *104*, 6471; P. L. Watson, G. W. Parshall, *Acc. Chem. Res.* **1985**, *18*, 51; E. Bunel, B. Burger, J. Bercaw, *J. Am. Chem. Soc.* **1988**, *110*, 976; L. Schock, T. Marks, *J. Am. Chem. Soc.* **1990**, *110*, 7701; B. Burger, M. Thompson, D. Cotter, J. Bercaw, *J. Am. Chem. Soc.* **1990**, *112*, 1566; K. McNeill, R. A. Andersen, R. Bergman, *J. Am. Chem. Soc.* **1995**, *117*, 3625.
- [4] For leading references on the insertion of an olefin in metal-alkyl bond, see: P. Cossee, *J. Catal.* **1964**, *3*, 80; H. Sinn, W. Kaminsky, *Adv. Organomet. Chem.* **1980**, *18*, 99; H. Sinn, W. Kaminsky, H.-J. Vollmer, R. Woldt, *Angew. Chem.* **1980**, *92*, 396; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 390; R. F. Jordan, *Adv. Organomet. Chem.* **1991**, *32*, 325; T. J. Marks, *Acc. Chem. Res.* **1992**, *25*, 57.
- [5] For the synthesis and characterization of $(\text{SiO})_2\text{Ta-H}$, see: V. Vidal, A. Théolier, J. Thivolle-Cazat, J.-M. Basset, J. Corker, *J. Am. Chem. Soc.* **1996**, *118*, 4595.
- [6] V. Vidal, A. Théolier, J. Thivolle-Cazat, J.-M. Basset, *Science* **1997**, *276*, 99; O. Maury, L. Lefort, V. Vidal, J. Thivolle-Cazat, J.-M. Basset, *Angew. Chem.* **1999**, *111*, 2121; *Angew. Chem. Int. Ed.* **1999**, *38*, 1952.
- [7] F. Quignard, C. Lecuyer, A. Choplin, D. Olivier, J.-M. Basset, *J. Mol. Cat.* **1992**, *74*, 353.
- [8] Nevertheless, since Ta-H is a d^2 surface complex, it is not possible to exclude processes that involve an increase in oxidation state, such as a direct oxidative addition of ethane or an α -alkyl transfer from the tantalum-ethyl fragment; the reverse step has been documented, see: P. R. Sharp, R. R. Schrock, *J. Organomet. Chem.* **1979**, *171*, 43; J. C. Hayes, G. D. N. Pearson, M. J. Cooper, *J. Am. Chem. Soc.* **1981**, *103*, 4648.

Unprecedented Formation of Five-, Six-, and Seven-Membered Metallacycles by Single and Double Insertion of Mono- and Disubstituted Alkynes into an Rh-O Bond**

Yasuhiro Yamamoto,* Xiao-Hong Han, and Jian-Fang Ma

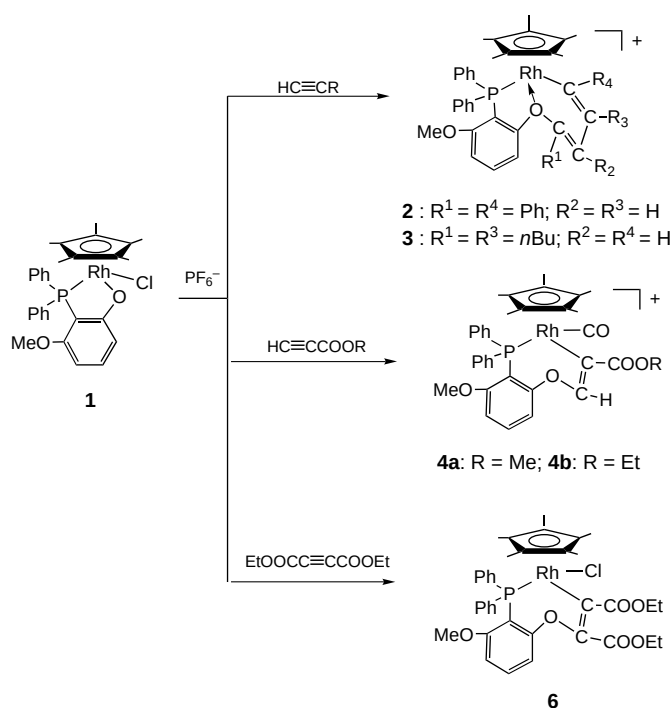
Metal alkynyl complexes are currently of great interest^[1, 2] since they are valuable for constructing vinylidene or carbene complexes for use in organic synthesis.^[3–8] Reactions of organotransition metal halides with 1-alkynes in the presence of anions such as PF_6^- , BF_4^- , and CF_3SO_3^- are representative methods for the preparation of vinylidene complexes.^[9] One *ortho*-methoxy group in (2,6-dimethoxyphenyl)diphenylphosphane (mdmpp) was demethylated in the reaction with the isoelectronic complexes $[(\eta^6\text{-arene})\text{RuCl}_2]_2$ (arene = C_6Me_6 , *p*-cymene, $\text{C}_6\text{H}_3\text{Me}_3$) and $[\text{Cp}^*\text{MCl}_2]_2$ (M = Rh, Ir) to give metal complexes with a (*P,O*)-chelating phosphane: $[(\eta^6\text{-arene})\text{RuCl}(\text{mdmpp-}\kappa\text{P},\kappa\text{O})]$ ^[10, 11] and $[\text{Cp}^*\text{MCl}(\text{mdmpp-}\kappa\text{P},\kappa\text{O})]$ ^[12, 13] ($\text{Cp}^* = \text{C}_5\text{Me}_5$). We recently reported the unprecedented insertion of tetracyanoethylene (tcne) and tetracyanoquinodimethane into the C-H bond adjacent to the M-O bonds of the above rhodium(III) and iridium(III) complexes to produce (*P,O*)-chelated $[\text{Cp}^*\text{MCl}[\text{PPh}_2\{2\text{-O-6-MeO-3-(CH(CN)}_2\text{C(CN)}_2\text{C}_6\text{H}_2)\}]]$ in the case of tcne.^[14]

Treatment of the ruthenium(III) complex with $\text{PhC}\equiv\text{CH}$ in the presence of NaPF_6 in acetone/ CH_2Cl_2 afforded the vinylidene complex $[(\eta^6\text{-arene})\text{Ru}(\text{mdmpp-}\kappa\text{P},\kappa\text{O})(=\text{C}=\text{CHPh})]\text{-PF}_6$.^[15] However, treatment of $[\text{Cp}^*\text{RhCl}(\text{mdmpp-}\kappa\text{P},\kappa\text{O})]$ (**1**)^[12] with 1-alkynes such as $\text{HC}\equiv\text{CCOOMe}$, $\text{PhC}\equiv\text{CH}$, and $n\text{BuC}\equiv\text{CH}$, and the disubstituted alkyne $\text{C}_2(\text{CO}_2\text{Et})_2$ in the presence of NaPF_6 or KPF_6 resulted in unusual reactions (Scheme 1). In the reaction with $\text{HC}\equiv\text{CCOOMe}$, extraction of CO from the ester group of one and insertion of another 1-alkyne molecule into the Rh-O σ bond occurred to produce a seven-membered metallacycle, and reactions with $\text{HC}\equiv\text{CR}$ led to the formation of complexes with five- and six-membered rings by double insertion of 1-alkynes into the Rh-O bond. The disubstituted alkyne also underwent single insertion into the Rh-O bond. Similar insertion of unsaturated molecules into the metal-oxygen bonds of metal alkoxides was achieved with cyclooctadiene^[16] and perfluoroolefins^[17] such as $\text{F}_2\text{C}=\text{CF}_2$ and hexafluorocyclobutene. The insertion of alkynes into the transition metal to oxygen σ bonds was achieved here for the first time.^[18] Interestingly, these unprecedented reactions allow single and double insertion of alkynes into the Rh-O bond of **1** to be controlled by means of the alkyne substituents.

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Scheme 1. Reactions of **1** with alkynes in the presence of PF_6^- .

Orange crystals, obtained from the reaction of **1** with an excess of ethynylbenzene were formulated as $[\text{Cp}^*\text{Rh}(\text{mdmpp-}\kappa\text{P},\kappa\text{O})(\text{PhC}\equiv\text{CH})_2]\text{PF}_6$ (**2a-PF₆**) on the basis of FAB mass spectrometric data. X-ray analysis showed the existence of two independent molecules, in which an Rh atom is surrounded by a novel tridentate ligand (P, O, C sites) derived from the head-to-head double-insertion of two $\text{PhC}\equiv\text{CH}$ molecules into the Rh-O bond (Figure 1). A similar

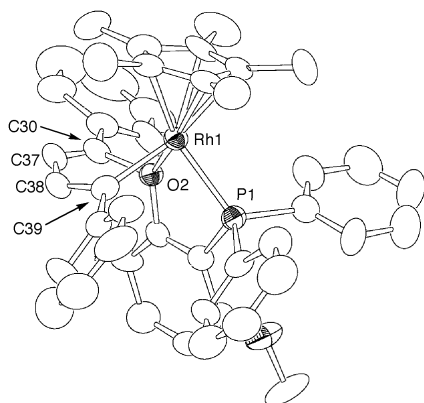


Figure 1. Perspective ORTEP view of the molecular structure of **2a** (showing one molecule; 50% probability thermal ellipsoids). Selected bond lengths [Å] and angles [°]: Rh1-P1 2.312(2), Rh1-O2 2.239(5), Rh1-C39 2.108(9), O2-C30 1.447(10), C30-C37 1.34(1), C37-C38 1.43(1), C38-C39 1.36(1); P1-Rh1-O2 78.4(2), P1-Rh1-C39 92.2(3), O2-Rh1-C39 78.0(3).

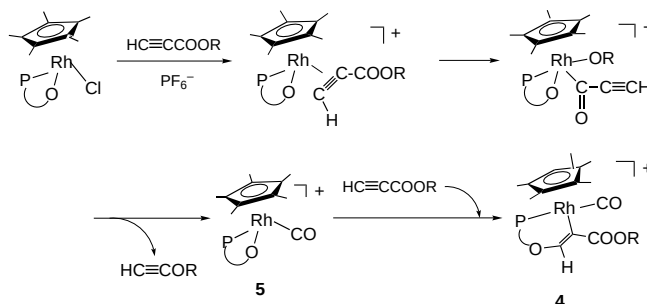
double insertion proceeded on treatment of **1** with 1-hexyne to give **3**, but X-ray analysis revealed that the structure resulted from a head-to-tail double insertion. The $n\text{Bu}$ -substituted terminal C atom of the diene was connected to

the O atom. The olefinic proton in the Rh-CH= moiety appeared as a doublet at $\delta = 5.69$ in the ^1H NMR spectrum due to coupling with ^{103}Rh . A similar insertion of two $\text{PhC}\equiv\text{CH}$ molecules into an Ru-N bond was recently reported for the triruthenium cluster $[\text{Ru}_3(\mu\text{-H})(\mu\text{-N}=\text{CPh}_2)(\text{CO})_{10}]$.^[20]

Complex **1** reacted readily with an excess of $\text{HC}\equiv\text{CCOOME}$ under similar conditions to give yellow-orange crystals of **4a-PF₆**. The IR bands at 2060 and 1699 cm^{-1} were assigned to terminal CO and methoxycarbonyl groups, respectively. The presence of a PF_6^- ion was confirmed by a $\nu(\text{PF})$ band at 837 cm^{-1} . The ^1H NMR spectrum showed three characteristic bands at $\delta = 1.54$ (d), 3.16 (s), and 3.76 (s), assigned to Cp^* , methoxy, and methoxycarbonyl protons, respectively. X-ray analysis revealed the Rh atom to be surrounded by a CO and a bidentate P,C ligand formed by insertion of methyl propiolate into the metal-oxygen bond. The carbon atom bearing the methoxycarbonyl group occupied a rhodium site regioselectively due to the polarity of the Rh-O and $\text{C}\equiv\text{C}$ bonds. A similar complex **4b** was obtained from the reaction of **1** with $\text{HC}\equiv\text{CCOOEt}$.

The formation of **4** in high yield in THF confirmed that the CO group originated from the propiolate. Abstraction of CO from ester groups is quite rare, although formation of $[\text{Ni}(\text{CO})(\text{PPh}_3)_3]$ ^[21] from the reaction of $[\text{Ni}(\text{cod})_2]$ (cod = 1,5-cyclooctadiene) with triphenylphosphane and phenyl propionate and of $[\text{Rh}(\text{OAr})(\text{CO})\text{L}_2]$ ^[22] from MeCOOAr and $[\text{Rh}(\text{H})\text{L}_4]$ (L = phosphane ligand) have been documented.

Since compound **5**, prepared independently,^[12] reacted readily with $\text{HC}\equiv\text{CCOOR}$ to give **4**, we assume that formation of **4** involves initial oxidative addition of the ester and extraction of CO from an acyl group to give the carbonyl complex **5** (Scheme 2). The reaction is completed by CO



Scheme 2. Possible pathway for the formation of **4** ($\text{P}^-\text{O} = \text{PPh}_2(2\text{-O-6-MeOC}_6\text{H}_3)$).

abstraction, accompanied by elimination of $\text{HC}\equiv\text{COR}$. The formation of $\text{HC}\equiv\text{CCOEt}$ and **4b** was confirmed by monitoring the reaction between **1** and $\text{HC}\equiv\text{CCOOEt}$ by NMR spectroscopy.

Reactions with internal alkynes such as $\text{MeC}\equiv\text{CPh}$ and $\text{EtOOC}\equiv\text{CCOOEt}$ were carried out in the presence of KPF_6 . The former failed to react, whereas the latter produced reddish orange **6** (Scheme 1). The IR spectrum showed bands at 1705 and 1589 cm^{-1} for a carbonyl group and a C-C double bond. However, there was no band at about 840 cm^{-1} for a PF_6^-

group. In the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum, no signal due to a PF_6 moiety was observed, and only a doublet at $\delta = 10.28$ ($J_{\text{Rh,P}} = 136.8$ Hz) appeared. In the ^1H NMR spectrum, methyl signals of the ester groups appeared at $\delta = 1.03$ and 1.27 as a triplet, and the methylene protons gave rise to two multiplets at $\delta = 4.03$ and 4.18 due to the nonequivalence of the methylene protons that results from the asymmetric coordination environment of the Rh atom. The spectroscopic data suggested that the complex was a seven-membered metallacycle derived from the insertion of $\text{EtOOC}\equiv\text{CCOOEt}$. The proposed structure was confirmed by X-ray analysis (Figure 2). When this reaction was performed without PF_6^- ions,

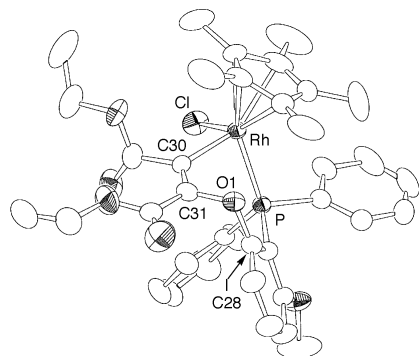


Figure 2. Perspective ORTEP view of the molecular structure of **6** (50% probability thermal ellipsoids). Selected bond lengths [Å] and angles [°]: Rh1–Cl1 2.399(1), Rh1–P1 2.302(1), Rh1–C30 2.044(6), C30–C31 1.324(8), C31–O1 1.415(7), O1–C28 1.394(6); P1–Rh1–Cl1 89.90(5), P1–Rh1–C30 85.3(1), O1–Rh1–C30 93.7(2).

the starting materials were recovered quantitatively. The presence of PF_6^- was indispensable for this reaction.

Further mechanistic studies and reactions with other small molecules are now in progress.

Experimental Section

2a-PF₆: An excess of NaPF_6 was added to a solution of **1** (50 mg, 0.086 mmol) and $\text{PhC}\equiv\text{CH}$ (0.1 mL) in CH_2Cl_2 (15 mL) and acetone (10 mL) at room temperature. After 4 h, the solvent was removed, and the residue was extracted with CH_2Cl_2 . The CH_2Cl_2 was removed to give an oily residue, which was purified by chromatography on silica gel with $\text{CH}_2\text{Cl}_2/n$ -hexane (1/5) as eluent. The reddish brown eluate was dried, and the residue was recrystallized from CH_2Cl_2 /diethyl ether to give orange crystals of **2a-PF₆** (27 mg, 35%). IR (Nujol): $\tilde{\nu} = 1591, 1568, 837\text{ cm}^{-1}$; UV (CH_2Cl_2): $\lambda_{\text{max}} \approx 360, 286\text{ nm}$; ^1H NMR (250 MHz, CDCl_3): $\delta = 1.35$ (d, $J(\text{P,H}) = 2.5$ Hz, Cp*, 15H), 3.30 (s, OMe, 3H), 6.3–7.6 (m, CH= and ArH, 25H); ^{31}P NMR (100 MHz, CDCl_3): $\delta = 36.9$ (d, $J(\text{Rh,P}) = 157.5$ Hz), –143.8 (sept, $J(\text{P,F}) = 717$ Hz); FAB MS (m/z of cationic part): 749 (749.7); C,H analysis calcd for $\text{C}_{45}\text{H}_{43}\text{O}_2\text{P}_2\text{F}_6\text{Rh}$: C 60.41, H 4.84; found: C 60.12, H 4.83. Crystal data: space group $P2_1/a$ (No. 14) with $a = 20.003(4)$, $b = 15.555(5)$, $c = 26.289(4)$ Å, $\beta = 90.24(2)^\circ$, $V = 8179(3)$ Å³ at 26 °C, $Z = 8$ (two independent molecules per unit cell). The structure was solved by Patterson methods and refined by full-matrix least-squares techniques for 14832 reflections having $I > 10.0\sigma(I)$ to $R = 0.133$, $R_w = 0.181$, $R1 = 0.066$ (for 7979 reflections); GOF 1.60.

3-PF₆: A solution of **1** (50 mg, 0.086 mmol), 1-hexyne (0.1 mL) and an excess of NaPF_6 in CH_2Cl_2 /acetone (15/10 mL) was stirred for 4 h. The solvent was removed, and the residue extracted with CH_2Cl_2 . After the solution was concentrated to 3 mL, diethyl ether was added to give orange crystals of **3-PF₆** (24 mg, 32%). IR (Nujol): $\tilde{\nu} = 1660, 1593, 837\text{ cm}^{-1}$; UV (CH_2Cl_2): $\lambda_{\text{max}} \approx 390$ (sh), 283 nm; ^1H NMR (250 MHz, CDCl_3): $\delta = 0.68$ (t, $J(\text{H,H}) = 6.8$ Hz, CH_3 , 3H), 1.03 (t, $J(\text{H,H}) = 6.8$ Hz, CH_3 , 3H), 1.39 (d,

$J(\text{P,H}) = 2.5$ Hz, Cp*, 15H), 1.2–2.8 (m, $(\text{CH}_2)_3$, 12H), 3.38 (s, OMe, 3H), 5.56 (s, CH=, 1H), 5.69 (d, $J(\text{Rh,H}) = 15.0$ Hz, 1H), 6.6–7.8 (m, ArH, 13H); ^{31}P NMR (100 MHz, CDCl_3): $\delta = 44.8$ (d, $J(\text{Rh,P}) = 157.5$ Hz), –143.8 ($J(\text{P,F}) = 712$ Hz); FAB MS (m/z of cation part): found: 709; calcd 707.7; C,H analysis calcd for $\text{C}_{41}\text{H}_{31}\text{O}_2\text{P}_2\text{F}_6\text{Rh}$: C 57.62, H 6.01; found: C 57.65, H 5.85. Crystal data: space group $P1$ (No. 2), $a = 11.575(4)$, $b = 16.67(1)$, $c = 10.800(5)$ Å, $\alpha = 90.17(5)^\circ$, $\beta = 99.46(3)^\circ$, $\gamma = 93.41(4)^\circ$, $V = 2051(1)$ Å³ at 26 °C, $Z = 2$. The structure was solved by Patterson methods and refined by full-matrix least-squares techniques for all unique reflections (5376) to $R = 0.231$, $R_w = 0.092$ and $R1 = 0.073$ (for 2310 reflections); GOF 1.25.

4a-PF₆: Yellow-orange crystals of **4a-PF₆** (61 mg, 40%) were obtained from **1** (110 mg, 0.189 mmol), $\text{HC}\equiv\text{CCOOEt}$ (0.1 mL), and an excess of NaPF_6 by a procedure similar to that for **3-PF₆**. IR (Nujol): $\tilde{\nu} = 2060, 1699, 1583, 837\text{ cm}^{-1}$; UV (CH_2Cl_2): $\lambda_{\text{max}} = 293\text{ nm}$; ^1H NMR (250 MHz, CDCl_3): $\delta = 1.54$ (d, $J(\text{P,H}) = 2.5$ Hz, Cp*, 15H), 3.16 (s, OMe, 3H), 3.76 (s, OMe, 3H), 6.7–7.8 (m, ArH, 13H), 7.96 (s, CH=, 1H); ^{31}P NMR (100 MHz, CDCl_3): $\delta = 3.12$ (d, $J(\text{Rh,P}) = 122.3$ Hz, 1P), –143.9 (sept, $J(\text{P,F}) = 712$ Hz, PF_6); C,H analysis calcd for $\text{C}_{34}\text{H}_{35}\text{O}_3\text{P}_2\text{F}_6\text{Rh}$: C 51.14, H 4.31; found: C 50.89, H 4.40. Crystal data: space group $P2_1/a$ (No. 14), $a = 15.410(5)$, $b = 22.199(4)$, $c = 10.602(4)$ Å, $\beta = 110.11(3)^\circ$, $V = 3405(1)$ Å³ at –100 °C, $Z = 4$ (at 26 °C: $b = 14.582(3)$, $c = 14.426(3)$ Å, $V = 3463(1)$ Å³, $Z = 4$). The structure was solved by direct methods (SIR92) and refined by full-matrix least-squares techniques for 5097 reflections having $I > 2.5\sigma(I)$ to $R = 0.071$, $R_w = 0.132$, $R1 = 0.049$ (for 5097 reflections); GOF 1.83.

4b-PF₆: Orange (32%): IR (Nujol): $\tilde{\nu} = 2060, 1695, 1585, 1564, 837\text{ cm}^{-1}$; UV (CH_2Cl_2): $\lambda_{\text{max}} = 310\text{ nm}$; ^1H NMR (250 MHz, CDCl_3): $\delta = 1.28$ (t, $J(\text{H,H}) = 7.5$ Hz, Me, 3H), 1.54 (d, $J(\text{P,H}) = 2.5$ Hz, Cp*, 15H), 3.16 (s, OMe, 3H), 4.22 (q, $J(\text{H,H}) = 7.5$ Hz, OCH_2 , 2H), 6.7–7.8 (m, ArH, 13H), 7.96 (s, CH=, 1H); ^{31}P NMR (100 MHz, CDCl_3): $\delta = 3.15$ (d, $J(\text{Rh,P}) = 124.9$ Hz, 1H), –143.9 ($J(\text{P,F}) = 711.0$ Hz, PF_6); C,H analysis calcd for $\text{C}_{35}\text{H}_{37}\text{O}_3\text{P}_2\text{F}_6\text{Rh}$: C 51.80, H 4.51; found: C 51.48, H 4.57.

6: Orange crystals (45 mg, 63%) were obtained from **1** (55 mg, 0.095 mmol), $\text{EtOOC}\equiv\text{CCOOEt}$ (0.1 mL), and an excess of NaPF_6 . IR (Nujol): $\tilde{\nu} = 1705$ (C=O), 1589 cm^{-1} (C=C); ^1H NMR (250 MHz, CDCl_3): $\delta = 1.03$ (t, $J(\text{H,H}) = 7.2$ Hz, Me, 3H), 1.27 (t, $J(\text{H,H}) = 7.2$ Hz, Me, 3H), 1.26 (d, $J(\text{P,H}) = 3.2$ Hz, Cp*, 15H), 3.03 (s, OMe, 3H), 4.03 (m, CH_2 , 2H), 4.18 (m, CH_2 , 2H), 7.0–7.9 (m, Ph, 13H); ^{31}P NMR (100 MHz, CDCl_3): $\delta = 10.28$ (d, $J(\text{Rh,P}) = 136.8$ Hz, 1P); C,H analysis calcd for $\text{C}_{37}\text{H}_{41}\text{O}_6\text{PClRh}$ · $0.5\text{CH}_2\text{Cl}_2$: C 56.76, H 5.34; found: C 56.56, H 5.22. Crystal data for **6**· $0.5\text{CH}_2\text{Cl}_2$: space group $Pna2_1$ (No. 33), $a = 16.466(3)$, $b = 14.582(3)$, $c = 14.426(3)$ Å, $V = 3463(1)$ Å³ at 25 °C, $Z = 4$. The structure was solved by direct methods (SIR92) and refined by full-matrix least-squares techniques for 3179 reflections having $I > 2.0\sigma(I)$ to $R = 0.039$, $R_w = 0.035$, $R1 = 0.029$ (for 2771 reflections); GOF 1.37.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-132821 (**4a**), -132822 (**3**), -132823 (**6**) and -132824 (**2a**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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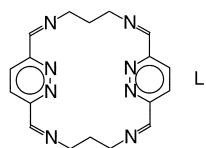
- [1] L. T. Scott, M. J. Cooney, D. Johnels, *J. Am. Chem. Soc.* **1990**, *112*, 4054.
- [2] M. H. Chisholm, *Angew. Chem.* **1991**, *103*, 690; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 673.
- [3] B. M. Trost, G. Dyker, R. J. Kulawiec, *J. Am. Chem. Soc.* **1990**, *112*, 7809.
- [4] L. S. Liebeskind, R. Chidambaram, D. Mitchell, B. S. Foster, *Pure Appl. Chem.* **1988**, *60*, 27.
- [5] A. G. M. Barrett, J. Mortier, M. Sabat, M. A. Sturgess, *Organometallics* **1988**, *7*, 2553.
- [6] R. Mahe, P. H. Dixneuf, S. Lecolier, *Tetrahedron Lett.* **1986**, *27*, 6333.
- [7] A. G. M. Barrett, N. E. Carpenter, *Organometallics* **1987**, *6*, 2249.
- [8] S. L. Buchwald, R. H. Grubbs, *J. Am. Chem. Soc.* **1983**, *105*, 5490.
- [9] M. I. Bruce, *Chem. Rev.* **1991**, *91*, 197, and references therein.

- [10] Y. Yamamoto, R. Sato, F. Matsuo, C. Sudoh, T. Igoshi, *Inorg. Chem.* **1996**, 35, 2329.
- [11] Y. Yamamoto, R. Sato, M. Ohshima, F. Matsuo, C. Sudoh, *J. Organomet. Chem.* **1995**, 489, C68.
- [12] X.-H. Han, Y. Yamamoto, *J. Organomet. Chem.* **1998**, 561, 157.
- [13] Y. Yamamoto, K. Kawasaki, S. Nishimura, *J. Organomet. Chem.* **1999**, 587, 49.
- [14] Y. Yamamoto, X.-H. Han, K. Sugawara, S. Nishimura, *Angew. Chem.* **1999**, 112, 1318; *Angew. Chem. Int. Ed.* **1999**, 38, 1242.
- [15] Y. Yamamoto, T. Tanase, C. Sudoh, T. Turuta, *J. Organomet. Chem.* **1998**, 569, 29.
- [16] B. de Bruin, M. J. Boerakker, J. A. Brands, J. J. M. Donners, M. P. J. Donners, R. de Gelder, J. M. M. Smits, A. W. Gal, A. L. Spek, *Chem. Eur. J.* **1999**, 5, 2921.
- [17] M. H. Chisholm, F. A. Cotton, M. W. Extine, W. W. Reichert, *J. Am. Chem. Soc.* **1978**, 100, 1727.
- [18] The insertion of alkynes into the Pt–OH bond is cited as H. Jin, PhD Thesis, The Australian National University, **1990** in ref. [19], but details were not reported.
- [19] M. A. Bennett, H. Jin, S. Li, L. M. Rendina, A. C. Willis, *J. Am. Chem. Soc.* **1995**, 117, 8335.
- [20] J. A. Cabeza, I. Rio, R. J. Franco, F. Grepioni, V. Riera, *Organometallics* **1997**, 16, 2763.
- [21] T. Yamamoto, J. Ishizu, T. Kohara, S. Komiya, A. Yamamoto, *J. Am. Chem. Soc.* **1980**, 102, 3758.
- [22] T. Yamamoto, S. Miyashita, Y. Naito, S. Komiya, T. Ito, A. Yamamoto, *Organometallics* **1982**, 1, 808.

A Grid Complex $[\text{Cu}_4\text{L}_2]^{4+}$ and a Mixed-Valent Complex $[\text{Cu}^{\text{II}}\text{Cu}^{\text{I}}\text{L}(\text{MeCN})_2]^{3+}$ of the Pyridazine-Containing Macrocycle L^{**}

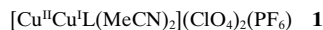
Sally Brooker,* Sarah J. Hay, and Paul G. Plieger

The Schiff base macrocycle **L**, obtained from the [2+2] condensation of 3,6-diformylpyridazine^[1–4] and 1,3-diaminopropane, has allowed the isolation of a wide range of transition metal complexes with intriguing properties, in particular redox^[1, 5, 6] and magnetic properties.^[5–7] To date, many pyridazine-bridged or phthalazine-bridged dicopper(II) complexes,^[8–10] but few copper(I) complexes^[11–15] and, to our knowledge, no mixed-valent copper complexes, have been isolated. The structure, magnetochemistry, and electrochemistry of the first example



complexes,^[8–10] but few copper(I) complexes^[11–15] and, to our knowledge, no mixed-valent copper complexes, have been isolated. The structure, magnetochemistry, and electrochemistry of the first example

of a macrocyclic pyridazine-bridged dicopper complex was reported recently.^[5] In contrast to other pyridazine-bridged dicopper complexes, two well-separated one-electron reductions were observed for this dicopper(II) complex, as is seen for related phenolate-bridged complexes,^[16] except that, as expected, they occur at more positive potentials due to the π -acceptor properties of **L**. We report here on the formation and characterization of the mixed-valent complex **1** and the air-stable tetracopper(I) grid complex $[\text{Cu}_4\text{L}_2](\text{PF}_6)_4$ (**2**) of the Schiff-base macrocyclic ligand **L**.



The mixed-valent $\text{Cu}^{\text{II}}\text{Cu}^{\text{I}}$ complex **1** is formed by a transmetalation reaction in acetonitrile (see the Experimental Section) and crystallized, in air, in 41 % yield, by diffusion of diethyl ether into the reaction solution. The IR spectrum of **1** shows that the macrocycle is intact. The structure determination of **1**·MeCN (Figure 1) reveals a similar overall

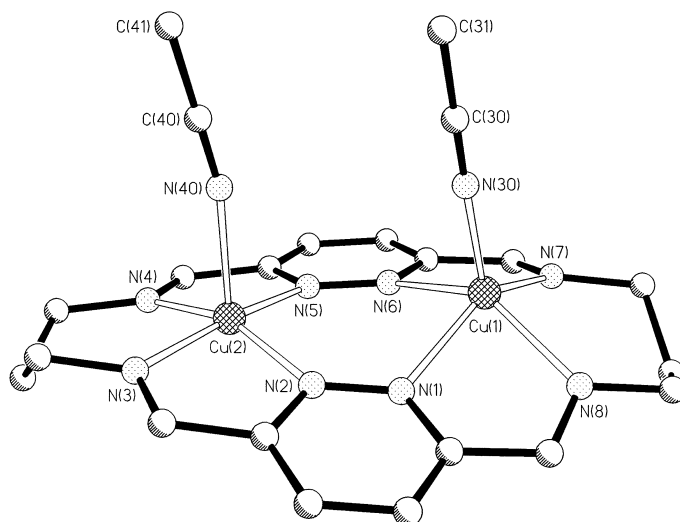


Figure 1. Perspective view of the cation of **1**, $[\text{Cu}^{\text{II}}\text{Cu}^{\text{I}}\text{L}(\text{MeCN})_2]^{3+}$. Selected interatomic distances [Å] and angles [°]: Cu(1)–N(30) 1.931(4), Cu(1)–N(7) 2.038(4), Cu(1)–N(8) 2.105(4), Cu(1)–N(1) 2.151(3), Cu(1)–N(6) 2.289(3), Cu(2)–N(3) 1.978(3), Cu(2)–N(5) 2.008(3), Cu(2)–N(4) 2.034(3), Cu(2)–N(2) 2.072(3), Cu(2)–N(40) 2.157(4), Cu(1)···Cu(2) 3.9599(9); N(30)–Cu(1)–N(7) 115.87(15), N(30)–Cu(1)–N(8) 115.62(14), N(7)–Cu(1)–N(8) 91.49(15), N(30)–Cu(1)–N(1) 102.05(14), N(7)–Cu(1)–N(1) 141.48(13), N(8)–Cu(1)–N(1) 77.37(13), N(30)–Cu(1)–N(6) 104.39(13), N(7)–Cu(1)–N(6) 75.18(14), N(8)–Cu(1)–N(6) 139.62(13), N(1)–Cu(1)–N(6) 89.64(12), N(3)–Cu(2)–N(5) 168.97(13), N(3)–Cu(2)–N(4) 92.85(14), N(5)–Cu(2)–N(4) 80.48(13), N(3)–Cu(2)–N(2) 80.42(13), N(5)–Cu(2)–N(2) 102.93(13), N(4)–Cu(2)–N(2) 160.04(13), N(3)–Cu(2)–N(40) 92.51(14), N(5)–Cu(2)–N(40) 97.09(14), N(4)–Cu(2)–N(40) 98.03(15), N(2)–Cu(2)–N(40) 101.00(14), C(30)–N(30)–Cu(1) 178.2(4), C(40)–N(40)–Cu(2) 168.1(4).

structure to that of the dicopper(II) analogue $[\text{Cu}_2^{\text{II}}\text{L}(\text{MeCN})_2]^{4+}$ (**3**).^[5] The copper centers are doubly bridged by the pyridazine groups and the macrocycle is fairly flat (the pyridazine ring planes intersect at 23.5(2)°; cf. 29.67(7)° for **3**). In each case four of the nitrogen donors come from the macrocycle (N_{macro}) and the fifth from an acetonitrile solvent molecule. However, the copper ions have distinct geometries consistent with the mixed-valent complex being class I

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(localized valence) at 173 K in the solid state (consistent with this, a four-line EPR spectrum is observed for **1** as a DMF glass at 133 K). The copper(I) ion, Cu(1), has a distorted five-coordinate geometry and is located 0.723(2) Å out of the plane of the four N_{macro} donors, whereas the copper(II) ion, Cu(2), has a distorted square-pyramidal geometry and is only 0.257(2) Å out of the plane of the four N_{macro} donors (cf. 0.317(2) Å for copper(II) in **3**). As expected, the Cu– N_{macro} bond lengths are longer (av Cu(1)– N_{macro} 2.146(4) Å) than those observed for Cu– N_{macro} (av Cu(2)– N_{macro} 2.023(4) Å; cf. 2.006(3) Å in **3**).^[8] The Cu– N_{MeCN} bond lengths reveal a Jahn–Teller distortion for the copper(II) ion in **1** (Cu(2)– N_{MeCN} 2.157(4) Å versus Cu(1)– N_{MeCN} 1.931(4) Å), as was observed in **3** (Cu– N_{MeCN} 2.128(3) Å).

In marked contrast to the dicopper mixed-valent product **1** obtained by transmetallation of a lead–macrocycle complex with copper(I), the product upon use of a copper(I) template in the reaction of the macrocycle components, 3,6-diformylpyridazine and 1,3-diaminopropane, is a tetracopper(I) grid complex, $[\text{Cu}_4\text{L}_2](\text{PF}_6)_4$ (**2**, 61% yield). An imine stretch is observed in the IR spectrum at 1627 cm^{−1} and there is no evidence of amine or carbonyl absorptions. Crystals of **2**·2MeCN·0.5Et₂O were grown from the acetonitrile reaction solution by diffusion of diethyl ether, and the X-ray structure determination was carried out (Figure 2). A square of four-

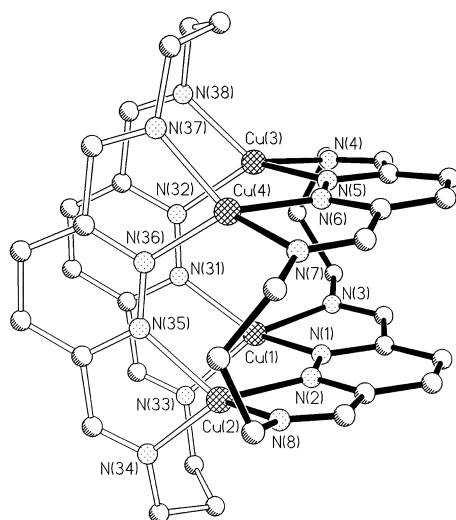


Figure 2. Perspective view of the cation of **2**, $[\text{Cu}_4\text{L}_2]^{4+}$. Selected interatomic distances [Å] and angles [°]: Cu(1)–N(1) 1.973(14), Cu(1)–N(31) 2.016(12), Cu(1)–N(3) 2.032(13), Cu(1)–N(33) 2.040(14), Cu(2)–N(8) 2.001(14), Cu(2)–N(2) 2.001(14), Cu(2)–N(34) 2.049(14), Cu(2)–N(35) 2.067(14), Cu(3)–N(32) 1.994(14), Cu(3)–N(5) 1.991(13), Cu(3)–N(4) 2.014(13), Cu(3)–N(38) 2.037(12), Cu(4)–N(36) 1.956(14), Cu(4)–N(6) 2.022(13), Cu(4)–N(7) 2.059(14), Cu(4)–N(37) 2.050(14), Cu(1)–Cu(2) 3.733(3), Cu(1)–Cu(3) 3.711(3), Cu(1)–Cu(4) 5.228(3), Cu(2)–Cu(3) 5.310(3), Cu(2)–Cu(4) 3.738(3), Cu(3)–Cu(4) 3.726(3); N(1)–Cu(1)–N(31) 128.6(5), N(1)–Cu(1)–N(3) 80.1(6), N(31)–Cu(1)–N(3) 120.0(5), N(1)–Cu(1)–N(33) 121.7(6), N(31)–Cu(1)–N(33) 79.7(6), N(3)–Cu(1)–N(33) 134.1(6), N(8)–Cu(2)–N(2) 81.2(6), N(8)–Cu(2)–N(34) 134.3(5), N(2)–Cu(2)–N(34) 123.7(5), N(8)–Cu(2)–N(35) 118.0(6), N(2)–Cu(2)–N(35) 123.9(6), N(34)–Cu(2)–N(35) 81.7(6), N(32)–Cu(3)–N(5) 130.6(5), N(32)–Cu(3)–N(4) 120.2(5), N(5)–Cu(3)–N(4) 80.3(5), N(32)–Cu(3)–N(38) 81.1(5), N(5)–Cu(3)–N(38) 119.6(5), N(4)–Cu(3)–N(38) 132.6(5), N(36)–Cu(4)–N(6) 129.4(5), N(36)–Cu(4)–N(7) 123.8(6), N(6)–Cu(4)–N(7) 79.9(5), N(36)–Cu(4)–N(37) 80.2(6), N(6)–Cu(4)–N(37) 118.9(5), N(7)–Cu(4)–N(37) 132.0(5).

coordinate copper(I) ions is ligated by two folded (2+2) macrocycles. Each macrocycle provides a bidentate $N_{\text{imine}}N_{\text{pyridazine}}$ donor set to each copper atom (bite angle 79.7(6)–81.7(6)°). For each copper atom the two bidentate chelate planes (Cu $N_{\text{imine}}N_{\text{pyridazine}}$, one bidentate donor set from each macrocycle) intersect at approximately right angles (79.8–101.4°). The pyridazine rings within each folded macrocycle lie over each other and are almost parallel (the pyridazine rings of N(1) and N(5) form an angle of 0.9(5)° and are separated by 3.58–3.61(2) Å; the pyridazine rings of N(31) and N(35) form an angle of 2.9(6)° and are separated by 3.57–3.72(2) Å). This is very similar to the arrangement of the pyridazine rings in the tetracopper(I) grid of the acyclic 3,6-bis(2'-pyridyl)pyridazine (dppn) ligand of Youinou and co-workers.^[15]

These findings, along with the results of deliberate syntheses of grids using related pyridazine ligands,^[15,17–20] indicate that the way is indeed clear for producing families of grid complexes from ligands related to **L**.^[17] The successful template synthesis of the Schiff base macrocycle **L** on this tetracopper(I) grid is a significant extension of these studies.

Experimental Section

1: To a refluxing suspension of $[\text{Pb}_2\text{L}](\text{ClO}_4)_4$ (0.199 g, 0.13 mmol; **L**' is the (4+4) analogue of the (2+2) macrocycle **L**)^[1] in dry, degassed acetonitrile (40 mL) was added solid $[\text{Cu}(\text{CH}_3\text{CN})_4](\text{PF}_6)_3$ (0.197 g, 0.53 mmol). The resulting brown mixture was refluxed under argon for 45 min, during which time the solution clarified. The argon atmosphere was removed, and complex **1** was isolated as a brown crystalline solid by vapor diffusion of diethyl ether into the reaction solution (0.090 g, 41%). Elemental analysis calcd for $\text{C}_{18}\text{H}_{20}\text{N}_8\text{Cl}_2\text{O}_8\text{PF}_6\text{Cu}_2$: C 26.4, H 2.5, N 13.7; found: C 27.0, H 2.7, N 14.0; IR (KBr): $\tilde{\nu}$ = 1645, 1101, 839, 623, 558 cm^{−1}; FAB-MS: m/z : 575 $[\text{Cu}_2\text{L}(\text{ClO}_4)]^+$, 474 $[\text{Cu}_2\text{L}]^+$; molar conductivity (CH_3CN): Λ_m = 383 mol^{−1}cm²Ω^{−1} (cf. 340–420 mol^{−1}cm²Ω^{−1} for a 3:1 electrolyte in CH_3CN)^[22]; UV/Vis (CH_3CN): λ_{max} (ϵ) = 449 (3730), 410 nm sh (3145 L mol^{−1}cm^{−1}).

Crystal data for **1** ($\text{C}_{24}\text{H}_{20}\text{Cl}_2\text{Cu}_2\text{F}_6\text{N}_{11}\text{O}_8\text{P}$): black oblong block, 0.88 × 0.58 × 0.37 mm, monoclinic, space group $P2_1/c$, a = 11.802(2), b = 30.042(6), c = 11.197(2) Å, β = 116.262(3)°, V = 3560(1) Å³, Z = 4, μ = 1.48 mm^{−1}. Of 45348 reflections collected, 7251 were independent and used in the structure analysis. The structure was solved by direct methods (SHELXS-97)^[23,24] and refined against all F^2 data (SHELXL-97)^[25] to $R1$ = 0.0516 for 5959 data with $F > 4\sigma(F)$; $wR2$ = 0.1405, GOF = 1.04 for all 7251 data (F^2); 526 parameters. All non-hydrogen atoms were anisotropic, and one perchlorate ion was disordered over two sets of oxygen atom sites.

2: To a yellow solution of 3,6-diformylpyridazine (0.095 g, 0.70 mmol)^[1] in dry degassed acetonitrile (20 mL) was added solid $[\text{Cu}(\text{CH}_3\text{CN})_4](\text{PF}_6)_3$ (0.261 g, 0.70 mmol) and 1,3-diaminopropane (0.052 g, 0.70 mmol) in dry degassed acetonitrile (10 mL). The resulting brown mixture was stirred under argon for 12 h, and the argon atmosphere removed. On vapor diffusion of diethyl ether into the reaction solution, **2** formed as a brown crystalline solid (0.163 g, 61%). Elemental analysis calcd for $\text{C}_{36}\text{H}_{40}\text{N}_{16}\text{P}_4\text{F}_{24}\text{Cu}_4$: C 28.3, H 2.6, N 14.6; found: C 28.5, H 2.4, N 14.5; IR (KBr): $\tilde{\nu}$ = 1627, 840, 558 cm^{−1}; ¹H NMR (CD_3NO_2 , referenced to solvent, 298 K): δ = 8.94, 8.35, ca. 4.3 (obscured by solvent), 4.17, 2.05; FAB-MS: m/z : 1385 $[\text{Cu}_4\text{L}_2(\text{PF}_6)_3]^+$, 1240 $[\text{Cu}_4\text{L}_2(\text{PF}_6)_2]^+$, 1095 $[\text{Cu}_4\text{L}_2(\text{PF}_6)]^+$, 474 $[\text{Cu}_2\text{L}]^+$; molar conductivity (CH_3CN): Λ_m = 550 mol^{−1}cm²Ω^{−1} (cf. 340–420 mol^{−1}cm²Ω^{−1} for a 3:1 electrolyte in CH_3CN)^[22]; UV/Vis (CH_3CN): λ_{max} (ϵ) = 410 nm (19170 L mol^{−1}cm^{−1}).

Crystal data for **2**·2MeCN·0.5Et₂O ($\text{C}_{42}\text{H}_{51}\text{Cu}_4\text{F}_{24}\text{N}_{18}\text{O}_{0.50}\text{P}_4$): red-black plate, 0.76 × 0.36 × 0.02 mm, monoclinic, space group Cc , a = 17.712(7), b = 18.520(7), c = 19.864(7) Å, β = 99.004(4)°, V = 6436(4) Å³, Z = 4, μ = 1.52 mm^{−1}. Of 40404 reflections collected, 10707 were independent and used in the structure analysis. The structure was solved by direct methods (SHELXS-97)^[23,24] and refined against all F^2 data (SHELXL-97)^[25] to

$R1 = 0.0945$ for 4546 data with $F > 4\sigma(F)$; $wR2 = 0.2583$, $GOF = 0.96$ for all 10707 data (F^2); 571 parameters; Flack $x = 0.07(3)$. All copper, acetonitrile, and PF_6^- atoms were anisotropic except the fluorine atoms on the disordered PF_6^- ion.

X-ray data were collected at 168 K for **1** and 173 K for **2** on a Bruker SMART diffractometer using graphite-monochromated MoK_{α} radiation. Reflections were collected in the range $4 < 2\theta < 53^\circ$, and a semiempirical absorption correction was applied. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137503 and -137504. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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- [1] S. Brooker, R. J. Kelly, *J. Chem. Soc. Dalton Trans.* **1996**, 2117.
- [2] S. Brooker, R. J. Kelly, G. M. Sheldrick, *J. Chem. Soc. Chem. Commun.* **1994**, 487.
- [3] R. H. Wiley, *J. Macromol. Sci. Chem.* **1987**, A24, 1183.
- [4] F. Abraham, M. Lagrenee, S. Sueur, B. Mernari, C. Bremard, *J. Chem. Soc. Dalton Trans.* **1991**, 1443.
- [5] S. Brooker, R. J. Kelly, B. Moubaraki, K. S. Murray, *Chem. Commun.* **1996**, 2579.
- [6] S. Brooker, R. J. Kelly, P. G. Plieger, *Chem. Commun.* **1998**, 1079.
- [7] S. Brooker, P. G. Plieger, B. Moubaraki, K. S. Murray, *Angew. Chem.* **1999**, 111, 424; *Angew. Chem. Int. Ed.* **1999**, 38, 408.
- [8] S. S. Tandon, L. Chen, L. K. Thompson, S. P. Connors, J. N. Bridson, *Inorg. Chim. Acta* **1993**, 213, 289.
- [9] L. Chen, L. K. Thompson, J. N. Bridson, *Inorg. Chem.* **1993**, 32, 2938.
- [10] S. S. Tandon, L. K. Thompson, R. C. Hynes, *Inorg. Chem.* **1992**, 31, 2210.
- [11] F. W. Hartstock, L. K. Thompson, *Inorg. Chim. Acta* **1983**, 72, 227.
- [12] S. K. Mandal, L. K. Thompson, E. J. Gabe, F. L. Lee, J.-P. Charland, *Inorg. Chem.* **1987**, 26, 2384; L. Chen, L. K. Thompson, J. N. Bridson, *Inorg. Chim. Acta* **1993**, 214, 67.
- [13] P. Hubberstey, C. E. Russell, *J. Chem. Soc. Chem. Commun.* **1995**, 959.
- [14] P. N. W. Baxter, H. Sleiman, J. M. Lehn, K. Rissanen, *Angew. Chem.* **1997**, 109, 1350; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1294.
- [15] M.-T. Youinou, N. Rahmouni, J. Fischer, J. A. Osborn, *Angew. Chem.* **1992**, 104, 771; *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 733.
- [16] See for example: R. R. Gagne, C. A. Koval, T. J. Smith, M. C. Cimolino, *J. Am. Chem. Soc.* **1979**, 101, 4571; R. R. Gagne, L. M. Henling, T. J. Kistenmacher, *Inorg. Chem.* **1980**, 19, 1226; S. K. Mandal, B. Adhikary, K. Nag, *J. Chem. Soc. Dalton Trans.* **1986**, 1175; S. S. Tandon, L. K. Thompson, J. N. Bridson, *Inorg. Chem.* **1993**, 32, 32; H. Okawa, M. Tadokoro, Y. Aratake, M. Ohba, K. Shindo, M. Mitsumi, M. Koikawa, M. Tomono, D. E. Fenton, *J. Chem. Soc. Dalton Trans.* **1993**, 253; K. K. Nanda, A. W. Addison, N. Paterson, E. Sinn, L. K. Thompson, U. Sakaguchi, *Inorg. Chem.* **1998**, 37, 1028.
- [17] S. Brooker, unpublished results.
- [18] H. Sleiman, P. N. W. Baxter, J. M. Lehn, K. Airola, K. Rissanen, *Inorg. Chem.* **1997**, 36, 4734.
- [19] F. J. Romero-Salguero, J.-M. Lehn, *Tetrahedron Lett.* **1999**, 40, 859.
- [20] J. Rojo, F. J. Romero-Salguero, J.-M. Lehn, G. Baum, D. Fenske, *Eur. J. Inorg. Chem.* **1999**, 1421.
- [21] G. J. Kubas, *Inorg. Synth.* **1991**, 68.
- [22] W. J. Geary, *Coord. Chem. Rev.* **1971**, 7, 81.
- [23] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, 46, 467.
- [24] G. M. Sheldrick, *Methods Enzymol.* **1997**, 276, 628.
- [25] G. M. Sheldrick, T. R. Schneider, *Methods Enzymol.* **1997**, 277, 319.

Intermolecular Trapping of the Nazarov Intermediate: Domino Electrocyclization/[3+2] Cycloadditions with Allylsilanes**

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Reactions that entail the stereoselective creation of multiple carbon–carbon bonds are highly valued for their synthetic utility.^[1] In particular, one-step processes that convert readily available, acyclic components into fused or bridged polycyclic products offer a powerful method for increasing molecular complexity.^[2] We have described several examples of the deliberate intramolecular trapping of oxyallyl cations generated during the Nazarov cyclization^[3] of 1,4-dien-3-ones, which has resulted in the high yield and stereoselective formation of diverse polycyclic products.^[4] These studies were predicated on the idea that a pendant alkene trap could intercept the oxyallyl cation intermediate at a rate competitive with the normal elimination pathway, a process that we have termed the “interrupted Nazarov” reaction. More surprising was our observation of the high-yield intermolecular trapping of the Nazarov intermediate using silyl hydride.^[5] This occurrence suggests a much broader potential for this process.

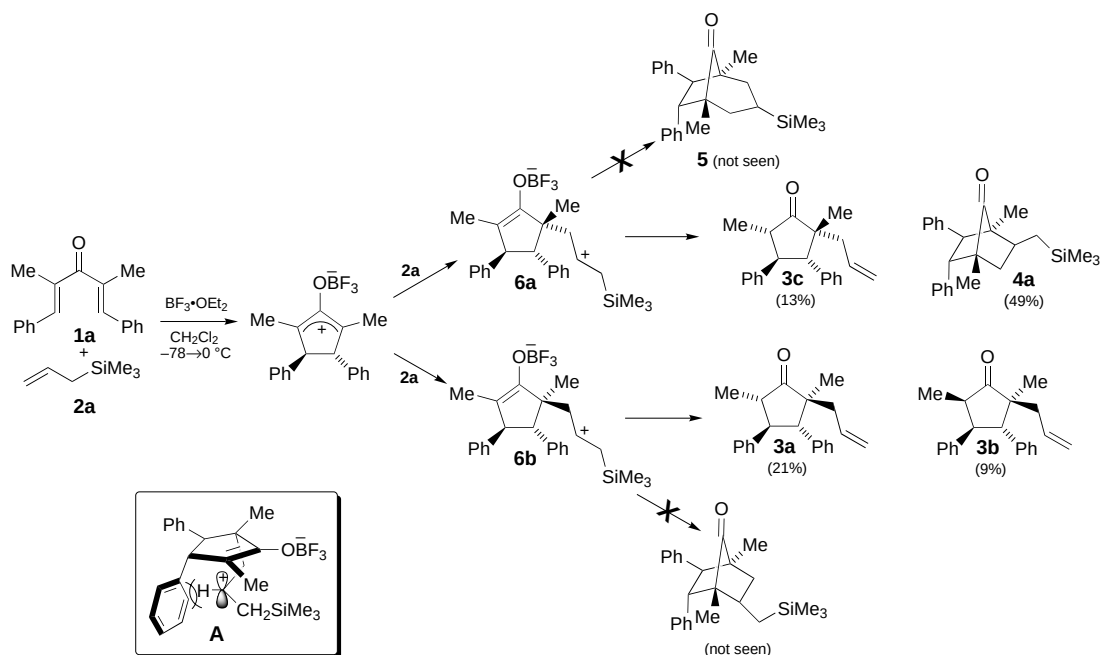
These results prompted us to investigate the use of simple allylic silanes in the intermolecular trapping of Nazarov intermediates.^[6] The well-precedented nucleophilic reactivity of allylsilanes^[7] and their relative stability under Lewis acidic conditions suggested that they might function as effective traps of the oxyallyl cation intermediate. The expected 2-allylcyclopentanones could serve as useful building blocks in the construction of a variety of cyclopentanoid targets. Surprisingly, simple allylation was found to be a minor pathway—the major products in most cases were unprecedented bicyclo[2.2.1]heptanones resulting from a formal [3+2] addition of the oxyallyl cation and the alkene.^[8, 9] Described below are the preliminary results of this study, which offers a novel, one-step preparation of bridged bicyclic systems from two simple, unsaturated fragments.

Initial experiments employed dienone **1a**^[10a,b] and allyltrimethylsilane (**2a**). Treatment of a mixture of these two compounds with $BF_3 \cdot OEt_2$ at low temperature resulted in the formation of several diastereomeric allylated cyclopentanones (**3a–c**) in a combined yield of 43 %, along with 49 % of an entirely different product (as a single isomer) lacking any unsaturation (Scheme 1). The presence of a trimethylsilyl group suggested that this product was either bicyclo[2.2.1]-heptanone **4a** or the isomeric bicyclo[3.2.1]octanone **5**, which result from either direct ring closure of the intermediate

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Scheme 1.

zwitterion **6a** or ring closure with concomitant sila-Wagner-Meerwein shift, respectively. Proton and carbon NMR data were more consistent with structure **4a** (with the indicated stereochemistry), and further support came from the unambiguous assignment of closely related structures by X-ray diffraction analysis (see below). Notably, no products derived from either the simple Nazarov cyclization of **1a** or the Hosomi–Sakurai addition of **2a** to **1a**^[11] were observed.

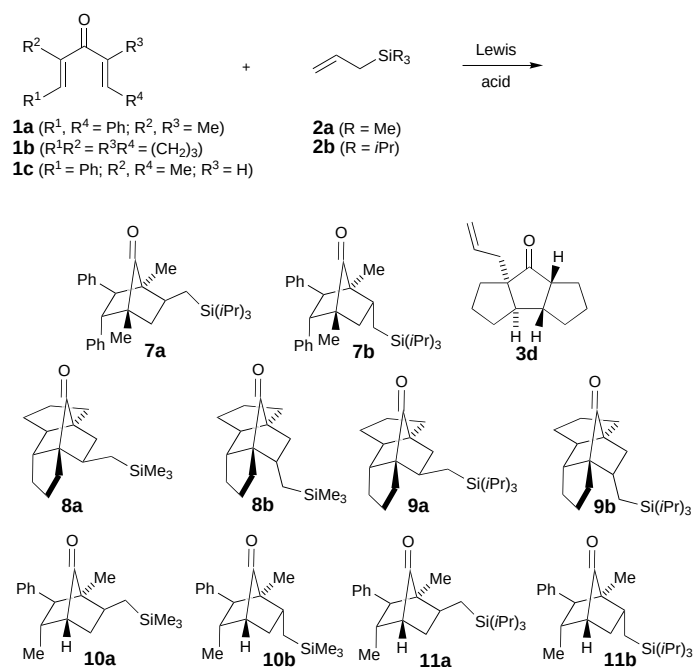
The formation of **4a** entails three successive carbon–carbon bond-forming steps: a) electrocyclic closure of the dienone–Lewis acid complex to generate the oxyallyl intermediate; b) nucleophilic attack of the allylsilane γ carbon atom at one of the oxyallyl termini to produce an adduct with a boron enolate and a silicon-stabilized carbocation; and c) collapse of this zwitterion to the bridged bicyclic product. The majority of the simple allylated material (**3a** + **3b**, 30%) resulted from **6b**, which was formed by the allylsilane approaching from the face opposite the adjacent phenyl group. In contrast, **4a** derives from **6a**, which is formed by the apparent attack of the allylsilane from the same face as the adjacent phenyl ring. The failure to observe any bicyclic products resulting from **6b** may be a result of unfavorable nonbonded interactions in the transition state (**A**) for ring closure.

These promising results prompted a more comprehensive examination of this novel domino process (Table 1). Preliminary screening of conditions established that $\text{BF}_3 \cdot \text{OEt}_2$ and SnCl_4 gave superior yields and selectivity, and subsequent reactions were carried out with only these Lewis acids. Variation of the stoichiometry was also informative: while the initial result was obtained using ten equivalents of allylsilane to ensure efficient trapping of the oxyallyl intermediate, it quickly became apparent that nearly comparable results were obtained with as little as two equivalents (entries 1 vs. 2, 5 vs. 6, 7 vs. 8, 10 vs. 11, and 12 vs. 13). We

were interested in examining the effect of a bulkier silyl group, as this change was expected to suppress desilylation and favor the [3+2] pathway.^[12] In the event, treatment of **1a** with allyltriisopropylsilane (**2b**) yielded approximately 50% of the [3+2] adduct with either $\text{BF}_3 \cdot \text{OEt}_2$ or SnCl_4 , with little or no accompanying allylation product **3** (entries 4–6). A remarkable dependence of the stereochemistry on the Lewis acid used was noted in this case: while $\text{BF}_3 \cdot \text{OEt}_2$ led to a 1:1 mixture of diastereomers **7a** and **7b**, SnCl_4 gave exclusively *exo* isomer **7b**, whose structure was unambiguously determined by X-ray diffraction analysis. As noted below, this was also seen in a number of other cases.

The nature of the dienone was also probed. Dienone **1a** was known to undergo rapid electrocyclization even at low temperature, and we wondered whether a less reactive dienone could also be used in this domino process. To that end, we next examined the behavior of dicyclopentenyl ketone (**1b**)^[10c] with **2a** and **2b** (entries 7–13).^[13] Cyclization of **1b** in the presence of **2a** and $\text{BF}_3 \cdot \text{OEt}_2$ provided the [3+2] adduct **8a** in 35% yield as a single (*endo*) diastereomer, while use of SnCl_4 led to diastereomeric **8b** in low yield. On the other hand, the corresponding [3+2] adducts **9a** and **9b** were obtained in 65 and 91% yields, respectively (entries 10–13), with allylsilane **2b**. The structure of **9b** was unambiguously determined by X-ray crystallography, and strong support for the structure assigned to **9a** was obtained through two-dimensional NMR analysis. As in the case of **8a** and **8b**, the stereochemistry of the [3+2] adducts was completely dependent upon the choice of Lewis acid. The initial attack of the allylsilane occurs from the less-hindered face (Scheme 2, path a) during the formation of **8a**, **b** and **9a**, **b**, which is in contrast to the results obtained with **1a**. This is likely a consequence of the unfavorable *trans*-diquinane ring fusion that would result from an attack analogous to that in the monocyclic cases (path b). However, a *trans* ring fusion is then

Table 1. Dienone + allylsilane domino [3+2] trapping reactions.^[a]

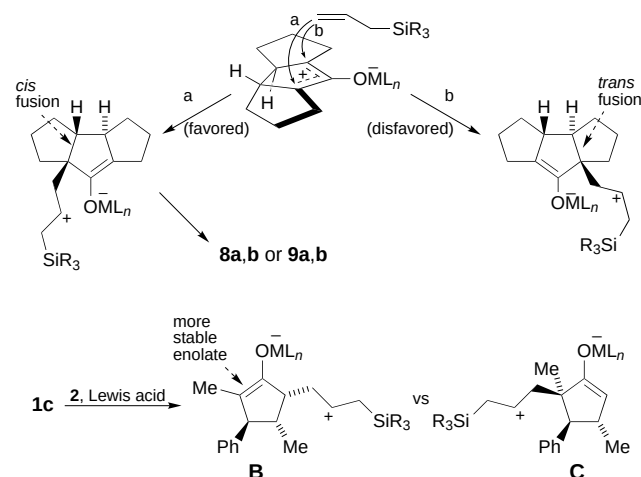


Entry	Dienone	Allylsilane (equiv)	Lewis acid	Products (% yield) ^[b]
1	1a	2a (10)	$\text{BF}_3 \cdot \text{OEt}_2$	3a-c (43) + 4a (49)
2	1a	2a (2)	$\text{BF}_3 \cdot \text{OEt}_2$	3a-c (35) + 4a (42)
3	1a	2a (10)	SnCl_4	3a-c (29) + 4a (29)
4	1a	2b (5)	$\text{BF}_3 \cdot \text{OEt}_2$	3a (28) + 7a (25) + 7b (25)
5	1a	2b (5)	SnCl_4	7b (51)
6	1a	2b (2)	SnCl_4	7b (47)
7	1b	2a (10)	$\text{BF}_3 \cdot \text{OEt}_2$	3d (30) + 8a (35)
8	1b	2a (2)	$\text{BF}_3 \cdot \text{OEt}_2$	3d (30) + 8a (32)
9	1b	2a (10)	SnCl_4	3d (35) + 8b (10)
10	1b	2b (10)	$\text{BF}_3 \cdot \text{OEt}_2$	9a (65)
11	1b	2b (2)	$\text{BF}_3 \cdot \text{OEt}_2$	9a (60)
12	1b	2b (5)	SnCl_4	9b (90)
13	1b	2b (2)	SnCl_4	9b (91)
14	1c	2a (10)	$\text{BF}_3 \cdot \text{OEt}_2$	10a
15	1c	2a (10)	SnCl_4	10a (37) + 10b (28)
16	1c	2b (10)	$\text{BF}_3 \cdot \text{OEt}_2$	11a (72)
17	1c	2b (10)	SnCl_4	11a (38) + 11b (61)

[a] See Experimental Section for a typical procedure. Further details can be found in the Supporting Information. [b] Yields of isolated products after chromatography.

unavoidable in the final ring closure. In light of the strain inherent in the products, the efficiency with which **9a** and **9b** are formed is quite remarkable.

Finally, the behavior of unsymmetrical dienone **1c** was studied to probe the regiochemical preferences of the domino process (entries 14–17). Cyclization in the presence of **2a** or **2b** using $\text{BF}_3 \cdot \text{OEt}_2$ led to the exclusive formation of *exo* products **10a** and **11a**, respectively, while SnCl_4 provided mixtures of *endo* and *exo* isomers with either allylsilane. In all four examples the initial attack on the unsymmetrical oxyallyl system occurred at the less-substituted terminus. On the basis of related intermolecular trapping reactions involving photochemically generated oxyallyls, we had expected attack to occur preferentially at the more-substituted end because of the greater positive-charge density.^[14] However, exclusive



Scheme 2.

bond formation at the less substituted end of the allyl system is consistent with earlier observations by Noyori et al.,^[15] and can be rationalized in terms of enolate stability, with preferred formation of the more substituted isomer **B** rather than **C** (Scheme 2). As with **1a**, the allylsilane approached the oxyallyl from the same face as the larger (methyl) group on the neighboring carbon atom.

We have described the first examples of intermolecular trapping of the Nazarov oxyallyl intermediate by carbon nucleophiles. This transformation creates three new carbon–carbon bonds and up to five contiguous stereocenters during the construction of densely functionalized bicyclo[2.2.1]heptanones from simple dienone and allylsilane precursors. Regioselectivity is complete in the case of an unsymmetrical dienone, as is the stereoselectivity of the initial trapping step in all cases. The final ring-closure step is highly sensitive to the Lewis acid used in the electrocyclization: in several cases complete selectivity for *exo*- or *endo*-disposed CH_2SiR_3 groups could be achieved by use of either $\text{BF}_3 \cdot \text{OEt}_2$ or SnCl_4 . Further studies of the scope of this process and the origins of the Lewis acid-dependence of the stereochemical outcome are underway and will be disclosed in due course.

Experimental Section

Cyclization of **1a** in the presence of **2a** (representative procedure): CH_2Cl_2 (10 mL) and **2a** (217 mg, 1.9 mmol) were added to a dry 50-mL round-bottom flask equipped with a magnetic stir bar, nitrogen inlet, and rubber septum, and the resulting solution was cooled to -78°C . $\text{BF}_3 \cdot \text{OEt}_2$ (30 mg, 0.21 mmol) was added by syringe followed by the dropwise addition of a solution of **1a** (50 mg, 0.19 mmol) in CH_2Cl_2 (10 mL) by cannula. The resulting slightly yellow-brown solution was allowed to slowly warm to 0°C and the reaction was quenched by the addition of H_2O (5 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (15 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated under reduced pressure to give a colorless oil which was purified by flash chromatography (silica gel, hexanes/EtOAc 30/1 $\rightarrow$ 25/1 $\rightarrow$ 20/1 $\rightarrow$ 15/1) to provide **4a** (35 mg, 49%) as a white solid, **3a** (12 mg, 21%) as a white solid and an inseparable mixture of **3b** and **3c** ($\sim$ 2/3; 12 mg, 21%) as a colorless oil. For purposes of characterization, **3c** could be obtained in pure form by carrying out the reaction using TiCl_4 in place of $\text{BF}_3 \cdot \text{OEt}_2$. **4a**: m.p. $115\text{--}116^\circ\text{C}$; $R_f = 0.56$ (hexanes/EtOAc 4/1); ^1H NMR (500 MHz, CDCl_3 , 25°C , TMS): $\delta = 7.39\text{--}7.01$ (m, 10H), 3.30 (d, $^3J(\text{H,H}) = 7.2$ Hz, 1H), 3.12 (dd, $^3J(\text{H,H}) = 7.2$, $^4J(\text{H,H}) = 2.2$ Hz, 1H), 2.21 (dd,

$^2J(\text{H,H}) = 12.9$, $^3J(\text{H,H}) = 10.1$ Hz, 1 H), 2.15–2.09 (m, 1 H), 1.02 (s, 3 H), 0.87 (ddd, $^2J(\text{H,H}) = 13.0$, $^3J(\text{H,H}) = 4.7$, $^4J(\text{H,H}) = 2.1$ Hz, 1 H), 0.83 (dd, $^2J(\text{H,H}) = 14.1$, $^3J(\text{H,H}) = 2.4$ Hz, 1 H), 0.70 (s, 3 H), 0.26 (dd, $^2J(\text{H,H}) = 13.9$, $^3J(\text{H,H}) = 13.3$ Hz, 1 H), 0.04 (s, 9 H); ^{13}C NMR (75 MHz, CDCl_3 , 25 °C, TMS): $\delta = 219.5$, 143.6, 138.3, 129.5, 128.6, 128.5, 128.5, 127.2, 126.9, 55.8, 55.6, 51.6, 48.3, 40.1, 35.0, 21.6, 14.4, 10.6, –0.3; IR (thin film): $\tilde{\nu} = 1756.8\text{ cm}^{-1}$; elemental analysis calcd for $\text{C}_{25}\text{H}_{32}\text{OSi}$: C 79.71, H 8.58; found: C 79.63, H 8.57. **3a**: m.p. 80–81 °C; $R_f = 0.50$ (hexanes/EtOAc 4/1); ^1H NMR (300 MHz, CDCl_3 , 25 °C, TMS): $\delta = 7.23$ –7.11 (m, 10 H), 5.87–5.73 (m, 1 H), 5.28–5.22 (m, 2 H), 3.69 (d, $^2J(\text{H,H}) = 12.2$ Hz, 1 H), 3.25 (dd, $^3J(\text{H,H}) = 12.0$, 12.0 Hz, 1 H), 2.53 (dddd, $^2J(\text{H,H}) = 14.3$, $^3J(\text{H,H}) = 5.6$, $^4J(\text{H,H}) = 1.7$, 1.7 Hz, 1 H), 2.26 (dq, $^3J(\text{H,H}) = 11.7$, 6.9 Hz, 1 H), 2.11 (dd, $^2J(\text{H,H}) = 14.4$, $^3J(\text{H,H}) = 13.9$ Hz, 1 H), 1.13 (d, $^3J(\text{H,H}) = 6.8$ Hz, 3 H), 0.76 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3 , 25 °C, TMS): $\delta = 221.5$, 140.9, 137.4, 134.6, 129.4, 128.8, 128.3, 127.9, 127.0, 126.8, 119.5, 53.5, 53.4, 52.8, 50.8, 41.2, 20.6, 13.2; IR (thin film): $\tilde{\nu} = 1734\text{ cm}^{-1}$; elemental analysis calcd for $\text{C}_{22}\text{H}_{22}\text{O}$: C 86.78, H 7.96; found: C 86.64, H 8.07. **3b**: $R_f = 0.46$ (hexanes/EtOAc 4/1); ^1H NMR (300 MHz, CDCl_3 , 25 °C, TMS): $\delta = 7.28$ –7.11 (m, 10 H), 5.85–5.70 (m, 1 H), 5.30–5.24 (m, 2 H), 4.15 (dd, $^2J(\text{H,H}) = 13.2$, 8.3 Hz, 1 H), 3.93 (d, $^3J(\text{H,H}) = 13.0$ Hz, 1 H), 2.84 (dq, $^3J(\text{H,H}) = 8.1$, 8.0 Hz, 1 H), 2.55 (dddd, $^2J(\text{H,H}) = 14.2$, $^3J(\text{H,H}) = 5.1$, $^4J(\text{H,H}) = 1.6$, 1.6 Hz, 1 H), 2.11 (dd, $^2J(\text{H,H}) = 14.0$, $^3J(\text{H,H}) = 9.0$ Hz, 1 H), 0.83 (s, 3 H), 0.74 (d, $^3J(\text{H,H}) = 7.8$ Hz, 3 H); ^{13}C NMR (75 MHz, CDCl_3 , 25 °C, TMS): $\delta = 223.3$, 138.4, 137.7, 135.0, 129.2, 129.0, 128.4, 126.9, 126.5, 119.4, 54.4, 47.1, 46.6, 44.8, 40.4, 20.7, 12.0 (one carbon resonance is obscured by an overlapping resonance from **3c**). **3c**: m.p. 96 °C; $R_f = 0.46$ (hexanes/EtOAc 4/1); ^1H NMR (300 MHz, CDCl_3 , 25 °C, TMS): $\delta = 7.28$ –7.11 (m, 10 H), 5.51 (dddd, $^3J(\text{H,H}) = 16.8$, 10.0, 7.3, 7.3 Hz, 1 H), 4.97 (dddd, $^3J(\text{H,H}) = 10.0$, $^2J(\text{H,H}) = 2.1$, $^4J(\text{H,H}) = 1.0$, 1.0 Hz, 1 H), 4.86 (dddd, $^3J = 16.8$, $^2J(\text{H,H}) = 2.2$, $^4J(\text{H,H}) = 1.4$, 1.1 Hz, 1 H), 3.44 (dd, $^3J(\text{H,H}) = 11.9$, 11.2 Hz, 1 H), 3.43 (d, $^3J(\text{H,H}) = 11.9$ Hz, 1 H), 2.38 (dq, $^3J(\text{H,H}) = 11.2$, 6.8 Hz, 1 H), 2.13 (dd, $^2J(\text{H,H}) = 14.1$, $^3J(\text{H,H}) = 7.1$ Hz, 1 H), 1.72 (dddd, $^2J(\text{H,H}) = 14.1$, $^3J(\text{H,H}) = 7.3$, $^4J(\text{H,H}) = 1.1$, 1.1 Hz, 1 H), 1.22 (s, 3 H), 1.19 (d, $^3J(\text{H,H}) = 6.9$ Hz, 3 H); ^{13}C NMR (75 MHz, CDCl_3 , 25 °C, TMS): $\delta = 220.7$, 141.0, 136.7, 133.6, 129.4, 128.8, 128.3, 127.9, 127.1, 127.0, 118.2, 58.9, 53.2, 52.3, 50.4, 37.8, 22.1, 13.7; IR (thin film): $\tilde{\nu} = 1734.7\text{ cm}^{-1}$; elemental analysis calcd for $\text{C}_{22}\text{H}_{22}\text{O}$: C 86.78, H 7.96; found: C 86.71, H 8.01.

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- [1] a) L. F. Fietze, *Chem. Rev.* **1996**, 96, 115–136; b) N. Hall, *Science* **1994**, 266, 32–34; c) T. L. Ho, *Tandem Reactions in Organic Synthesis*, Wiley-Interscience, New York, **1992**.
- [2] a) T. Hudlicky, *Chem. Rev.* **1996**, 96, 3–30; b) T. Hudlicky, M. G. Natchus in *Organic Synthesis: Theory and Applications*, Vol. 2 (Ed.: T. Hudlicky), JAI Press, Greenwich, CT, **1993**, pp. 1–26; c) P. A. Wender, B. L. Miller in *Organic Synthesis: Theory and Applications*, Vol. 2 (Ed.: T. Hudlicky), JAI Press, Greenwich, CT, **1993**, pp. 27–66.
- [3] Reviews: a) K. L. Habermas, S. E. Denmark, T. K. Jones, *Org. React. (N.Y.)* **1994**, 45, 1–158; b) S. E. Denmark in *Comprehensive Organic Synthesis*, Vol. 5 (Eds.: B. M. Trost, I. Fleming), Pergamon, Oxford, **1991**, pp. 751–784.
- [4] a) J. A. Bender, A. E. Blize, C. C. Browder, S. Giese, F. G. West, *J. Org. Chem.* **1998**, 63, 2430–2431; b) Y. Wang, A. M. Arif, F. G. West, *J. Am. Chem. Soc.* **1999**, 121, 876–877; c) C. C. Browder, F. G. West, *Synlett* **1999**, 1363–1366; d) J. A. Bender, A. M. Arif, F. G. West, *J. Am. Chem. Soc.* **1999**, 121, 7443–7444.
- [5] S. Giese, F. G. West, *Tetrahedron Lett.* **1998**, 39, 8393–8396.
- [6] Reviews on allylsilanes: a) I. Fleming, A. Barbero, D. Walter, *Chem. Rev.* **1997**, 97, 2063–2192; b) I. Fleming, J. Dunoguès, R. Smithers, *Org. React. (N.Y.)* **1989**, 37, 57–575.
- [7] a) M. F. Gotta, H. Mayr, *J. Org. Chem.* **1998**, 63, 9769–9775; b) H. Mayr, M. Patz, *Angew. Chem.* **1994**, 106, 990–1010; *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 938–957.
- [8] Recent examples of [3+2] annulations employing allylsilanes: a) H. Monti, D. Rizzotto, G. Léandri, *Tetrahedron* **1998**, 54, 6725–6738; b) H.-J. Knölker, P. G. Jones, G. Wanzl, *Synlett* **1998**, 613–616; c) G. M. Choi, S. H. Yeon, J. Jin, B. R. Yoo, I. N. Jung, *Organometallics* **1997**, 16, 5158–5162; d) W. S. Murphy, D. Neville, *Tetrahedron Lett.*

- 1997**, 38, 7933–7936; e) H.-J. Knölker, N. Foitzik, H. Goesmann, R. Graf, P. G. Jones, G. Wanzl, *Chem. Eur. J.* **1997**, 3, 538–551; f) R. L. Danheiser, T. Takahashi, B. Bertók, B. R. Dixon, *Tetrahedron Lett.* **1993**, 34, 3845–3848; g) R. L. Danheiser, B. R. Dixon, R. W. Gleason, *J. Org. Chem.* **1992**, 57, 6094–6097.
- [9] Other oxyallyl + alkene [3+2] annulations: a) H. Mizuno, K. Domon, K. Masuya, K. Tanino, I. Kuwajima, *J. Org. Chem.* **1999**, 64, 2648–2656; b) S. A. Hardinger, C. Bayne, E. Kanorowski, R. McClellan, L. Larres, M.-A. Nuesse, *J. Org. Chem.* **1995**, 60, 1104–1105; c) Y. Hayakawa, K. Yokoyama, R. Noyori, *J. Am. Chem. Soc.* **1978**, 100, 1799–1806.
- [10] a) C. W. Shoppee, B. J. A. Cooke, *J. Chem. Soc. Perkin Trans. 1* **1973**, 1026–1030; b) P. Yates, N. Yoda, W. Brown, B. Mann, *J. Am. Chem. Soc.* **1958**, 80, 202–205; c) P. E. Eaton, C. Giordano, G. Schloemer, U. Vogel, *J. Org. Chem.* **1976**, 41, 2238–2241.
- [11] a) A. Hosomi, H. Sakurai, *J. Am. Chem. Soc.* **1977**, 99, 1673–1675; b) H. Sakurai, A. Hosomi, J. Hayashi, *Org. Synth.* **1984**, 62, 86–94.
- [12] a) H.-J. Knölker, *J. Prakt. Chem.* **1997**, 339, 304–314; b) H.-J. Knölker, N. Foitzik, H. Goesmann, R. Graf, *Angew. Chem.* **1993**, 105, 1104–1106; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 1081–1083; c) G. Hagen, H. Mayr, *J. Am. Chem. Soc.* **1991**, 113, 4954–4961.
- [13] Previous studies with hydride trapping indicated that electrocyclization of **1b** requires warming the reaction mixture to 0–10 °C.^[5]
- [14] F. G. West, P. V. Fisher, G. U. Gunawardena, S. Mitchell, *Tetrahedron Lett.* **1993**, 34, 4583–4586.
- [15] R. Noyori, F. Shimizu, K. Fukuta, H. Takaya, Y. Hayakawa, *J. Am. Chem. Soc.* **1977**, 99, 5196–5198.

Reaction of $[\{\text{PhP}(\text{Se})(\mu\text{-Se})\}_2]$ with Dialkyl Cyanamides: X-ray Crystal Structures of the Phosphorus-Containing Triselenapentalenes $[\text{Me}_2\text{N}-\text{C}(\text{Se})=\text{N}]_2\text{P}(\text{Se})\text{Ph}$ and $[\text{O}(\text{CH}_2\text{CH}_2)_2\text{N}-\text{C}(\text{Se})=\text{N}]_2\text{P}(\text{Se})\text{Ph}^{**}$

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The chemistry of phosphorus–selenium heterocycles $(\text{RP})_x\text{Se}_y$ ($\text{R} = \text{alkyl or aryl}$), which are generally prepared by oxidation of the parent homocyclic polyphosphanes $(\text{RP})_n$ with stoichiometric quantities of elemental selenium, has received scant attention.^[1–7] The studies undertaken on the reactivity of these molecules have to date been directed towards the development of new selenating reagents for the transformation of carbonyl groups in organic molecules to selenocarbonyls.^[1, 8] In this regard, an analogue is sought of the commercially available 2,4-bis(*p*-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide $[\{(p\text{-MeOC}_6\text{H}_4)\text{P}(\text{S})(\mu\text{-S})\}_2]$ (Lawesson's reagent), a valuable tool for the conversion of carbonyl compounds to their thiocarbonyl counterparts.^[9]

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The oxidation of pentaphenylcyclopentaphosphane, (PhP)₅, with ten molar equivalents of selenium gives an insoluble red solid which has been characterized as 2,4-diphenyl-1,3-disele-nadiphosphetane-2,4-diselenide [[PhP(Se)(μ-Se)]₂].^[4, 5] Few in-vestigations into the reactivity of this compound have been carried out. Hill and co-workers have reported its use, prepared in situ from (PPh)₃/Se, for the conversion of molybdenum and tungsten ketenyl complexes to their selenoketenyl analogues.^[8] Additionally, treatment of *cis*-[PtCl₂(PR₃)₂] (R = ½ dppe, PET₃, PMe₂PH or PPh₂Me) with [[PhP(Se)(μ-Se)]₂] in THF leads to rupture of the four-membered P₂(μ-Se)₂ ring to afford [Pt(Se₃PPh-Se,Se')(PR₃)₂].^[10] The facile heterocycle formation from [[FcP(S)(μ-S)]₂] (Fc = ferrocenyl) using dialkyl cyana-mides, to give thiadiazaphosphorines,^[11] led us to investigate the reactivity of cyanamides with [[PhP(Se)(μ-Se)]₂], the results of which we report here.

A refluxing toluene solution of [[PhP(Se)(μ-Se)]₂] with a ten-fold excess of R₂NCN (R₂ = Me₂, -CH₂CH₂OCH₂CH₂-, or -(CH₂)₅-) changes from red to lime green over 18 h, with precipitation of small quantities of elemental selenium. Following column chromatography and recrystallization from dichloromethane/*n*-hexane, the 1,6,6λ⁴-triseleno-3a-phospha-3,4-diazapentalenes **1–3** are available as air- and moisture-stable yellow solids, albeit in poor yields (about 5 %), together with R₂NC(Se)NH₂. Compounds **1–3** represent the first phosphorus-containing triselenapentalenes; two of them, **1** and **2**, have been crystallographically characterized (Figures 1 and 2).^[12]

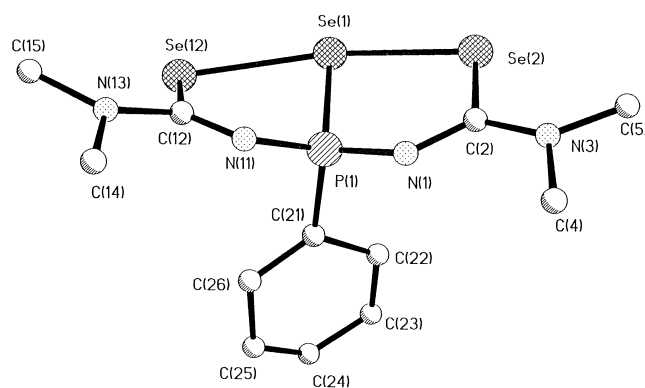
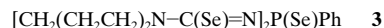
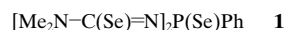


Figure 1. Molecular structure of **1** (C–H bonds omitted for clarity). Selected bond lengths [Å] and angles [°]: Se(1)–P(1) 2.229(1), Se(1)–Se(12) 2.703(1), Se(1)–Se(2) 2.578(1), P(1)–N(11) 1.620(3), P(1)–N(1) 1.609(3), N(1)–C(2) 1.308(5), N(11)–C(12) 1.318(4), C(2)–Se(2) 1.921(4), C(12)–Se(12) 1.913(4), N(3)–C(2) 1.329(5), N(13)–C(12) 1.332(5); P(1)–Se(1)–Se(12) 78.66(3), P(1)–Se(1)–Se(2) 86.25(3), Se(12)–Se(1)–Se(2) 164.27(2), N(11)–P(1)–N(1) 110.4(2), N(11)–P(1)–Se(1) 110.08(12), N(1)–P(1)–Se(1) 109.37(12), C(2)–N(1)–P(1) 124.8(3), C(12)–N(11)–P(1) 119.5(3), C(2)–Se(2)–Se(1) 93.35(12), C(12)–Se(12)–Se(1) 90.86(12).

The structures of **1** and **2** contain a central core of two fused five-membered PSe₂NC rings with the amine substituents lying in the same approximate plane as the five-membered

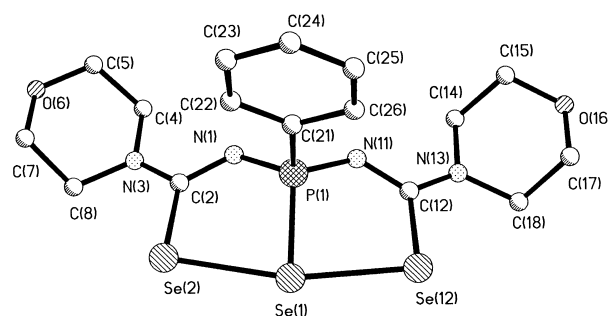


Figure 2. Molecular structure of **2** (C–H bonds omitted for clarity). Selected bond lengths [Å] and angles [°]: Se(1)–P(1) 2.224(2), Se(1)–Se(12) 2.621(1), Se(1)–Se(2) 2.648(1), P(1)–N(11) 1.605(5), P(1)–N(1) 1.608(5), N(1)–C(2) 1.320(8), N(11)–C(12) 1.315(8), C(2)–Se(2) 1.913(6), C(12)–Se(12) 1.925(6), N(3)–C(2) 1.332(9), N(13)–C(12) 1.328(8); P(1)–Se(1)–Se(12) 83.37(5), P(1)–Se(1)–Se(2) 82.31(5), Se(12)–Se(1)–Se(2) 165.21(3), N(11)–P(1)–N(1) 109.3(3), N(11)–P(1)–Se(1) 109.7(2), N(1)–P(1)–Se(1) 110.3(2), C(2)–N(1)–P(1) 122.5(5), C(12)–N(11)–P(1) 123.3(4), C(2)–Se(2)–Se(1) 92.5(2), C(12)–Se(12)–Se(1) 92.2(2).

ring with which they are associated, with the phenyl sub-stituent on the central phosphorus approximately orthogonal to the central core. In contrast to triselenapentalenes with carbon- and nitrogen-containing backbones,^[13–15] the tetra-hedral geometry at P(1) in **1** and **2** imparts substantial non-planarity to the pentalene ring system in these compounds. For **2**, the mean deviations of the P(1)–Se(1)–C(2)–N(1)–Se(2) and P(1)–Se(1)–C(12)–N(11)–Se(12) planes are 0.19 Å and 0.18 Å, respectively, while in **1** these values are 0.09 Å and 0.26 Å. The Se(2)–Se(1)–Se(12) angles are similar in each structure (164.27(2)° for **1**, 165.21(3)° for **2**); the Se–Se distances in **2** are approximately equal (Se(1)–Se(12) 2.621(1), Se(1)–Se(2) 2.648(1) Å), although there is a pro-nounced asymmetry in the corresponding bond length values for **1** (Se(1)–Se(12) 2.703(1) Å, Se(1)–Se(2) 2.578(1) Å).

In **1**, a short intermolecular distance between Se(12) and a phenyl ring hydrogen atom in an adjacent molecule (Se(12)⋯H(22a') 3.08 Å) may make a small contribution to lengthening of the Se(1)–Se(12) vector compared with that in **2**, however, the absence of any other significant intermolecular contacts such as π-stacking of the phenyl rings or Se⋯Se interactions in either structure makes the disparate Se–Se lengths in **1** more perplexing. The P(1)–Se(1) distances in **1** and **2** (2.229(1) and 2.224(2) Å, respectively) are substantially longer than the more typical lengths of 2.08–2.12 Å for selenides containing P^V=Se bonds,^[4, 16] but still about 0.06 Å shorter than reported for heterocycles containing P^V–Se single bonds.^[1, 2, 4] In conjunction with the short P–N bond lengths (1.605(5)–1.620(3) Å), this indicates extensive elec-tron delocalization within the pentalene framework. The remaining bonding parameters in **1** and **2** are in good agreement with each other and to related triselenapentalene structures.^[13–15]

The weakness of the P(1)–Se(1) bond in the molecular structures of **1** and **2** is highlighted by the abnormally low ¹J(P–Se) couplings observed in the ³¹P{¹H} NMR spectra of **1–3** (317–324 Hz), values more comparable with the ¹J(P–Se) couplings in the triphospholane (PhP)₃Se₂ (260–305 Hz) which contains P^{III}–Se single bonds.^[5] No ²J(P–Se) couplings

are resolved for **1–3**. The results described here suggest that there is a potentially large and interesting cycloaddition chemistry associated with phosphorus–selenium heterocycles.

Experimental Section

General procedure for **1–3**: A toluene solution (10 mL) of $[(\text{PhP}(\text{Se})-(\mu\text{-Se}))_2]$ (0.15 mmol) and $\text{R}_2\text{N-CN}$ (1.5 mmol) was heated at reflux for 18 h during which time the solution changed color from red to lime green, with the precipitation of a small quantity of selenium. Upon cooling to room temperature, the solvent was removed in vacuo and the products extracted into dichloromethane (2 mL). Column chromatography using silica (dichloromethane eluant) gave **1–3** as yellow solids. Yellow crystals of **1** and **2** were isolated upon layering of a dichloromethane (1 mL) solution of each compound with *n*-hexane at room temperature.

IR: KBr disk; $^{31}\text{P}\{^1\text{H}\}$ NMR: CDCl_3 solvent, 121.5 MHz; MS: FAB^+ , 3-nitrobenzyl alcohol (3-NOBA) matrix.

1: IR: $\tilde{\nu}=1528\text{ s }(\nu_{\text{CN}})$, $1109\text{ s }(\nu_{\text{PC}})$, $990\text{ s }(\nu_{\text{PN}})$, $524\text{ m cm}^{-1}(\nu_{\text{PSe}})$; MS: 486 $[M^+]$; $^{31}\text{P}\{^1\text{H}\}$ NMR: $\delta=82.7$ ($^1J(\text{P,Se})=317\text{ Hz}$).

2: IR: $\tilde{\nu}=1502\text{ s }(\nu_{\text{CN}})$, $1112\text{ s }(\nu_{\text{PC}})$, $997\text{ s }(\nu_{\text{PN}})$, $521\text{ s cm}^{-1}(\nu_{\text{PSe}})$; MS: 570 $[M^+]$; $^{31}\text{P}\{^1\text{H}\}$ NMR: $\delta=83.9$ ($^1J(\text{P,Se})=324\text{ Hz}$).

3: IR: $\tilde{\nu}=1500\text{ s }(\nu_{\text{CN}})$, $1109\text{ m }(\nu_{\text{PC}})$, $984\text{ s }(\nu_{\text{PN}})$, $512\text{ m cm}^{-1}(\nu_{\text{PSe}})$; MS: 566 $[M^+]$; $^{31}\text{P}\{^1\text{H}\}$ NMR: $\delta=81.7$ ($^1J(\text{P,Se})=318\text{ Hz}$).

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- [1] D. L. An, N. Higeta, K. Toyota, M. Yoshifuji, *Chem. Lett.* **1998**, 17, and references therein.
- [2] S. W. Hall, M. J. Pilkington, A. M. Z. Slawin, D. J. Williams, J. D. Woollins, *Polyhedron* **1991**, 10, 261.
- [3] J. T. Shore, W. T. Pennington, M. C. Noble, A. W. Cordes, *Phosphorus Sulfur Silicon Relat. Elem.* **1988**, 39, 153.
- [4] J. C. Fitzmaurice, D. J. Williams, P. T. Wood, J. D. Woollins, *J. Chem. Soc. Chem. Commun.* **1988**, 741.
- [5] P. T. Wood, J. D. Woollins, *J. Chem. Soc. Chem. Commun.* **1988**, 1190.
- [6] K. Eckstein, K. Karaghiosoff, *Phosphorus Sulfur Silicon Relat. Elem.* **1993**, 75, 257.
- [7] K. Eckstein, K. Karaghiosoff, R. Motzer, *Phosphorus Sulfur Silicon Relat. Elem.* **1994**, 93–94, 185.
- [8] I. Baxter, A. F. Hill, J. M. Malget, A. J. P. White, D. J. Williams, *Chem. Commun.* **1997**, 2049.
- [9] M. P. Cava, M. I. Levinson, *Tetrahedron* **1985**, 41, 5061.
- [10] I. P. Parkin, M. J. Pilkington, A. M. Z. Slawin, D. J. Williams, J. D. Woollins, *Polyhedron* **1990**, 9, 987.
- [11] M. R. St. John Foreman, R. J. Mortimer, A. M. Z. Slawin, J. D. Woollins, *J. Chem. Soc. Dalton Trans.* **1999**, 3419.
- [12] X-ray diffraction studies on crystals of **1** and **2**, grown from dichloromethane/*n*-hexane, were performed using a Bruker SMART diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation ($\lambda=0.71073\text{ \AA}$). The structures were solved by direct methods, the non-hydrogen atoms were refined^[17] with anisotropic displacement parameters, hydrogen atoms bound to carbon were idealized and fixed (C–H 0.95 $\text{\AA}$). Structural refinements were performed with the full-matrix least-squares method on F^2 using the program SHELXTL-PC.^[17] Crystal data for **1**: $\text{C}_{12}\text{H}_{17}\text{N}_4\text{PSe}_3$, yellow needles, $0.3 \times 0.2 \times 0.2\text{ mm}^3$, $M_r=485.15$, monoclinic, $a=9.537(1)$, $b=12.523(1)$, $c=14.178(2)\text{ \AA}$, $\beta=95.049(2)^\circ$, $F(000)=936$, $V=1687.5(3)\text{ \AA}^3$, $T=298\text{ K}$, space group $P2_1/n$, $Z=4$, $\mu(\text{MoK}\alpha)=6.629\text{ mm}^{-1}$, $2\theta_{\text{max}}=46^\circ$. Of 7191 measured data 2409 were unique reflections ($R_{\text{int}} 0.0300$) to give $R1 [I > 2\sigma(I)]=0.0265$ and $wR2=0.0568$. Crystal data for **2**: $\text{C}_{16}\text{H}_{21}\text{N}_4\text{O}_2\text{PSe}_3$, yellow needles, $0.2 \times 0.1 \times 0.1\text{ mm}^3$, $M_r=569.22$, monoclinic, $a=6.807(1)$, $b=16.023(1)$, $c=18.983(1)\text{ \AA}$, $\beta=94.787(1)^\circ$, $F(000)=1112$, $V=2063.28(7)\text{ \AA}^3$, $T=293\text{ K}$, space group $P2_1/c$, $Z=4$, $\mu(\text{MoK}\alpha)=5.444\text{ mm}^{-1}$, $2\theta_{\text{max}}=46^\circ$. Of 10306 measured data 2970 were unique reflections ($R_{\text{int}} 0.2251$) to give $R1 [I > 2\sigma(I)]=0.0618$ and $wR2=0.1017$. Crystallographic data (excluding structure

factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-138177 for **1** and -138178 for **2**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [13] R. Richter, J. Sieler, L. K. Hansen, R. Köhler, L. Beyer, E. Hoyer, *Acta. Chem. Scand.* **1991**, 45, 1.
- [14] A. Hordvik, J. A. Porter, *Acta. Chem. Scand.* **1973**, 27, 485.
- [15] A. Hordvik, K. Julshamn, *Acta. Chem. Scand.* **1971**, 25, 2507.
- [16] P. Bhattacharyya, J. Novosad, J. R. Phillips, A. M. Z. Slawin, D. J. Williams, J. D. Woollins, *J. Chem. Soc. Dalton Trans.* **1995**, 1607.
- [17] G. M. Sheldrick, SHELXTL, version 5.03, Program for Crystal Structure Solution, University of Göttingen, **1994**.

Highly Regioselective Allylic Alkylation of Dienyl Acetates and Enynyl Acetates Catalyzed by an Iridium Complex**

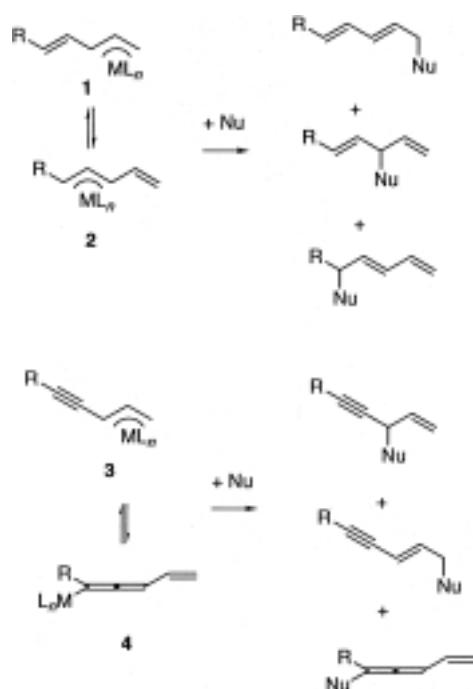
Ryo Takeuchi* and Keisuke Tanabe

Allylic substitution catalyzed by transition metal complexes that proceed via a π -allylmetal complex is one of the most efficient reactions for selective carbon–carbon and carbon–heteroatom bond formation.^[1] The substituent on the π -allyl ligand has a considerable effect on the reactivity and the selectivity. Alkenyl and alkynyl groups are particularly important as substituents on the π -allyl ligand. 1-(1-Alkenyl)- π -allylmetal intermediate **1** isomerizes to 1-alkyl-3-vinyl- π -allylmetal intermediate **2** through a σ - π - σ interconversion.^[2] Similarly, 1-(1-alkynyl)- π -allylmetal intermediate **3** isomerizes to the 1-alkyl-3-vinyl- σ -allenylmetal intermediate **4** (Scheme 1).^[3] Selective reaction of these intermediates with a nucleophile increases the importance of this type of transformation in organic synthesis. We first reported that an iridium complex was a new and efficient catalyst for allylic alkylation, and showed a different regio- and stereoselectivity compared to those of π -allylpalladium chemistry.^[4] We describe here the allylic alkylation of dienyl acetates and enynyl acetates catalyzed by an iridium complex [Eq. (1) and (2)].^[5]

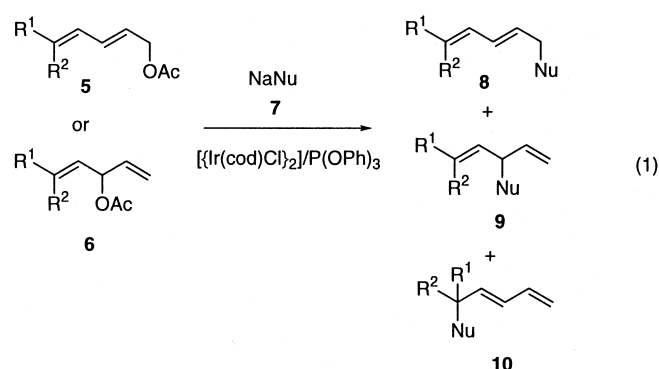
The results of the allylic alkylation of **5** and **6** are summarized in Table 1. Acetate **5a** reacted with two equivalents of **7a–c** in the presence of a catalytic amount of

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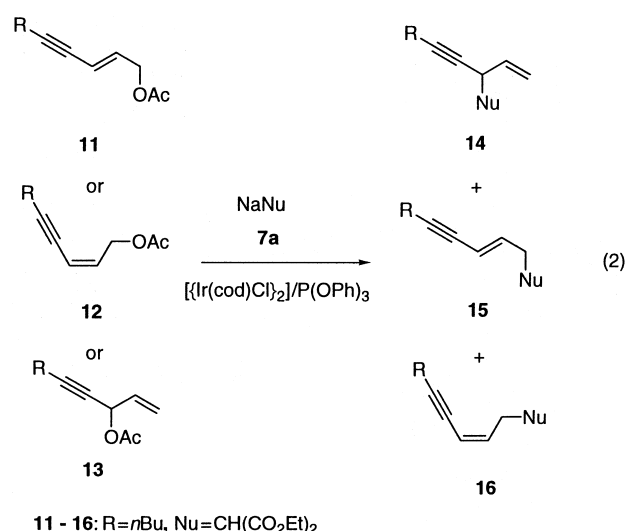
[**] This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (No. 283, “Innovative Synthetic Reactions”) from the Ministry of Education, Science, Sports, and Culture, Government of Japan.



Scheme 1. Reaction of intermediates **1** and **3** with a nucleophile. M = metal (Ir, Pd), L = ligands.



$[\text{Ir}(\text{cod})\text{Cl}]_2$ (Ir atom 4 mol %; cod = cycloocta-1,5-diene) and $\text{P}(\text{OPh})_3$ (P/Ir = 2) at room temperature to give **9aa**, **9ab**, and **9ac**, respectively, in yields of 87–94 % (Table 1, entries 1–3). The reaction occurred with perfect regioselectivity



11 - **16**: R = *n*Bu, Nu = $\text{CH}(\text{CO}_2\text{Et})_2$

at the substituted allylic terminus. No other product was obtained. The reaction of **5a** with **7d**, which is a more bulky nucleophile than **7a**, gave a 6:94 mixture of **8ad** and **9ad** in 84 % yield (Table 1, entry 4). Alkylation of **5a** with **7d** was more regioselective at the substituted allylic terminus than that of 2-hexenyl acetate with **7d**. The reaction of 2-hexenyl acetate with **7d** gave a product resulting from alkylation at the unsubstituted allylic terminus in 71 % selectivity.^[4b]

The $[\text{Pd}(\text{PPh}_3)_4]$ -catalyzed reaction of **5a** with **7b** was reported to give a 66:33:1 mixture of **8ab**, **9ab**, and **10ab**, respectively, in 81 % yield.^[2b] In contrast to the reaction catalyzed by an Ir complex, that catalyzed by a Pd complex gave **8ab** as the major product. This result shows that the iridium catalyst is a useful complement to the palladium catalyst.

Acetate **6a**, which is a regioisomer of **5a**, reacted with **7a**–**d** at room temperature to give products in 81–84 % yields. Reactions with **7a**, **7b**, and **7c** occurred with perfect regioselectivity at the substituted allylic terminus (Table 1, entries 5–7). The reaction with **7d** gave a 1:99 mixture of **8ad** and **9ad** in 83 % yield (Table 1, entry 8). These results strongly suggest the intermediacy of the 1-alkenyl- π -allyliridium intermediate **1** (M = Ir).

Table 1. Allylic alkylation of **5** and **6** catalyzed by the Ir complex.^[a]

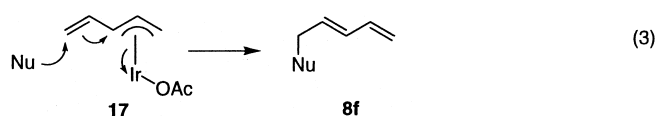
Entry	Substrate	Nu	t [h]	Product	Yield [%] ^[b]	8 : 9 : 10 ^[c]
1	R ¹ = Me, R ² = H (5a)	CH(CO ₂ Et) ₂ (7a)	3	8aa , 9aa , 10aa	94	0:100:0
2	R ¹ = Me, R ² = H (5a)	CH(CO ₂ Me) ₂ (7b)	3	8ab , 9ab , 10ab	87	0:100:0
3	R ¹ = Me, R ² = H (5a)	CHAcCO ₂ Et (7c)	16	8ac , 9ac , 10ac	93	0:100:0
4	R ¹ = Me, R ² = H (5a)	CMe(CO ₂ Et) ₂ (7d)	8	8ad , 9ad , 10ad	84	6:94:0
5	R ¹ = Me, R ² = H (6a)	CH(CO ₂ Et) ₂ (7a)	3	8aa , 9aa , 10aa	84	0:100:0
6	R ¹ = Me, R ² = H (6a)	CH(CO ₂ Me) ₂ (7b)	3	8ab , 9ab , 10ab	81	0:100:0
7	R ¹ = Me, R ² = H (6a)	CHAcCO ₂ Et (7c)	16	8ac , 9ac , 10ac	84	0:100:0
8	R ¹ = Me, R ² = H (6a)	CMe(CO ₂ Et) ₂ (7d)	3	8ad , 9ad , 10ad	83	1:99:0
9	R ¹ = Ph, R ² = H (6b)	CH(CO ₂ Et) ₂ (7a)	2	8b , 9b , 10b	92	0:100:0
10	R ¹ = <i>n</i> Pr, R ² = H (6c)	CH(CO ₂ Et) ₂ (7a)	2	8c , 9c , 10c	93	0:100:0
11	R ¹ = Me, R ² = Me (6d)	CH(CO ₂ Et) ₂ (7a)	3	8d , 9d , 10d	90	0:100:0
12	R ¹ = (<i>E</i>)- <i>n</i> BuCH = CH, R ² = H (6e)	CH(CO ₂ Et) ₂ (7a)	4	8e , 9e , 10e	92	0:100:0
13	R ¹ = R ² = H (6f)	CH(CO ₂ Et) ₂ (7a)	4	8f , 9f	81	19:81:–

[a] A mixture of **5** or **6** (2 mmol), NaNu (4 mmol), $[\text{Ir}(\text{cod})\text{Cl}]_2$ (0.04 mmol), $\text{P}(\text{OPh})_3$ (0.16 mmol), and THF (10 mL) was stirred under Ar at room temperature. [b] Yield of isolated product based on **5** or **6**. [c] Determined by gas chromatography and NMR spectroscopy.

Acetates **6b–e** smoothly underwent alkylation with **7a** to give **9b–e** as a single regioisomer in yields of 90–93% (Table 1, entries 9–12). A trienyl system also gave a single product (entry 12). In contrast, allylic alkylation of **6f** with **7a** gave a mixture of regioisomeric products (Table 1, entry 13). This result requires some discussion (see below).

We found that alkylation of a π -allyliridium intermediate monosubstituted at the terminal position occurred with high regioselectivity at the substituted allylic terminus when the substituent is an alkyl or aryl group.^[4a–c] Since an alkenyl group is more electron-withdrawing than an alkyl group, an allylic terminus substituted with an alkenyl group is more electron-deficient than an allylic terminus substituted with an alkyl group.^[6] Therefore, alkylation occurs at the substituted allylic terminus of intermediate **1** ($M = Ir$) with perfect regioselectivity. It is clear that oxidative addition of **5** gives intermediate **1** ($M = Ir$), which then reacts with a nucleophile to give **9** prior to isomerization to **2**. These results show that nucleophilic attack is much faster than isomerization. Oxidative addition of **6** can give either **1** or **2** (Scheme 1). Since a metal complex can approach a terminal alkene more easily than an internal alkene because of steric effect,^[7] intermediate **1** ($M = Ir$) is formed preferentially.

Although oxidative addition of **6f** gives **17** as the only intermediate, alkylation of **6f** gives a mixture of **8f** and **9f**. Formation of **8f** can be explained by a S_N2' -type nucleophilic attack at a vinyl substituent on the π -allyliridium intermediate **17** [Eq. (3)].^[2b] Such a S_N2' -type nucleophilic attack to



intermediate **1** ($R = Me$, $M = Ir$) can occur, but no corresponding product (**10aa**) is obtained. Steric hindrance from a substituent on a vinylic carbon atom would retard the S_N2' -type nucleophilic attack.

The isomerization of **9** to **8** by the catalytic effect of the palladium complex under alkylation conditions has been reported.^[2a,b] We examined the allylic alkylation of **5a** with **7a** under more severe conditions. The reaction of **5a** with two equivalents of **7a** in the presence of $[\{Ir(cod)Cl\}_2]/P(OPh)_3$ (Ir atom 4 mol %, $P(OPh)_3$ 8 mol %) in refluxing THF for 24 h gave **9aa** in 94% yield as a single product. Product **9** did not isomerize to **8** in the presence of a catalytic amount of the iridium complex.

Following the success with the dienyl system we investigated the allylic alkylation of an enynyl system.^[8] We examined the allylic alkylation of **11**, **12**, and **13** with **7a**.^[9] The structure of the compounds and the ligand used had a considerable effect on the selectivity (Table 2). When $P(OPh)_3$ was used as a ligand the reactions proceeded at room temperature to give products in yields of 83–87% (Table 2, entries 1–3). It is noteworthy that the reaction of **12** with **7a** gave **16**, in which a retention of the *Z* geometry was observed, in 95% selectivity (Table 2, entry 2). Reactions

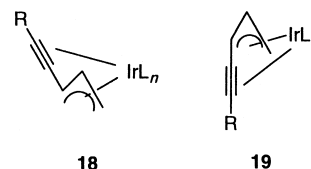
Table 2. Allylic alkylation of **11**, **12**, and **13** with **7a** catalyzed by the Ir complex.^[a]

Entry	Substrate	Ligand	Conditions	Yield [%] ^[b]	14 : 15 : 16 ^[c]
1	11	$P(OPh)_3$	RT, 4 h	87	65:33:2
2	12	$P(OPh)_3$	RT, 7 h	86	2:3:95
3	13	$P(OPh)_3$	RT, 19 h	83	40:9:51
4	11	$P(OEt)_3$	THF, reflux, 21 h	43	21:77:2
5	12	$P(OEt)_3$	THF, reflux, 21 h	16	8:8:84
6	13	$P(OEt)_3$	THF, reflux, 2 h	91	92:8:0

[a] A mixture of **11**, **12**, or **13** (2 mmol), **7a** (4 mmol), $[\{Ir(cod)Cl\}_2]$ (0.04 mmol), $P(OPh)_3$ or $P(OEt)_3$ (0.16 mmol), and THF (10 mL) was stirred under Ar. [b] Yield of isolated product based on **11**, **12**, or **13**. [c] Determined by gas chromatography and NMR spectroscopy.

using $P(OEt)_3$ required more forcing conditions to give products. Acetate **13** reacted with **7a** to give products in 91% yield (Table 2, entry 6). Product **14**, resulting from alkylation at the substituted allylic terminus, was obtained with 92% selectivity.

Alkynes coordinate with metals more strongly than alkenes. To explain the product distributions of the reactions of **11–13**, we must consider the coordination of an alkynyl group to an Ir center. We previously proposed that the electron-withdrawing property of $P(OPh)_3$ promoted the carbonium ion character at the substituted allylic terminus, thus directing a nucleophile to this position.^[4a,b] With intermediate **18**, coordina-



tion of an alkynyl group would reduce the carbonium ion character at the substituted allylic terminus and so decrease the selectivity in forming **14**.^[10] Therefore, nucleophile attack occurs to some extent at the unsubstituted allylic terminus to give **15** and **16**. Oxidative addition of **12** gives *anti*- π -allyl intermediate **19**. We previously disclosed that the nucleophilic attack to an *anti*- π -allyliridium intermediate occurred at the unsubstituted allylic terminus to give a *Z*-configured product in high selectivity.^[4b,d] Similarly, nucleophilic attack on **19** occurs at the unsubstituted allylic terminus to give **16** in high selectivity. Alkylation of 1-alkyl- or aryl-2-propenyl acetate suggested that a *syn*- π -allyliridium intermediate was formed preferentially.^[4a,b] The reaction of **13** gave **14** and **16**, which suggests the formation of **18** as well as **19**. The increased stability of **19** arising from the coordination of an alkynyl group facilitates its formation. The reaction of **11** and **12** did not go to completion when $P(OEt)_3$ was used as a ligand. In contrast, the reaction of **13** gave products in 91% yield. Acetate **13** is more reactive than **11** and **12** as a result of the presence of a terminal alkene, which is approached by the metal complex more easily than an internal alkene. The reaction of **13** using $P(OEt)_3$ as a ligand gave **14** regioselectively. Although it is not yet clear why $P(OEt)_3$ gives a good result, it might retard the coordination of an alkynyl group to the Ir center.

Further mechanistic study and the synthetic application of 1-(1-alkenyl)- π -allyliridium and 1-(1-alkynyl)- π -allyliridium intermediates are underway.

Experimental Section

9aa: 5a (280 mg, 2.0 mmol), triphenylphosphite (49.6 mg, 0.16 mmol), and $[\text{Ir}(\text{cod})\text{Cl}]_2$ (26.9 mg, 0.04 mmol) were stirred in THF (5.0 mL) under an Ar atmosphere. In a separate flask, diethyl malonate (641 mg, 4.0 mmol) was added to a slurry of sodium hydride (96 mg, 4.0 mmol) in THF (5.0 mL). The resulting solution was added to the former by syringe and the combined mixture was stirred at room temperature for 3 h. After **5a** was consumed, diethyl ether and H_2O were added to the mixture, and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried (MgSO_4). The solvent was evaporated in vacuo. Purification of the residue by column chromatography (*n*-hexane/ AcOEt = 98/2) gave **9aa** as a colorless oil (452 mg; yield 94 %).

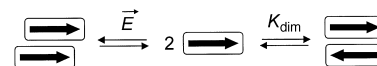
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- [1] a) Pd: J. Tsuji, *Palladium Reagents and Catalysts*, Wiley, Chichester, **1995**, p. 290; b) Mo: F. Glorius, A. Pfaltz, *Org. Lett.* **1999**, *1*, 141; c) Mo: B. M. Trost, I. Hachiya, *J. Am. Chem. Soc.* **1998**, *120*, 1104; d) W: R. Pretot, G. C. Lloyd-Jones, A. Pfaltz, *Pure Appl. Chem.* **1998**, *70*, 1035; e) Rh: R. Takeuchi, N. Kitamura, *New J. Chem.* **1998**, *22*, 659; f) Rh: P. A. Evans, J. D. Nelson, *J. Am. Chem. Soc.* **1998**, *120*, 5581; g) Ir: B. Bartels, G. Helmchen, *Chem. Commun.* **1999**, 741; h) Ru: Y. Morisaki, T. Kondo, T. Mitsudo, *Organometallics* **1999**, *18*, 4742.
- [2] a) Pd: Y. I. M. Nilsson, P. G. Andersson, J. E. Backvall, *J. Am. Chem. Soc.* **1993**, *115*, 6609; b) Pd: B. M. Trost, R. C. Bunt, *J. Am. Chem. Soc.* **1998**, *120*, 70; c) Mo: B. M. Trost, S. Hildbrand, K. Dogra, *J. Am. Chem. Soc.* **1999**, *121*, 10416.
- [3] For the isomerization of a σ -propargylic complex to a σ -allenyl complex, see a) S. Ogoshi, Y. Fukunishi, K. Tsutsumi, H. Kurosawa, *J. Chem. Soc. Chem. Commun.* **1995**, 2485; b) S. Ogoshi, Y. Fukunishi, K. Tsutsumi, H. Kurosawa, *Inorg. Chim. Acta.* **1997**, 265, 9.
- [4] a) R. Takeuchi, M. Kashio, *Angew. Chem.* **1997**, *109*, 268; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 263; b) R. Takeuchi, M. Kashio, *J. Am. Chem. Soc.* **1998**, *120*, 8647; c) R. Takeuchi, *Polyhedron* **2000**, *19*, 557; d) for allylic amination, see R. Takeuchi, N. Shiga, *Org. Lett.* **1999**, *1*, 265.
- [5] R. Takeuchi, K. Tanabe, *Abstr. Pap. 76th Annu. Meet. Chem. Soc. Jpn.* (Yokohama, Japan) **1999**, p. 2B734.
- [6] The electronegativity of an alkenyl group is bigger than that of an alkyl group, see N. Inamoto, S. Masuda, *Chem. Lett.* **1982**, 1003.
- [7] For the stability of an alkenemetal complex, see J. P. Collman, L. S. Hegedus, J. R. Norton, R. G. Finke, *Principles and Applications of Organotransition Chemistry*, University Science Books, Mill Valley, **1987**, p. 149.
- [8] The alkylation of enynyl acetates catalyzed by a Pd complex has been reported, see E. Keinan, E. Bosch, *J. Org. Chem.* **1986**, *51*, 4006.
- [9] A stereodivergent synthesis of (*E*)- and (*Z*)-2-alken-4-yn-1-ols from 2-propynoic acid has been reported, see R. Takeuchi, K. Tanabe, S. Tanaka, *J. Org. Chem.* **2000**, *65*, 1558.
- [10] When the central metal of an alkyne complex is in a high oxidation state, donation of electrons from an alkyne to the metal should be predominant over back donation from the metal to an alkyne. Oxidative addition of an allylic ester to an iridium(I) species gives an iridium(III) species. The Ir centers in intermediates **18** and **19** would be electron-rich because of the donation of electrons from a coordinated alkyne group.

Dipolar Dye Aggregates: A Problem for Nonlinear Optics, but a Chance for Supramolecular Chemistry**

Frank Würthner* and Sheng Yao

The development of nonlinear optical materials requires the optimization of dyes with respect to their molecular electrooptical properties, for example, polarizabilities α , β , and γ ,^[1] as well as their supramolecular organization.^[2] The most investigated $\chi^{(2)}$ nonlinear optical^[3] and photorefractive^[4] polymeric materials require macroscopic noncentrosymmetry, which is achieved through orientation ("poling") of the dipolar dyes by strong external electric fields. Although large dipole moments are considered to be favorable for a high poling efficiency, a large fraction of the materials based on well-designed chromophores with large dipole moments and excellent (hyper)polarizabilities failed to provide the expected electrooptic response.^[5] A possible explanation might be the formation of dye aggregates. According to this concept dipolar dyes are considered to aggregate in an antiparallel fashion as a result of internal electrostatic forces (Scheme 1, right) that counteract the external forces imparted by the electric field during the poling process (Scheme 1, left).^[6, 7]



Scheme 1. Model for the competition between internal organization of dipolar dyes by dipole-dipole interactions (right) and external organization by electric-field-induced dipole orientation leading to a noncentrosymmetric metastable dye arrangement as desired for nonlinear optical applications (left).

Herein we will give proof of this mechanism for merocyanine dyes **1** and **2**, which were recently introduced as the most suitable candidates for photorefractive applications on the basis of their molecular properties.^[8] However, their incorporation into polymers was critical and led us to use less-dipolar dyes based on aminothiophene and indoline electron-donor groups, for example, dyes **3** and **4**.^[9]

The first evidence for strong homoaggregation of dyes **1** and **2**, respectively, came from concentration-dependent UV/Vis spectra, which showed distinct hypsochromically shifted aggregate bands ("H-aggregates") already in dilute solution for solvents with low polarity (Figure 1). The well-defined isosbestic points over the whole accessible concentration range suggest the formation of dimer aggregates, which could be further confirmed by vapor-pressure osmometry (VPO).^[10] From the large hypsochromic shifts of about 70 nm and 117 nm (**2**) in the absorption bands we deduce the (**1a-h**)

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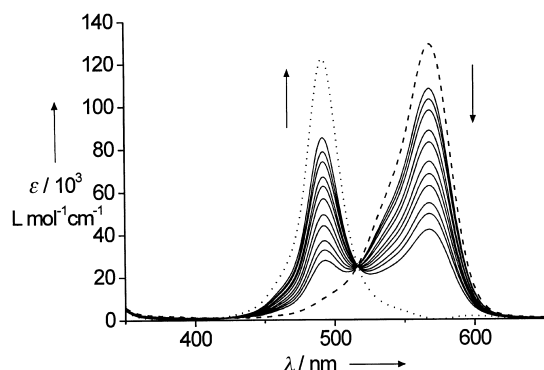
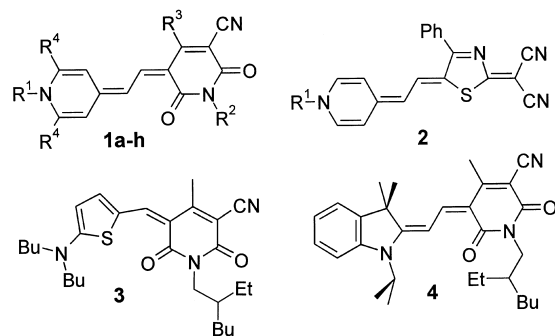


Figure 1. Concentration-dependent UV/Vis spectra of dye **1a** in dioxane. The arrows indicate increasing concentrations from 1.5×10^{-6} to $3 \times 10^{-5} \text{ mol L}^{-1}$. The apparent extinction coefficients ϵ were calculated according to the Beer–Lambert law, and the spectra of the monomer (---, $\lambda_{\text{max}} = 569 \text{ nm}$) and the dimer (···, $\lambda_{\text{max}} = 492 \text{ nm}$) were obtained from the data at two different concentrations and the calculated binding constant. Almost identical spectral changes are observed for dyes **1b–h**.

existence of closely packed dyes with almost parallel transition dipole moments, which is in accordance with exciton coupling theory.^[11]

Interestingly these dimer aggregates proved to be energetically and structurally very stable supramolecular motifs. The Gibbs free dimerization energies of dyes **1** and **2** are comparable to the highest interaction energies reported for supramolecular systems in low-polarity environments and the values in more-polar solvents, such as chloroform, still exceed those of the often used triple-hydrogen bonds (Table 1).^[12] This interaction energy may be reduced significantly if several sterically demanding substituents are attached to the central part of dyes **1** (R^3 , R^4).^[13] On the other hand, even the sterically most demanding *tert*-butyl substituent did not reduce the dimerization constant if it was attached at the outer pyridine nitrogen atom (R^1).

The structural characterization of the dimer aggregates was accomplished by dipole moment and two-dimensional NMR measurements.^[13] Figure 2 shows the results of dipole moment determinations for dye **1h** in comparison to the weakly aggregating dye **3**. From the slopes of the linear regression analyses the dipole moments are calculated^[14] as 12 D for **3** but only about 5 D for **1h**. The value for **3** is in reasonable agreement with the value of 14 D obtained from electro-optical absorption measurements (EOAM) in highly dilute solutions,^[9] whereas the value for **1h** is contrary to the EOAM

Table 1. Dimerization constants K_{dim} and Gibbs free dimerization energies ΔG^0 for dyes **1a–h** and **2–4** from UV/Vis dilution studies at 20 °C in various solvents.^[a,b]

Dye	R^1	R^2	R^3	R^4	Solvent	$K_{\text{dim}} [\text{L mol}^{-1}]$	$-\Delta G^0 [\text{kJ mol}^{-1}]$
1a	Hex	Hex	Me	H	dioxane	105 000	28.2
1b	Bu	Hex	Me	H	dioxane	121 000	28.5
1c	sBu	Hex	Me	H	dioxane	52 000	26.5
1d	tBu	Hex	Me	H	dioxane	116 000	28.4
1e	sBu	iPr	Pr	H	dioxane	9700	22.4
1f	Dodec	sPent	Pr	iPr	dioxane	900	16.6
1g	Dodec	Dodec	Me	H	CCl ₄	2×10^7	41
					dioxane	132 000	28.7
					CHCl ₃	520 ^[c]	15.2
					THF	65	10.2
1h	Ethex	Ethex	Me	H	dioxane	47 000	26.2
2	Hex				dioxane	8×10^6	39
					CHCl ₃	36 000	25.5
3					dioxane	50	9.5
4					dioxane	< 5	< 4 ^[d]

[a] Average from nonlinear regression analyses at four to six different wavelengths of the monomer and the dimer absorption band. The standard deviations are about $\pm 0.2 \text{ kJ mol}^{-1}$ for ΔG^0 . Each dye was studied at a minimum of eight different concentrations in cells of appropriate pathlengths (0.01 to 50 mm). [b] sPent = 2-pentyl; Ethex = 2-ethylhexyl; Dodec = 1-dodecyl. [c] The same value was obtained in a NMR dilution experiment in CDCl₃ as determined by the change in ¹H chemical shifts. [d] No aggregation was observed up to a concentration of $2 \times 10^{-2} \text{ mol L}^{-1}$.

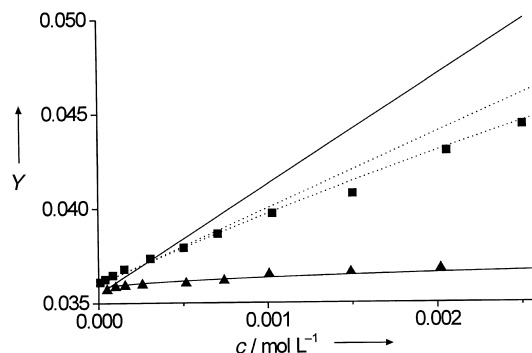


Figure 2. Dipole moment measurements of dyes **1h** (▲) and **3** (■) in dioxane with $Y = (\epsilon_r - 1)/(\epsilon_r + 2) - (n^2 - 1)/(n^2 + 2)$. The lines show the calculations according to Equation (1) and for the nonaggregating dyes with $\mu = 17 \text{ D}$ (**1h**, —) and for $\mu = 14 \text{ D}$ (**3**, ···), respectively.

value of 17 D.^[8b] Clearly, only an antiparallel orientation of the dipolar dyes in the dimer aggregate can account for this observation and indeed a calculation according to Equation (1) reproduced the experimental data for both dyes **1h** and **3** fairly well if dipole moments of zero were assumed for the dimers:

$$\mu^2 = \frac{1}{c_T} (c_M \mu_M^2 + c_D \mu_D^2) \quad (1)$$

where μ denotes the dipole moment, c the concentration of the monomeric (M) and the dimeric (D) species as calculated from the binding data given in Table 1, and c_T is the total concentration of dye. A major conclusion can be drawn from Figure 2: highly dipolar dyes **1h** do not orient in the given external electric field because of the much larger internal field within the dipolar aggregate (Scheme 1).

The strong coupling of the optical transitions, the antiparallel orientation of the dipole moments, and the insensitivity of the binding constant towards the size of the substituent at the pyridine nitrogen atom suggest a slightly slipped aggregate structure (Figure 3).^[11] This structural model was further

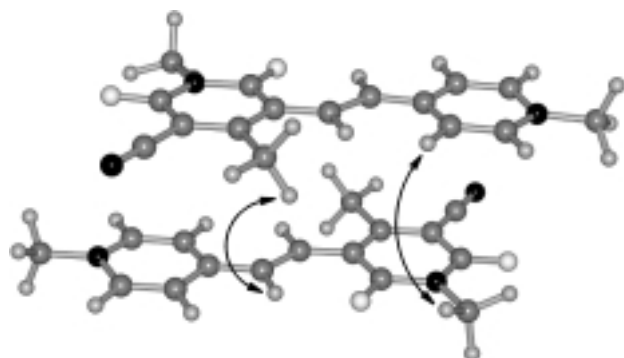


Figure 3. Proposed structure of **1a–h** dimer aggregates based on NMR, UV/Vis, and dipole moment data. The arrows indicate spatial proximities as evidenced by ROESY NMR cross peaks in $[D_8]$ dioxane at 20°C. The drawing was generated from AM1 geometry optimized molecules **1** with all its alkyl substituents replaced by methyl groups for simplicity.^[15]

supported by two-dimensional NMR experiments for **1h** in $[D_8]$ dioxane, which revealed distinct ROESY cross peaks between two pairs of protons that are at remote positions of the individual dye molecules but in spatial proximity in the dimer aggregate (~ 3 Å according to molecular modeling studies^[15]).

Finally, it seemed interesting to take a closer look at the effects of the solvent on the dimerization equilibrium and so provide evidence for the intermolecular forces involved. Therefore, dimerization energies ΔG^0 were determined from concentration-dependent UV/Vis spectra of dye **1g** in a number of solvents and linear free energy relationships were explored between ΔG^0 values and empirical solvent scales ($E_T - 30$, Z , π^*) as well as the Kirkwood–Onsager polarity function ($\epsilon_r - 1$)/(2 $\epsilon_r + 1$), which is based on the macroscopic permittivity ϵ_r of the solvent.^[16] By far the best fit was obtained using the Kirkwood–Onsager function (Figure 4). Here, the only solvent with a significant deviation was dioxane,^[17] whereas all the other solvents could be fitted by linear regression analysis with an impressive correlation

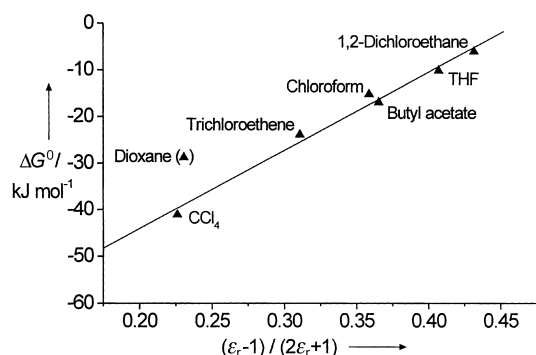


Figure 4. Dependence of the Gibbs dimerization energy for **1g** on the Kirkwood–Onsager polarity function of the solvent.

coefficient of 0.993, a mean standard deviation of only 1.7 kJ mol⁻¹ for the respective ΔG^0 values, and a point of intersection with the y axis at $\Delta G^0 = -78$ kJ mol⁻¹, which corresponds to the gas phase dimerization energy ($\epsilon_r = 1$). Since the Kirkwood–Onsager function considers only electrostatic contributions to describe the solvent polarity, these results provide strong evidence that the main intermolecular forces involved in the dimerization process are indeed electrostatic dipole–dipole interactions as suggested by Scheme 1. Accordingly, the dimerization mechanism and the solvent dependence clearly distinguish these dipolar dye aggregates from well-known aggregates of ionic dyes in water where aggregation is mainly driven by dispersion interactions and the hydrophobic effect.^[18]

In conclusion, the dimer aggregates studied in this work support an often assumed mechanism used to explain unexpected low nonlinear optical susceptibilities in polymeric materials based on electric-field-poled dipolar dyes. The high magnitude of the dimerization constants observed for dyes **1** and **2** are a very promising starting point for a number of projects directed towards functional supramolecular structures such as tweezers and macrocyclic hosts for encapsulation of dipolar molecules, as well as supramolecular polymers.

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- [1] a) J. J. Wolff, R. Wortmann, *Adv. Phys. Org. Chem.* **1999**, *32*, 121–217; b) T. Verbiest, S. Houbrechts, M. Kauranen, K. Clays, A. Persoons, *J. Mater. Chem.* **1997**, *7*, 2175–2189; c) D. R. Kanis, M. A. Ratner, T. J. Marks, *Chem. Rev.* **1994**, *94*, 195–242.
- [2] a) C. Bosshard, F. Pan, M. S. Wong, S. Manetta, R. Spreiter, C. Cai, P. Günter, V. Gramlich, *Chem. Phys.* **1999**, *245*, 377–394; b) S. Yitzchaik, S. Di Bella, P. M. Lundquist, G. K. Wong, T. J. Marks, *J. Am. Chem. Soc.* **1997**, *119*, 2995–3002; c) P. J. A. Kenis, O. F. J. Noordman, H. Schönherr, E. G. Kerver, B. H. M. Snellink-Ruel, G. J. van Hummel, S. Harkema, C. P. J. M. van der Vorst, J. Hare, S. J. Picken, J. F. J. Engbersen, N. F. van Hulst, G. J. Vansco, D. N. Reinhoudt, *Chem. Eur. J.* **1998**, *4*, 1225–1234.
- [3] a) D. M. Burland, R. D. Miller, C. A. Walsh, *Chem. Rev.* **1994**, *94*, 31–75; b) H.-T. Man, H. N. Yoon, *Adv. Mater.* **1992**, *4*, 159–168.
- [4] a) W. E. Moerner, S. M. Silence, *Chem. Rev.* **1994**, *94*, 127–155; b) K. Meerholz, *Angew. Chem.* **1997**, *109*, 981–985; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 945–948.
- [5] a) M. Dörr, R. Zentel, R. Dietrich, K. Meerholz, C. Bräuchle, J. Wichern, S. Zippel, P. Boldt, *Macromolecules* **1998**, *31*, 1454–1465; b) R. Hagen, O. Zobel, O. Sahr, M. Biber, M. Eckl, P. Strohmriegel, C.-D. Eisenbach, D. Haarer, *J. Appl. Phys.* **1996**, *80*, 3162–3166; c) It may be assumed that many studies have not been published if aggregation led to completely inactive materials. This was also the case for a number of polymers based on azo and methine dyes in our own earlier work.
- [6] a) L. R. Dalton, A. W. Harper, B. H. Robinson, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4842–4847; b) T. Katoh, Y. Inagaki, R. Okazaki, *J. Am. Chem. Soc.* **1998**, *120*, 3623–3628.
- [7] a) C. P. J. M. van der Vorst, S. J. Picken in *Polymers as electrooptical and photooptical active media* (Ed.: V. P. Shibaev), Springer, Berlin, **1996**, pp. 173–211; b) C. F. J. Böttcher, *Theory of electric polarization*, Elsevier, New York, **1973**.
- [8] a) S. Beckmann, K.-H. Etzsch, P. Krämer, K. Lukaszuk, R. Matschiner, A. J. Schmidt, P. Schuhmacher, R. Sens, G. Seybold, R. Wortmann, F. Würthner, *Adv. Mater.* **1999**, *11*, 536–541; b) R. Wortmann, F. Würthner, A. Sautter, K. Lukaszuk, R. Matschiner, K. Meerholz, *Proc. SPIE* **1998**, *3471*, 41–49.
- [9] a) F. Würthner, R. Wortmann, R. Matschiner, K. Lukaszuk, K. Meerholz, Y. De Nardin, R. Bittner, C. Bräuchle, R. Sens, *Angew. Chem.* **1997**, *109*, 2933–2936; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*,

- 2765–2768; b) K. Meerholz, Y. De Nardin, R. Bittner, R. Wortmann, F. Würthner, *Appl. Phys. Lett.* **1998**, *73*, 4–6.
- [10] VPO measurements (concentration range from 1 to 5 mmol L⁻¹ in dioxane at 60°C using benzil for calibration) afforded a molecular weight of 950 for compound **1h** (calculated for dimer: 955.4).
- [11] The small absorption band which is revealed in the calculated spectrum of the dimer at longer wavelengths indicates a small deviation from parallelism, see M. Kasha, H. R. Rawls, M. Ashraf El-Bayoumi, *Pure Appl. Chem.* **1965**, *11*, 371–392.
- [12] F. Würthner, C. Thalacker, A. Sautter, *Adv. Mater.* **1999**, *11*, 754–758, and references therein.
- [13] The syntheses of **1a–h** followed the procedure given in F. Würthner, *Synthesis* **1999**, 2103–2113. The purity of all new compounds was confirmed by ¹H NMR spectroscopy and elemental analysis. The ¹H and ¹³C NMR signals for **1h** were assigned on the basis of HMQC and HMBC experiments and the configuration was determined by NOESY and ROESY experiments: ¹H NMR (200 MHz, CDCl₃, 25°C, TMS): δ = 7.74 (d, *J* = 14.8 Hz, 1H, methine-H), 7.59 (d, *J* = 14.8 Hz, 1H, methine-H), 7.57 (d, *J* = 8.4 Hz, 2H, pyridine-2,6-H), 7.24 (d, *J* = 8.4 Hz, 2H, pyridine-3,5-H), 4.05 (d, *J* = 7.4 Hz, 2H, pyridine-NCH₂), 3.91 (m, 2H, NCH₂), 2.32 (s, 3H, CH₃), 1.81 (m, 2H, CH), 1.40–1.29 (m, 16H, CH₂), 0.98–0.85 (m, 12H, CH₃); ¹³C NMR (125 MHz, [D₈]THF, 25°C, 25.3/67.4): δ = 164.3 (C=O), 163.6 (C=O), 157.8 (q, pyridine-4-C), 156.0 (q), 141.9 (pyridine-2,6-CH), 141.0 (methine-CH), 120.2 (pyridine-3,5-CH), 119.4 (CN), 113.4 (methine-CH), 107.0 (q), 89.7 (q), 62.8 (pyridine-NCH₂), 43.3 (NCH₂), 41.7 (CH), 38.6 (CH), 31.9 (CH₂), 30.8 (CH₂), 29.7 (CH₂), 29.2 (CH₂), 25.8 (CH₂), 24.9 (CH₂), 24.1 (CH₂), 23.8 (CH₂), 18.5 (CH₃), 14.5 (CH₃), 14.3 (CH₃), 11.1 (CH₃), 10.6 (CH₃); UV/Vis (CH₂Cl₂) λ_{max} (ε) = 553 nm (119 000 L mol⁻¹ cm⁻¹); m.p. 179°C; elemental analysis calcd for C₃₀H₄₃N₃O₂ (477.7): C 75.43, H 9.07, N 8.80; found: C 75.13, H 8.80, N 8.84.
- [14] a) E. A. Guggenheim, *Trans. Faraday Soc.* **1949**, *45*, 714–720; b) G. M. Janini, A. H. Katrib, *J. Chem. Educ.* **1983**, *60*, 1087–1088.
- [15] CAChe for Windows, Version 3.2, Oxford Molecular Group, Inc., USA, **1999**.
- [16] C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, 2nd ed., VCH, Weinheim, **1990**.
- [17] It is well established that the microscopic solvent polarity of dioxane is badly described by its macroscopic permittivity ε_r because of a substantial quadrupole moment, see W. Baumann in *Physical Methods of Chemistry*, Vol. 3B (Eds.: B. W. Rossiter, J. F. Hamilton), Wiley, New York, **1989**, p. 45.
- [18] a) A. H. Herz, *Adv. Colloid Interface Sci.* **1977**, *8*, 237–298; b) D. Möbius, *Adv. Mater.* **1995**, *7*, 437–444; c) H. Nakahara, K. Fukuda, D. Möbius, H. Kuhn, *J. Phys. Chem.* **1986**, *90*, 6144–6148; d) F. Mizutani, S. Iijima, K. Tsuda, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1295–1299; e) M. Kussler, H. Balli, *Helv. Chim. Acta* **1989**, *72*, 17–28.

Chiral 1,1'-Diphosphetanylferrocenes: New Ligands for Asymmetric Catalytic Hydrogenation of Itaconate Derivatives**

Ulrich Berens, Mark J. Burk,* Arne Gerlach, and William Hems

The attainment of both high catalytic efficiency and high enantioselectivity remains a formidable challenge in asymmetric catalysis.^[1] Bidentate ligands composed of *trans*-2,5-disubstituted phospholane groups have been shown to be useful in asymmetric catalytic hydrogenation reactions.^[2] Despite high enantioselectivities, practical application of this technology frequently requires enhancement of catalyst activity and productivity. To augment the turnover frequencies of catalysts bearing bis(phospholane) ligands, we introduced more flexible backbones (for example, 1,3-propandiyl and 1,1'-ferrocene bridges).^[3] Whereas catalytic rates were greatly improved in these systems, enantioselectivities were found to plummet. We now have found that the combination of efficiency and selectivity may be realized through use of phosphetanes. Here we outline the synthesis of new 1,1'-diphosphetanylferrocene ligands (**1**; FerroTANE)^[4] and demonstrate the superiority of these ligands over known systems in the highly efficient and enantioselective Rh-catalyzed hydrogenation of itaconate derivatives.

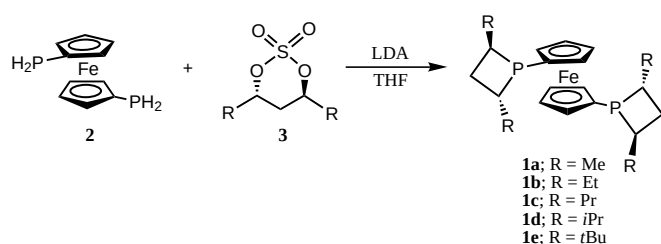
The first optically active phosphetanes previously were described in seminal reports by Marinetti and Ricard.^[5] More recently, we^[6] and the group of Marinetti and Genêt^[7] have independently prepared and examined enantiomerically pure 2,4-disubstituted phosphetanes for use as ligands in asymmetric catalysis. The chiral 2,4-disubstituted phosphetane moiety may be constructed from readily available enantiomerically pure 1,3-diols. The requisite 1,3-diols were prepared conveniently through asymmetric hydrogenation of 1,3-diketones using well-documented procedures involving biaryldi-phosphane–Ru catalysts.^[8] Subsequently, the diols were converted to 1,3-diol cyclic sulfates **3** through treatment with thionyl chloride followed by Ru-catalyzed oxidation with sodium periodate.^[6,7] As shown in Scheme 1, the reaction between the cyclic sulfates **3** and the known diphosphanylferrocene **2**^[3b] provided facile access to the desired ligands **1a–e**, which were isolated as yellow to orange crystalline solids in moderate to good overall yields. A wide range of different 2,4-disubstituted FerroTANE ligands may be obtained through this procedure. The facility with which ligand **1e** (R = *t*Bu) was formed is particularly surprising considering

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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Preparation of FerroTANE ligands.

that assembly required four independent neopentyl nucleophilic substitution reactions.

The new ligand series **1** initially was surveyed for relative utility in the Rh-catalyzed asymmetric hydrogenation of the standard olefinic substrate dimethyl itaconate **4**. The results of these studies (Table 1) reveal that cationic catalysts bearing

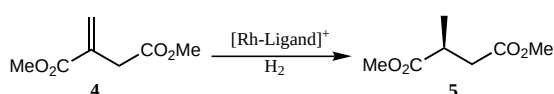


Table 1. Asymmetric hydrogenation of dimethyl itaconate **4**.^[a]

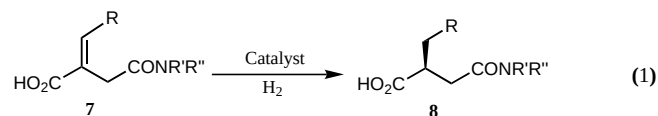
Entry	Ligand	ee [%] ^[b] (config.) ^[c]
1	(<i>R,R</i>)-Me-FerroTANE, 1a	91 (<i>S</i>)
2	(<i>S,S</i>)-Et-FerroTANE, 1b	98 (<i>R</i>)
3	(<i>R,R</i>)-Pr-FerroTANE, 1c	97 (<i>S</i>)
4	(<i>S,S</i>)- <i>i</i> Pr-FerroTANE, 1d	78 (<i>S</i>)
5	(<i>S,S</i>)- <i>t</i> Bu-FerroTANE, 1e	1 (<i>S</i>)
6	(<i>S,S</i>)- 6a	70 (<i>S</i>)
7	(<i>S,S</i>)- 6b	66 (<i>S</i>)

[a] All reactions were performed in MeOH (1M in **4**) at 20 °C, S/C = 200, and 0.55 MPa H₂ using the catalyst precursors [Rh(Ligand)(cod)]BF₄ (cod = 1,5-cyclooctadiene). All reactions were complete after 1 h reaction time. [b] Enantiomeric excess was determined by chiral GC chromatography using a Chiraldex G-TA column. [c] Absolute configuration was ascertained by comparison of sign of optical rotation of product with that reported for configurationally assigned (*R*)-**5**: [α]_D²⁰ = +6.11 (neat). See reference [10].

the Et-FerroTANE and Pr-FerroTANE ligands (**1b** and **1c**, respectively), are very effective for this transformation, affording the product **5** with enantioselectivity comparable to that achieved with the Et-DuPHOS-Rh catalyst (97% ee).^[11] Striking is the difference observed between results achieved with the FerroTANE ligands **1a–e** and the structurally analogous 1,1'-bis(2,5-dialkylphospholanyl)ferrocene derivatives^[3b] **6a** (alkyl = Me) and **6b** (alkyl = Et) (entries 6–7). The advantages conferred by the phosphetane ligands are evident, although the reason for such a significant increase in selectivity upon moving from a five- to a four-membered phosphorus heterocycle is unclear at present.

Specifically protected succinamide derivatives of type **8** have been shown to serve as versatile peptidomimetic intermediates in the design of active drugs.^[12] We envisaged that asymmetric catalytic hydrogenation of the monoamido itaconates **7** could provide direct and economic access to a diverse array of valuable analogues. Despite the attractiveness of this approach, little success has been reported thus far.

No general catalyst has yet been developed and shown to furnish a range of peptidomimetics **8** through efficient hydrogenation of substrates of type **7** [Eq. (1)].^[13, 14]



Initially, we examined the effectiveness of numerous cationic rhodium catalysts for asymmetric hydrogenation of the known substrate **9**.^[15] Screening studies were performed under a standard set of conditions (1M MeOH solution, 0.55 MPa (80 psi) H₂, substrate–catalyst ratio (S/C) = 1000, 1 h). We selected the Et-FerroTANE-Rh catalyst for comparison against other cationic rhodium catalyst systems.^[16] Ruthenium catalysts, such as Ru-BINAP, previously have been shown inadequate for hydrogenation of monoamido itaconate substrates analogous to **9** (BINAP = 2,2'-Bis(diphenylphosphanyl)-1,1'-binaphthyl).^[13c] The results of our screening experiments are shown in Table 2.

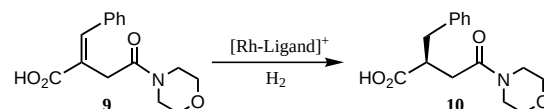


Table 2. Asymmetric hydrogenation of amido itaconate **9**.^[a]

Entry	Ligand	Conv. [%] ^[b]	ee [%] ^[c] (config.) ^[d]
1	(<i>S,S</i>)-Et-FerroTANE, 1b	100	98 (<i>R</i>)
2	(<i>S,S</i>)-Et-DuPHOS	5	85 (<i>R</i>)
3	(<i>R,R</i>)- 6b	55	70 (<i>S</i>)
4	(<i>R,R</i>)-DIPAMP	10	87 (<i>R</i>)
5	(<i>S,S</i>)-BPPM	75	79 (<i>S</i>)
6	(<i>S</i>)-Tol-BINAP	11	43 (<i>S</i>)
7	(<i>S</i>)-PHANEPHOS	60	62 (<i>S</i>)

[a] All reactions were performed in MeOH (1M in **9**) at 20 °C, S/C = 1000, and 0.55 MPa H₂ using the catalyst precursors [Rh(Ligand)(cod)]BF₄. [b] Conversion after 1 h reaction time. [c] Enantiomeric excess was determined by SFC chromatography using a Chiralpak AD HPLC column. [d] Absolute configuration was ascertained by comparison of sign of optical rotation of product with that reported for configurationally defined **10**. See reference [12f].

The data demonstrate that the Et-FerroTANE-Rh catalyst is far superior to all other catalysts tested for hydrogenation of substrate **9**, both in terms of rate as well as enantioselectivity. In fact, under the conditions described using the Et-FerroTANE-Rh catalyst, hydrogen uptake ceased within 15 min reaction time (see also Table 3). No other catalyst was found to effect complete conversion under these conditions. Moreover, enantioselectivities achieved were significantly lower with all other catalysts in comparison with that observed with the Et-FerroTANE-Rh catalyst (98% ee). The BPPM-Rh and analogous catalysts previously were considered the best available for this transformation.^[13c] Surprisingly, the Et-DuPHOS-Rh catalyst, which is so effective for hydrogenation of a wide range of Stobbe-derived itaconate derivatives,^[11] was

found relatively unavailing for substrate **9**. In general, impractically low rates were observed with the Et-Du-PHOS-Rh catalysts in hydrogenation of substrates of type **7**.

To explore the scope of the Et-FerroTANE-Rh catalyst, we have examined a variety of substrates **7** possessing different β -substituents and a morpholine-derived amido group. The results of these studies are shown in Table 3. As can be seen,

Table 3. Asymmetric catalytic synthesis of amido succinates **8**.^[a]

Entry	R in 8	t [min] ^[b]	ee [%] ^[c]
1	Ph	3 h ^[d]	98 ^[d]
2	<i>p</i> -FC ₆ H ₄	10	96
3	<i>p</i> -BrC ₆ H ₄	30	95
4	<i>p</i> -MeSC ₆ H ₄	15	97
5	thienyl	30	97
6	<i>n</i> Bu	30	92
7	<i>i</i> Pr	30	94
8	<i>t</i> Bu	60	99

[a] All reactions were performed in MeOH (0.2–0.5 M in **7**) at 20 °C, S/C = 1000, 0.55 MPa H₂, using the catalyst precursor [Rh((*S,S*)-**1b**)(cod)]BF₄. [b] Reaction time allowed for complete conversion. [c] Enantiomeric excesses were determined by either SFC chromatography using a Chiralpak AD HPLC column or by chiral GC using a CP-Chirasil L-Val column. [d] Reaction was performed at S/C = 20000.

the Et-FerroTANE-Rh catalysts were found to be very effective for rapid enantioselective synthesis of multifarious monoamido succinates **8**. High enantioselectivities were achieved in the hydrogenation of substrates **7** bearing assorted β -aryl groups, as well as linear and branched β -alkyl substituents. Importantly, the practical utility of the Et-FerroTANE-Rh catalyst is demonstrated effectively in entry 1, where complete conversion to **10** was achieved over 3 h at S/C = 20000 (turnover frequency = 7000 cycles h⁻¹). Enantioselectivity was found to be independent of catalyst loading.

In our final analysis, we have surveyed the effect of varying the amide functionality. Different amide groups may be incorporated readily into substrates **7** through direct reaction between a cyclic itaconic anhydride and a primary or secondary amine.^[15] Accordingly, we have prepared a range of secondary and tertiary amide derivatives analogous to morpholine amide **9** (Table 4). Preliminary results achieved upon using the Et-FerroTANE-Rh catalyst suggest broad tolerance to the nature of the amide functionality, and indicate that a diverse selection of monoamido itaconates may be hydrogenated very efficiently and with high enantio-

Table 4. Asymmetric catalytic synthesis of monoamido succinates **8** (R = Ph).^[a]

Entry	Amide group in 8	t [min] ^[b]	ee [%] ^[c]
1	piperidine	20	98
2	pyrrolidine	20	88
3	benzylamine	60	97
4	cyclohexylamine	60	97
5	O-Bn-hydroxylamine	60	96

[a] All reactions were performed in MeOH (0.2–0.4 M in **7**) at 20 °C, S/C = 1000, 0.55 MPa H₂, using the catalyst precursors [Rh((*S,S*)-**1b**)(cod)]BF₄. [b] Reaction time allowed for complete conversion. [c] Enantiomeric excesses were determined by SFC chromatography using Chiralpak AD or OD HPLC columns.

selectivities using this catalyst system. Of particular interest is the hydroxamic acid derivative (entry 5), as enantiomerically pure hydroxamic succinates are widely employed as intermediates for production of collagenase and metalloproteinase inhibitors.^[17]

Overall, we have highlighted the design and synthesis of a new family of 1,1'-diphosphetanylferrocene (FerroTANE) ligands **1**.^[4, 18] Further uses of these ligands and catalysts will be reported in due course.

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- [1] a) I. Ojima, *Catalytic Asymmetric Synthesis*, VCH, New York, **1993**; b) R. Noyori, *Asymmetric Catalysis in Organic Synthesis*, Wiley, New York, **1993**; c) E. N. Jacobsen, A. Pfaltz, H. Yamamoto, *Comprehensive Asymmetric Catalysis*, Springer, New York, **1999**, Vol. 1–3.
- [2] M. J. Burk, *Acc. Chem. Res.* **2000**, in press.
- [3] a) M. J. Burk, J. E. Feaster, R. L. Harlow, *Tetrahedron: Asymmetry* **1991**, 2, 569; b) M. J. Burk, M. F. Gross, *Tetrahedron Lett.* **1994**, 35, 9363.
- [4] FerroTANE is a trademark of Chirotech Technology Ltd. and is used to refer to the class of ligands of general formula 1,1'-diphosphetanylferrocene.
- [5] a) A. Marinetti, L. Ricard, *Tetrahedron* **1993**, 49, 10291; b) A. Marinetti, *Tetrahedron Lett.* **1994**, 35, 5861; c) A. Marinetti, L. Ricard, *Organometallics* **1994**, 13, 3956.
- [6] a) U. Berens, M. J. Burk, A. Gerlach (Chirotech Technology Ltd.), PCT Int. Appl. No. GB99/03637, November **1999**; b) U. Berens (Chiroscience Ltd.) U.S. Patent 5936109, **1999**; c) for preliminary publication of FerroTANE ligand synthesis, see: U. Berens (Chirotech Technology Ltd.) PCT Int. Pat. Appl. WO99/24444, May **1999**, *Chem. Abs.* **2000**, 130, 338253.
- [7] a) A. Marinetti, V. Kruger, F.-X. Buzin, *Tetrahedron Lett.* **1997**, 38, 2947; b) A. Marinetti, J.-P. Genêt, S. Jus, D. Blanc, V. Vidal, *Chem. Eur. J.* **1999**, 5, 1160; c) A. Marinetti, F. Labrue, J.-P. Genêt, *Synlett* **1999**, 1975.
- [8] a) M. Kitamura, T. Ohkuma, S. Inoue, N. Sayo, H. Kumabayashi, S. Akutagawa, T. Ohta, H. Takaya, R. Noyori, *J. Am. Chem. Soc.* **1988**, 110, 629; b) H. Kawano, Y. Ishii, M. Saburi, Y. Uchida, *J. Chem. Soc. Chem. Commun.* **1988**, 87; c) L. Shao, H. Kawano, M. Saburi, Y. Uchida, *Tetrahedron* **1993**, 49, 1997; d) S. D. Rychnovsky, G. Griesgraber, S. Zeller, D. J. Skaltitzky, *J. Org. Chem.* **1991**, 56, 5161.
- [9] M. J. Burk, J. E. Feaster, W. A. Nugent, R. L. Harlow, *J. Am. Chem. Soc.* **1993**, 115, 10125.
- [10] R. Rossi, P. Diversi, S. Ingrosso, *Gazz. Chim. Ital.* **1968**, 98, 1391.
- [11] M. J. Burk, F. Bienewald, M. Harris, A. Zanotti-Gerosa, *Angew. Chem.* **1998**, 110, 2034; *Angew. Chem. Int. Ed.* **1998**, 37, 1931.
- [12] a) T. D. Octain, M. Abou-Gharbia, *Drugs Future* **1991**, 16, 27; b) W. M. Moore, C. A. Spilburg, *Biochemistry* **1986**, 25, 5189; c) P. Buhlmayer, A. Caselli, W. Fuhrer, R. Goschke, V. Rasetti, H. Rueger, J. L. Stanton, L. Criscione, J. M. Wood, *J. Med. Chem.* **1988**, 31, 1839; d) A. A. Plattner, P. A. Marcotte, H. D. Kleinert, H. H. Stein, J. Greer, G. Bolis, A. K. Fung, B. A. Bopp, J. R. Luly, H. L. Sham, D. J. Kempf, S. H. Rosenberg, J. F. Dellaria, B. De, J. Merits, T. J. Perun, *J. Med. Chem.* **1988**, 31, 2277; e) K. Iizuka, T. Mamijo, T. Kubota, K. Akahane, H. Umeyama, Y. Koso, *J. Med. Chem.* **1988**, 31, 704; f) T. Nishi, M. Sakurai, S. Sato, M. Kataoka, Y. Morisawa, *Chem. Pharm. Bull.* **1989**, 37, 2200; g) H. Harada, T. Yamaguchi, A. Iyobe, A. Tsubaki, T. Kamijo, K. Iizuka, K. Ogura, Y. Kiso, *J. Org. Chem.* **1990**, 55, 1679; h) T. Morimoto, M. Chiba, K. Achiwa, *Tetrahedron Lett.* **1990**, 31, 261; i) H. Heitsch, R. Henning, H.-W. Kleemann, W. Linz, W.-U. Nickel, D. Ruppert, H. Urbach, A. Wagner, *J. Med. Chem.* **1993**, 36, 2788.
- [13] a) W. C. Christopf, B. D. Vineyard, *J. Am. Chem. Soc.* **1979**, 101, 4406; b) K. Inoguchi, T. Morimoto, K. Achiwa, *J. Organomet. Chem.* **1989**, 370, C9; c) H. Jendralla, *Synthesis* **1994**, 494.
- [14] a) U. Lerch, H. Jendralla, B. Seuring, R. Henning, Hoechst AG, US 5,321,139, **1994**; b) J.-P. Lecooue, C. Figier, J.-C. Souvie, ADIR, WO 99/01430, **1999**, *Chem. Abs.* **2000**, 130, 110156.

- [15] All substrates of type **7** (including **9**) may be prepared readily through Stobbe condensation, followed by cyclic anhydride formation and selective nucleophilic opening of the anhydride with amine nucleophiles; see: a) H. Jendralla, R. Henning, B. Seuring, J. Herchen, B. Kulitzscher, J. Wunner, *Synlett* **1993**, 155; b) H. Harada, T. Yamaguchi, A. Iyobe, A. Tsubaki, T. Kamijo, K. Iizuka, K. Ogura, Y. Kiso, *J. Org. Chem.* **1990**, 55, 1679.
- [16] Available ligands employed in this study: Tol-BINAP = 2,2'-bis(ditolylphosphanyl)-1,1'-binaphthyl; see: R. Noyori, H. Takaya, *Acc. Chem. Res.* **1990**, 23, 345. DIPAMP = 1,2-ethanediylbis[(o-methoxyphenyl)-phenylphosphane]; see: B. D. Vineyard, W. S. Knowles, M. J. Sabacky, G. L. Bachman, D. J. Weinkauff, *J. Am. Chem. Soc.* **1977**, 99, 5946. PHANEPHOS = 4,12-bis(diphenylphosphanyl)-[2,2]-paracyclophane; see: P. J. Pye, K. Rossen, R. A. Reamer, N. N. Tsou, R. P. Volante, P. J. Reider, *J. Am. Chem. Soc.* **1997**, 119, 6207. BPPM = *N*-(tert-butoxycarbonyl)-4-(diphenylphosphanyl)-2-[(diphenylphosphanyl)-methyl]pyrrolidine; see: K. Achiwa, *J. Am. Chem. Soc.* **1976**, 98, 8265.
- [17] a) M. C. Fournie-Zaluski, A. Coulad, R. Bouboutou, P. Chaillet, J. Devin, G. Waksman, J. Costentin, B. P. Roques, *J. Med. Chem.* **1985**, 28, 1158; b) W. M. Moore, C. A. Spilburg, *Biochem. Biophys. Res. Commun.* **1986**, 136, 390; c) B. Wirz, T. Weisbrod, H. Estermann, *Chim. Oggi* **1996**, 37; d) M. Whittaker, C. D. Floyd, P. Brown, A. J. H. Gearing, *Chem. Rev.* **1999**, 99, 2735.
- [18] The FerroTANE ligands and rhodium catalysts are available for both research and commercial use through Chirotech Technology Ltd.

[K⁺[Mo₆(μ-CN)₉(CO)₁₈]]⁸⁻: A Trigonal-Prismatic Cyanometalate Cage**

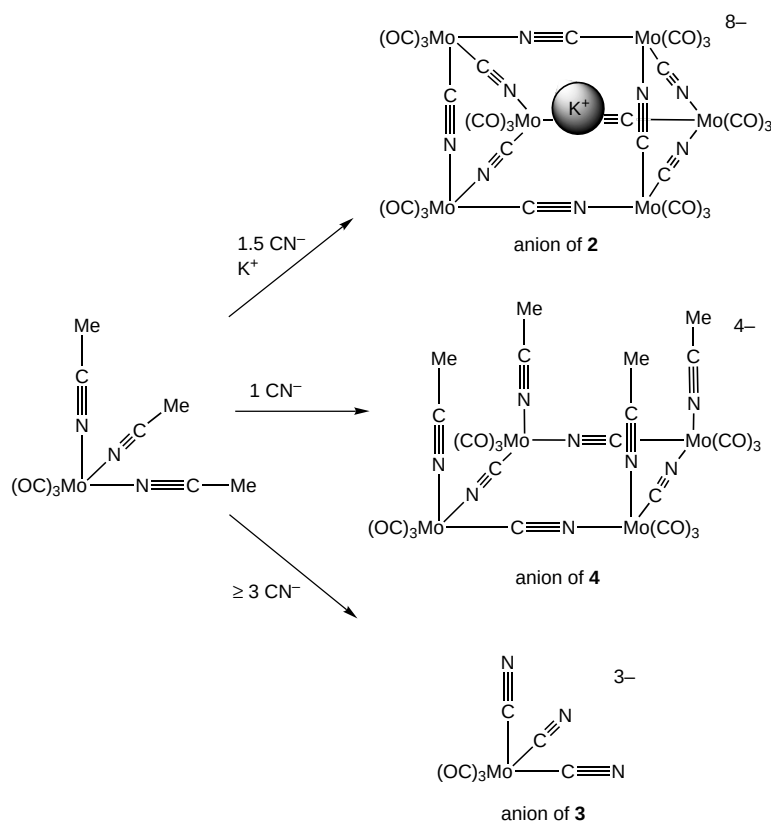
Stephen M. Contakes and
Thomas B. Rauchfuss*

*Dedicated to Professor Heinrich Vahrenkamp
on the occasion of his 60th birthday*

The preeminent cyanometalate is Prussian Blue. Prussian Blue and its many analogues feature cubic or incomplete cubic arrays of metals linked by μ-CN units.^[1, 2] The Prussian Blue motif is the basis of a new generation of high *T_c* magnets,^[3, 4] molecular bowls and boxes^[5, 6] with novel ion-binding properties,^[7] and unusual coordination polymers.^[8] Isoelectronic analogies between [L_nFe^{II}CN] and [L_nMo⁰CN] suggest that it should be possible to prepare families of cages based on Prussian Blue employing cyano derivatives of the Group 6 metal–carbonyl complexes [M(CO)₆].^[9] Relevant to

this plan is the well-recognized ability of cyanide to accommodate high negative charge, for example [Ni(CN)₄]⁴⁻.^[10, 11]

We have examined the reaction of (Et₄N)CN in MeCN with [Mo(Mes)(CO)₃] (**1**, Mes = mesitylene = 1,3,5-Me₃C₆H₃), the latter serving as a convenient source of [Mo(CO)₃-(MeCN)₃].^[7] When solutions of **1** and (Et₄N)CN in MeCN are combined in a 6:9 ratio in the presence of KPF₆, one obtains (Et₄N)₈[K⁺[Mo₆(μ-CN)₉(CO)₁₈]] (**2**) as yellow microcrystals in quantitative yield (Scheme 1). Crystallographic



Scheme 1. Synthesis of **2–4**.

analysis reveals that **2** consists of a trigonal-prismatic Mo₆(CN)₉ cage with idealized *D*_{3h} symmetry (Figure 1). Eight Et₄N⁺ ions are evident in the asymmetric unit. At the center of the cage lies a potassium cation. The potassium is formally 18-coordinate, but the K...C/N bonding is ionic. The potassium atom is 3.37 and 3.20 Å from the C/N atoms of the triangular and square faces, respectively. The Mo centers are octahedral with all OC-Mo-CO angles of about 84° and C/N-Mo-CO of about 96°. The average C/N-Mo-C/N angle within the square faces is 85°, and within the triangular faces it is 80°. The ring strain associated with the 60° Mo...Mo...Mo angles is also responsible for the acute Mo-C-N/Mo-N-C angles of 169° observed for the triangular faces (versus 178° for the square faces). Because of disorder between the C and N sites, the Mo–C/N distance of 2.23 Å represents an average of Mo–N(C) and Mo–C(N) distances. In similar compounds, the (CO)₃Mo⁰–[μ-NC]₃ distance is about 2.2 Å.^[7] This implies that Mo–CN and Mo–NC distances are similar, especially in view of the small thermal parameters for C/N atoms. The Cs⁺ analogue of **2** was also crystallographically characterized,

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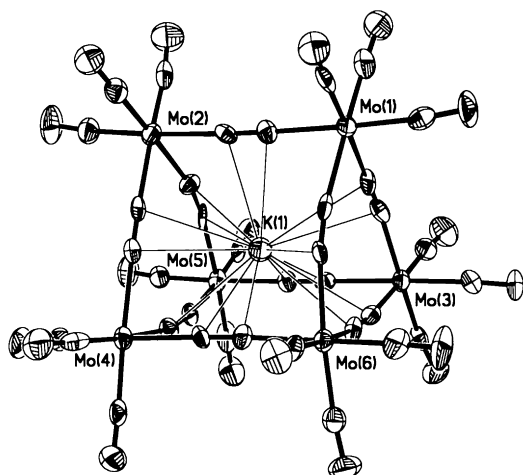
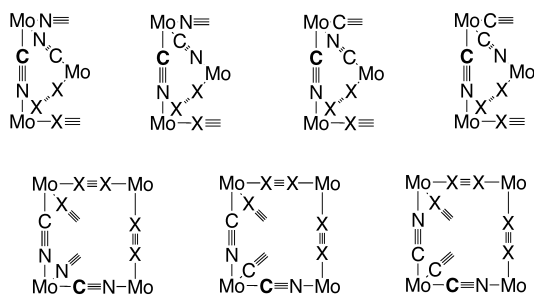


Figure 1. Structure of the anion in **2**, with thermal ellipsoids drawn at the 50% probability level. Selected average bond lengths [Å] and angles [°]: Mo-CN/NC (triangle) 2.24, Mo-CN/NC (square) 2.23, Mo-CO 1.95, C-N 1.17, C-O 1.19, K-C/N (triangle) 3.37, K-C/N (square) 3.20, Mo...Mo (within triangular faces) 5.56, Mo...Mo (between triangular faces) 5.63; C/N-Mo-C/N (triangle) 80, C/N-Mo-C/N (square) 85, Mo-C-N/Mo-N-C (triangle) 169, Mo-C-N/Mo-N-C (square) 178, OC-Mo-CO 84, OC-Mo-CN 96.

although the refinement suffered from disorder involving the Et_4N^+ ions.

The ^{13}C NMR measurements on the Cs^+ analogue of **2**, prepared from a single crystal that was ^{13}C -enriched (33%) at CN^- , revealed a series of seven broad peaks at $\delta = 169.0$, 170.0, 170.0, 171.5, 172.0, 173.0, and 174.0 in the $\mu\text{-CN}$ region.^[12] The occurrence of several CN signals reflects the structural complexity of **2**, which exists as a mixture of isostructural linkage isomers. In fact, when only the local environment about each Mo center in **2** is considered, there are seven different CN coordination sites (Scheme 2).



Scheme 2. Coordination sites for CN units in **2**. The relevant C atom is always shown in boldface.

While solutions of **1** react rapidly with $(\text{Et}_4\text{N})\text{CN}$ at all stoichiometries—the reaction with more than 3 equiv provides $(\text{Et}_4\text{N})_3[\text{Mo}(\text{CO})_3(\text{CN})_3]$ (**3**, Scheme 1)—we obtained a spectroscopically pure material only for the 1:1 reaction (in the absence of alkali metal templating ions!). This 1:1 product is the square $(\text{Et}_4\text{N})_4[\text{Mo}_4(\mu\text{-CN})_4(\text{CO})_{12}(\text{MeCN})_4]$ (**4**). In **4**, the four MeCN ligands are disposed on the same side of the ring, which is unusual (Figure 2). The packing diagram shows

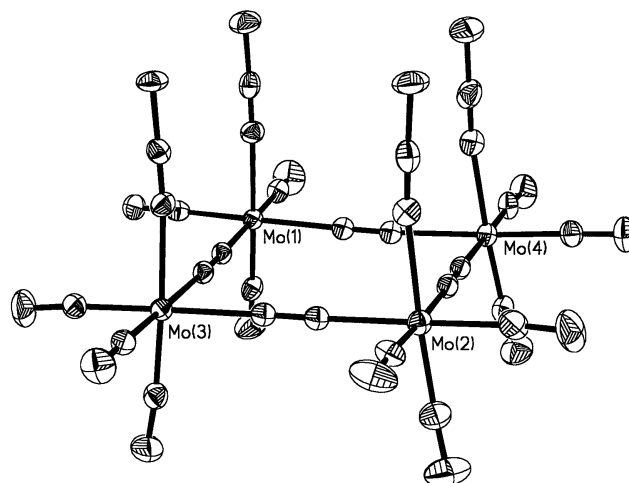


Figure 2. Structure of the anion in **4**, with thermal ellipsoids drawn at the 50% probability level. Selected average bond lengths [Å] and angles [°]: Mo-C/N 2.24, Mo-CO 1.94, Mo-NCMe 2.26, C-N 1.15, C-O 1.17, Mo...Mo 5.62; C/N-Mo-C/N 86, C/N-Mo-NCMe 85, Mo-C-N 177, Mo-N-CMe 176.

that the squares pack as dimers, such that one NCMe group of one square inserts into the $(\text{MeCN})_4\text{Mo}_4(\text{CN})_4$ “nest” of a partner square (Figure 3). We propose the “all-up” isomer is stabilized by this nesting interaction. With its preorganized

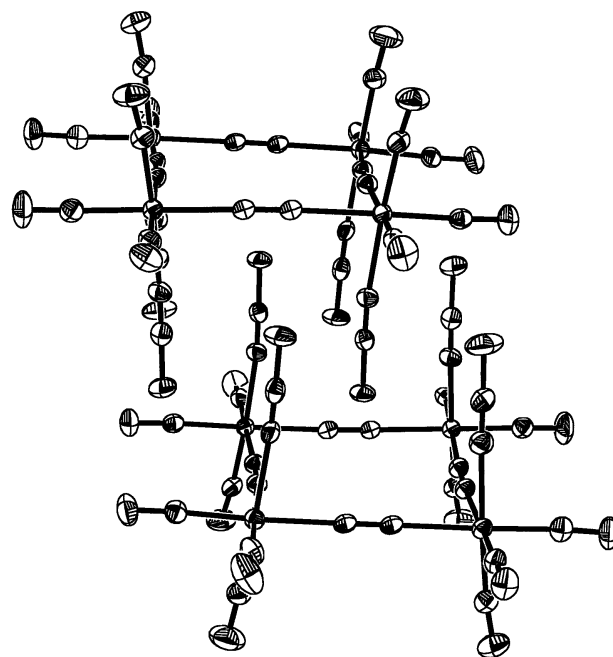


Figure 3. Packing of two anions in **4**, with thermal ellipsoids drawn at the 50% probability level. The distance between the two parallel $\text{Mo}_4(\mu\text{-CN})_4$ planes is 7.10 Å, and the Mo-NC-Me distance is 4.86 Å.

square subunit, **4** is a probable precursor to **2**. The four MeCN ligands in **4** are tilted towards the interior of the square, which will favor the formation of triangular faces; other M-CN triangles are known.^[13–15]

In summary, this work establishes a new cage geometry, the trigonal prism, and a novel coordination environment for potassium. More generally, these results demonstrate that

classical metal–carbonyl complexes are promising precursors to cyanometalate cages.

Experimental Section

2: A solution of KPF₆ (13 mg, 0.071 mmol), (Et₄N)CN (100 mg, 0.641 mmol), and **1** (128 mg, 0.427 mmol)^[16] in MeCN (10 mL) was stirred at room temperature for 1 h. The solvent was reduced to about 5 mL, and Et₂O (15 mL) was added to precipitate yellow microcrystals; yield: 149 mg (88 %). IR (KBr): $\tilde{\nu}_{\text{C}\equiv\text{N}}$ = 2094, 2085, 1998, 1934, 1881, 1756 cm⁻¹; elemental analysis calcd for C₉₁H₁₆₀KMo₆N₁₇O₁₈ (found): C 45.63 (44.14), H 6.73 (7.21), N 9.94 (10.33), Mo 24.03 (23.63), K 1.63 (1.45). The low carbon microanalysis is attributed to the formation of refractory KMo₆C phases, as indicated by elemental analysis and thermogravimetric analysis (TGA). The Cs⁺ analogue of **2** was prepared similarly. Crystals of **2** were grown from MeCN/Et₂O.

3: A suspension of **1** (100 mg, 0.333 mmol) and (Et₄N)CN (156 mg, 1.00 mmol) in MeCN (20 mL) was heated at reflux for 3 h. The solvent was reduced to about 5 mL, and Et₂O (30 mL) was added to precipitate the colorless product, which was washed with Et₂O; yield: 196 mg (91 %). IR (KBr): $\tilde{\nu}_{\text{C}\equiv\text{N}}$ = 2093, 2067, 1935, 1879, 1763 cm⁻¹; IR (MeCN): $\tilde{\nu}_{\text{C}\equiv\text{N}}$ = 2086, 1884, 1870, 1760 cm⁻¹; IR (CD₂Cl₂): $\tilde{\nu}_{\text{C}\equiv\text{N}}$ = 2076, 2066, 1867, 1740 cm⁻¹; ¹³C NMR (¹³C-labeled (33 %) sample, 100 MHz, CD₂Cl₂): δ = 163.45 (s); elemental analysis calcd for C₃₀H₆₀MoN₆O₃ (found): C 51.27 (50.92), H 9.47 (9.20), N 11.96 (12.23), Mo 14.79 (15.00). The K⁺ salt of [Mo(CO)₃(CN)₃]³⁻ has been previously described by Hieber et al.^[17]

4: A solution of (Et₄N)CN (52 mg, 0.333 mmol) and **1** (100 mg, 0.333 mol) in MeCN (15 mL) was stirred for 4 h. Filtration followed by addition of Et₂O (25 mL) to the golden solution gave a white powder, which was washed with Et₂O; yield: 90 mg (72 %). IR (MeCN): $\tilde{\nu}_{\text{C}\equiv\text{N}}$ = 2290, 2100, 1902, 1888, 1772 cm⁻¹; elemental analysis calcd for C₃₆H₉₄Mo₄N₁₂O (found): C 44.04 (44.12), H 6.20 (6.07), N 11.01 (11.05).

Crystals of **2** and **4**, mounted on glass fibers using Paratone-N (Exxon), were analyzed on a Siemens Platform/CCD automated diffractometer at 198 K. The data were processed with SHELXTL. The structures were solved using direct methods and refined using full-matrix least squares on *F*² with the program SHELXL-93. Hydrogen atoms were fixed in idealized positions with thermal parameters 1.5 × those of the attached carbon atoms. Data were corrected for absorption on the basis of Ψ scans.

Crystal data for **2** (C₉₉H₁₆₂N₂₁O₁₈Mo₆K): *M*_r = 2543.19, monoclinic, space group *P*2₁/*c*, *a* = 14.8474(9), *b* = 28.8113(18), *c* = 27.6760(17) Å, β = 91.157(2)°, *V* = 11836.6(13) Å³, *Z* = 4, ρ_{calcd} = 1.427 Mg m⁻³, *F*(000) = 5268, 1408 parameters; *R*₁ = 0.0664, *R*_w = 0.1277, GOF = 0.881 for all 20 849 data (*I* > 2σ(*I*)); max./min. residual electron density 1.050/−0.680 e[−] Å^{−3}.

Crystal data for **4** (C₆₈H₁₁₀N₁₈O₁₂Mo₄): *M*_r = 1755.50, triclinic, space group *P*1̄, *a* = 16.0533(6), *b* = 16.3657(7), *c* = 19.9581(8) Å, α = 67.8040(10), β = 68.2200(10), γ = 67.7400(10)°, *V* = 4331.2(3) Å³, *Z* = 2, ρ_{calcd} = 1.346 Mg m⁻³, *F*(000) = 1816, 991 parameters; *R*₁ = 0.0415, *R*_w = 0.0929, GOF = 0.841 for all 19 722 data (*I* > 2σ(*I*)); max./min. residual electron density 0.706/−0.433 e[−] Å^{−3}.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-133912 (**2**), -137914 (the Cs⁺ analogue of **2**), -133859 (**3**), and -137913 (**4**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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- [1] K. R. Dunbar, R. A. Heintz, *Prog. Inorg. Chem.* **1997**, *45*, 283.
- [2] A. G. Sharpe, *The Chemistry of Cyano Complexes of the Transition Metals*, Academic Press, London, **1976**.
- [3] S. M. Holmes, G. S. Girolami, *J. Am. Chem. Soc.* **1999**, *121*, 5593.
- [4] T. Mallah, C. Auberger, M. Verdaguer, P. Veillet, *J. Chem. Soc. Chem. Commun.* **1996**, 61.
- [5] K. K. Klausmeyer, S. R. Wilson, T. B. Rauchfuss, *Angew. Chem.* **1998**, *110*, 1808–1810; *Angew. Chem. Int. Ed.* **1998**, *37*, 1808.
- [6] J. L. Heinrich, P. A. Berseth, J. R. Long, *Chem. Commun.* **1998**, 1231.
- [7] K. K. Klausmeyer, S. R. Wilson, T. B. Rauchfuss, *J. Am. Chem. Soc.* **1999**, *121*, 2705.
- [8] T. Iwamoto, S. Nishikiori, T. Kitazawa, H. Yuge, *J. Chem. Soc. Dalton Trans.* **1997**, 4197.
- [9] W. P. Fehlhammer, M. Fritz, *Chem. Rev.* **1993**, *93*, 1243.
- [10] R. del Resario, L. S. Stuhl, *J. Am. Chem. Soc.* **1984**, *106*, 1160.
- [11] R. Nast, H. Schultz, H.-D. Moerler, *Chem. Ber.* **1970**, *103*, 777.
- [12] S. M. Contakes, K. K. Klausmeyer, R. M. Milberg, S. R. Wilson, T. B. Rauchfuss, *Organometallics* **1998**, *19*, 3633.
- [13] H. M. Dawes, M. B. Hursthouse, A. A. del Paggio, E. L. Muetterties, A. W. Parkins, *Polyhedron* **1985**, *4*, 379.
- [14] F. Calderazzo, U. Mazzi, G. Pampaloni, R. Poli, F. Tisato, P. F. Zanazzi, *Gaz. Chim. Ital.* **1989**, *119*, 241.
- [15] H. Vahrenkamp, A. Geiß, G. N. Richardson, *J. Chem. Soc. Dalton Trans.* **1997**, 3643.
- [16] G. S. Girolami, T. B. Rauchfuss, R. J. Angelici, *Synthesis and Technique in Inorganic Chemistry*, University Science Books, Mill Valley, CA, **1999**.
- [17] W. Hieber, W. Abeck, H. K. Platzter, *Z. Anorg. Allgem. Chem.* **1955**, *280*, 252.

Gd₈Se₁₅—A 24-Fold Superstructure of the ZrSSi Type**

Enkhtsetseg Dashjav, Oliver Oeckler, Thomas Doert,* Hansjürgen Mattausch, and Peter Böttcher

In the structures of the rare-earth polychalcogenides LnX_n (Ln = Sc, Y, La, Ce–Lu; X = S, Se, Te) homonuclear bonds are frequently observed between the chalcogen atoms for $n \geq 1.5$. All of these compounds can be derived from one common structural pattern: Puckered [LnX] double sheets sandwiched by planar-quadratic [X] layers. This principal pattern is also found in the isopointal, tetragonal compounds of the ZrSSi, PbFCl, (anti-)Fe₂As, and (anti-)Cu₂Sb types. We prefer the ZrSSi type as basic type because of its striking similarities to the LnX₂ compounds. The structures of the compounds LnTe_{2.5} (=Ln₂Te₅) and LnTe₃ can be regarded as stacking variants of the LnX₂ structure. The LnX₂ compounds have been under studied since the mid-1950s. Hints at chalcogen-deficient phases LnX_{2-δ} ($0 \leq \delta \leq 0.5$), accompanied by the occurrence of superstructures, have since been given in a large number of contributions. Chalcogen deficiencies have only been observed in the planar quadratic [X] layers.^[1]

Up to now, only one completely ordered superstructure of the type LnX_{2-δ} has been solved. The compound CeSe_{1.9} is a ten-fold superstructure of the ZrSSi type ($a = \sqrt{5}a_0$, $b = \sqrt{5}a_0$, $c = 2c_0$; a_0 , c_0 : unit cell dimensions of the basic structure), in which one out of ten Se atoms in the [Se] layer is missing, one Se atom appears as an isolated Se²⁻ ion, and the remaining eight Se atoms form four pairs of Se₂²⁻ dumbbells, which are arranged as an eight-membered ring around the vacant site.^[2]

The 24-fold supercell of the ZrSSi type observed for Gd₈Se₁₅ ($a = 3a_0$, $b = 4a_0$, $c = 2c_0$, space group *Amm*2) is the second ordered superstructure of the rare-earth polychalcogenides LnX_{2-δ} which could be solved by X-ray investigations. First hints on this material were obtained by Wang and Steinfink during their investigation of the structure of ErSe₂ but the superstructure was not determined.^[3] The same 24-fold supercell (space group *Amm*2) has been chosen to describe the superstructure of NdTe_{1.90} but a certain amount of disorder remains in the structure model.^[4] A closely related monoclinic 24-fold supercell (space group *A*2₁/*m*11) describes DyS_{1.76} and Dy₆S₁₁, both with substantial disorder.^[5, 6] A related 24-fold supercell was suggested for YSe_{1.83} but without a structural solution.^[7] Finally, the structure of Dy₁₁Se₂₀ has been refined in a model which is based on a 66-fold supercell.^[8] The crystal structure of Gd₈Se₁₅ given in this

work is, therefore, the first structural refinement of the ErSe₂ type, in which the deficient [Se] layer can be described without disorder in the 24-fold supercell of the ZrSSi type.^[9]

Figure 1 shows the structural similarities of Gd₈Se₁₅ with the ZrSSi type: Slightly puckered [GdSe] double sheets are sandwiched by almost planar [Se] layers along the *c* axis. The

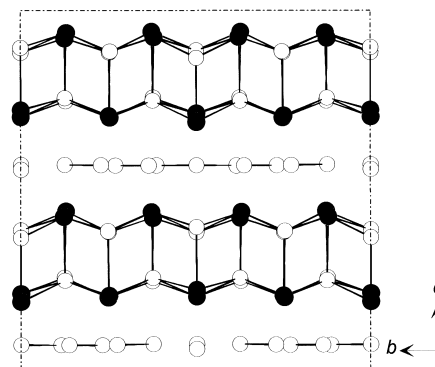


Figure 1. Projection of the crystal structure of Gd₈Se₁₅ along [100]. One unit cell is depicted; Gd atoms: black; Se atoms: white.^[10]

Gd–Se distances are in the range 2.90–3.18 Å and occur in the [GdSe] sheets as well as between the Gd and the Se atoms of the selenium layers. These distances are in the range of the sum of the ionic radii (Gd³⁺: 0.94–1.11 Å; Se²⁻: 1.98 Å). Overall, there are ten crystallographically independent Gd³⁺ centers: four with coordination number (CN) nine (capped tetragonal antiprism; $d_{\text{Gd-Se}}$: 2.93–3.18 Å); five with CN = 8 (bicapped trigonal prism; $d_{\text{Gd-Se}}$: 2.91–3.05 Å); and one with CN = 7 (capped trigonal prism; $d_{\text{Gd-Se}}$: 2.90–3.00 Å). All Gd–Gd distances are larger than 3.94 Å; the doubled radius of Gd in the metal, $2r_{\text{met}}$, is 3.57 Å.

As can be seen from Figure 2, as a result of the occurrence of ordered defects in the selenium layer, only 21 out of 24 possible positions are occupied. Besides three vacancies, three isolated Se²⁻ anions, five Se₂²⁻ dumbbells, and one Se₈⁸⁻ ring is observed. The shortest Se–Se distances occur in the two crystallographically independent dumbbells (2.42 and

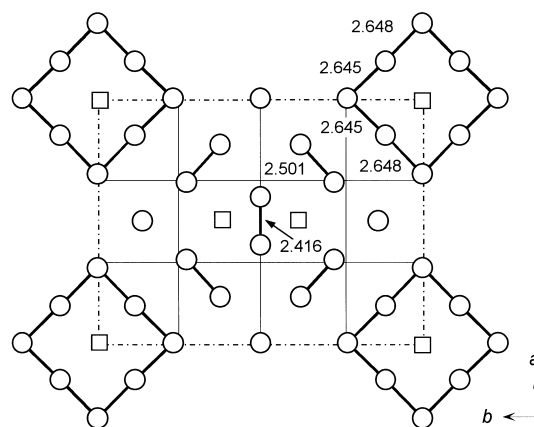


Figure 2. Projection of one of the selenium layers onto (001); the supercell is depicted as a dash-dot line, subcells indicated; vacancies: white squares, Se atoms: white circles. Selected Se–Se distances are given [Å].^[11]

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2.50 Å). They are considerably larger than the sum of the covalent radii (2.34 Å), nevertheless this is often observed for such dimers (M_2Se_2 ($M = Na, K$): 2.40–2.43 Å;^[10] MSe_2 ($M = Ba, La, Ce, Eu$): 2.40–2.47 Å;^[11] $LnSe_{1.9}$ ($Ln = La, Ce, Pr, Nd, Sm$): 2.49 Å).^[2, 12] The Se–Se distances in the almost planar eight-membered ring (torsion angles 174.9(1)° and 175.1(1)°) are already 2.65 Å. Additionally, two relatively short Se...Se contacts of 2.94 and 3.05 Å are observed between the Se^{2-} anions and the longer Se_2^{2-} dumbbells (sum of the van der Waals radii of selenium is 3.80 Å).

Considering the charge on the rare-earth metal as Gd^{3+} and the selenium atoms in the puckered double sheets as Se^{2-} , the overall charge in the unit cell is +24 per $[GdSe]$ double sheet. Thus, 21 selenium atoms of the former square layer must carry 24 negative charges. This requirement is fulfilled by three vacancies, three isolated Se^{2-} anions, five Se_2^{2-} dumbbells and one eight-membered selenium ring, represented as Se_8^{8-} . Upon counting electronic charges in this way, the Se atoms on the corners of the eight-membered ring carry two lone pairs, whereas the ones on the middles of the edges carry three lone pairs (hypervalence). These selenium atoms show slightly elongated displacement parameters along the edges compared to those of the corners. This displacement parameter should at least be additionally twice as large, if the eight-membered ring was built of statistically disordered Se_2^{2-} dumbbells with Se–Se distances of 2.50 Å. We therefore compare the linear triatomic Se units of the Se_8 ring with molecular anions like I_3^- or with the linear triatomic tellurium units in $NaTe$ or Tl_2Te_3 .^[13] All of these groups can be symmetrical or slightly asymmetrical. In every case, even in the eight-membered Se ring in Gd_8Se_{15} , one calculates (Pauling^[14]) bond orders of about 0.30 ± 0.05 ($d_n = d_1 - 0.6 \log n$; d_n = observed bond distance, d_1 = covalent single-bond distance).

To our knowledge, such isolated Se_8 units with eight nearly equivalent Se–Se distances have never been observed before, although a certain similarity to the arrangement of the four Se_2^{2-} dumbbells of the $CeSe_{1.9}$ type exists.^[2, 12]

Experimental Section

All preparations were carried out under an argon atmosphere. Mixtures of gadolinium and selenium (molar ratios $Gd:Se = 8:15$) were heated to 950 °C in an evacuated and sealed quartz ampoule for seven days. After cooling, a chemical transport reaction (850 °C–650 °C, I_2 as carrier) was performed. Dark-metallic, almost tetragonal platelets of Gd_8Se_{15} grow on the cooler site of the ampoule. X-ray diffraction patterns of the powder can be indexed completely and unambiguously with the results of the single crystal-structure determination of the superstructure. Striking differences occur, however, compared to calculated diagrams based on a structure model in the substructure ($ZrSSi$ type). The composition of several crystals was checked by energy-dispersive X-ray microanalysis (EDX); no deviation from the composition found by X-ray structure determination was found.

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- [1] P. Böttcher, *Angew. Chem.* **1988**, 100, 781–794; *Angew. Chem. Int. Ed. Engl.* **1988**, 27, 759–772; P. Böttcher, T. Doert, *Phosphorus Sulphur Silicon* **1998**, 136–138, 255–282; P. Böttcher, T. Doert, H. Arnold, R. Tamazyan, *Z. Kristallogr.* **2000**, 215, 246–253, and references therein.

- [2] P. Plambeck-Fischer, W. Abriel, W. Urland, *J. Solid State Chem.* **1989**, 78, 164–169; W. Urland, P. Plambeck-Fischer, M. Grupe, *Z. Naturforsch. B* **1989**, 44, 261–264; M. Grupe, W. Urland, *J. Less-Common Met.* **1991**, 170, 271–275.
- [3] R. Wang, H. Steinfink, *Inorg. Chem.* **1967**, 6, 1685–1692.
- [4] K. Stöwe, 7th Annual Meeting of the German Crystallographic Society (DGK), Leipzig, **1999**, p. 97.
- [5] R. Tamazyan, V. Molchanov, G. Kuzmicheva, I. Vasileva, *Russ. J. Inorg. Chem.* **1994**, 39, 397–402.
- [6] N. V. Podberezhskaya, D. Y. Naumov, I. G. Vasilieva, N. V. Pervukhina, S. A. Magarill, S. V. Borisov, *J. Struct. Chem.* **1998**, 39, 710–719.
- [7] S.-J. Kim, H.-J. Oh, *Bull. Korean Chem. Soc.* **1995**, 16, 515–518.
- [8] A. van der Lee, L. M. Hoistad, M. Evain, B. F. Foran, S. Lee, *Chem. Mater.* **1997**, 9, 218–226.
- [9] Crystal structure analysis of Gd_8Se_{15} : crystal dimensions: $0.09 \times 0.05 \times 0.02 \text{ mm}^3$; data collection at ambient temperature; STOE IPDS-Diffractometer, $Mo_{K\alpha}$ radiation, dynamic reflection profiles, $2\theta_{\max} = 60^\circ$, 23906 reflections (4878 independent); orthorhombic, space group *Amm2* (No. 38), $a = 12.172(1)$, $b = 16.212(1)$, $c = 16.624(1)$ Å, $V = 3280.4(4)$ Å³, $Z = 6$; $F(000)$: 6132. Correction for Lorentz and polarization effects, numerical absorption correction (STOE X-SHAPE and X-RED, $\mu = 48.844$, $T_{\min} = 0.3058$, $T_{\max} = 0.9458$); structure solved by direct methods.^[15] The structure model was refined as an inversion twin (Flack parameter: 0.55(8)) with 188 parameters and converged at $R_1 = 0.035$ and $wR_2 = 0.063$ for 3418 observed reflections ($I > 2\sigma(I)$), and $R_1 = 0.059$ and $wR_2 = 0.069$ for all reflections (SHELXL-97). Min./max. residual electron density $-1.895/+2.212 \text{ e Å}^{-3}$; weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.0270P)^2]$ with $P = (F_o^2 + 2F_c^2)/3$.^[15] Further details on the crystal structure may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; E-mail: crysdata@fiz-karlsruhe.de) on quoting the depository number CSD-411069.
- [10] P. Böttcher, J. Getzschmann, R. Keller, *Z. Anorg. Allg. Chem.* **1993**, 619, 476–488.
- [11] F. Hulliger, T. Siegrist, *Z. Naturforsch. B* **1981**, 36, 14–15; S. Bénazeth, D. Carré, P. Laruelle, *Acta Crystallogr. B* **1982**, 38, 33–39; J.-P. Marcon, R. Pascard, *C. R. Seances Acad. Sci. Ser. C* **1968**, 266, 270–273; J. A. Aitken, J. A. Cowen, M. G. Kanatzidis, *Chem. Mater.* **1998**, 10, 3928–3935.
- [12] E. Dashjav, T. Doert, P. Böttcher, H. Mattausch, O. Oeckler, *Z. Kristallogr. NCS*, in press.
- [13] K.-F. Tebbe, U. Georgy, *Acta Crystallogr. C* **1986**, 42, 1675–1678; P. Böttcher, R. Keller, *J. Less-Common Met.* **1985**, 101, 311–321; T. Doert, R. Cardoso-Gil, P. Böttcher, *Z. Anorg. Allg. Chem.* **1999**, 625, 2160–2163.
- [14] L. Pauling, *Die Natur der Chemischen Bindung*, Verlag Chemie, Weinheim, **1962**.
- [15] SHELXS-97, Program for Crystal Structure Solution, G. Sheldrick, Göttingen, **1997**; SHELXL-97, Program for Crystal Structure Refinement, G. Sheldrick, Göttingen, **1997**.
- [16] DIAMOND, Visual Information System for Crystal Structures, G. Bergerhoff, Bonn, **1996**.

Table 1. Selected physical and spectroscopic data for **TCP-TG** and **6–9**.

6: ^1H NMR (400 MHz, CDCl_3): δ = 3.49 (s, 2H, SCH_2CO), 2.79 (q, J = 7.3 Hz, CH_2), 1.33 (t, J = 7.3 Hz, CH_3); HR-MS ($\text{C}_4\text{H}_8\text{O}_2\text{S}_2$): calcd: 151.9966; found: 151.9977; elemental analysis: calcd: C 31.56, H 5.30; found: C 31.49, H 5.32.

7: ^1H NMR (400 MHz, CDCl_3): δ = 8.82 (d, J = 4.4 Hz, 1H, pyrrole β -H), 8.79 (d, J = 4.9 Hz, 1H, pyrrole β -H), 8.77 (d, J = 4.4 Hz, 1H, pyrrole β -H), 8.76 (d, J = 4.9 Hz, 1H, pyrrole β -H), 8.62 (d, J = 4.9 Hz, 1H, pyrrole β -H), 8.55 (d, J = 4.4 Hz, 1H, pyrrole β -H), 8.30 (d, J = 4.4 Hz, 1H, pyrrole β -H), 8.27 (d, J = 4.9 Hz, 1H, pyrrole β -H), 7.88–6.50 (m, 46H, arom H), 6.43 (d, J = 8.8 Hz, 1H, arom H), 6.34 (d, J = 8.3 Hz, 1H, arom H), 6.10 (d, J = 8.3 Hz, 1H, arom H), 6.08 (d, J = 5.9 Hz, 1H, arom H), 5.87 (m, 2H, arom H), 5.34–4.70 (m, 11H, benzyl CH_2), 4.34 (d, J = 14.2 Hz, 1H, benzyl CH_2), 4.22 (d, J = 13.7 Hz, 1H, benzyl CH_2), 4.21 (d, J = 13.2 Hz, 1H, benzyl CH_2), 2.66 (d, J = 13.2 Hz, 1H, benzyl CH_2), 2.52 (d, J = 14.2 Hz, 1H, benzyl CH_2), 0.73 (s, 9H, piv CH_3), 0.68 (s, 9H, piv CH_3), 0.33 (s, 9H, piv CH_3), 0.23 (s, 9H, piv CH_3), –0.44 (m, 1H, SCH_2CH_3), –0.50 (t, J = 6.8 Hz, 3H, SCH_2CH_3), –1.20 (m, 1H, SCH_2CH_3), –2.15 (d, J = 18.6 Hz, 1H, COCH_2S), –2.72 (d, J = 18.6 Hz, 1H, COCH_2S), –2.94 (s, 2H, NH); UV/Vis (CH_2Cl_2): λ_{max} ($\epsilon \cdot 10^{-3}$) = 325 (19.1), 338 (18.3), 402 (39), 422 (211.6), 518 (11.8), 587 (4), 640 nm ($1.4 \text{ M}^{-1} \text{ cm}^{-1}$); IR (neat): $\tilde{\nu}$ = 3458, 3333, 3305, 3056, 2958, 2926, 2875, 2853, 1747, 1585, 1457, 1367, 1260, 1231, 1110, 1082, 1025, 796, 748, 719 cm^{-1} ; HR-MS ($\text{C}_{156}\text{H}_{125}\text{O}_{21}\text{N}_4\text{S}_2$): calcd: 2453.8278; found: 2453.8284.

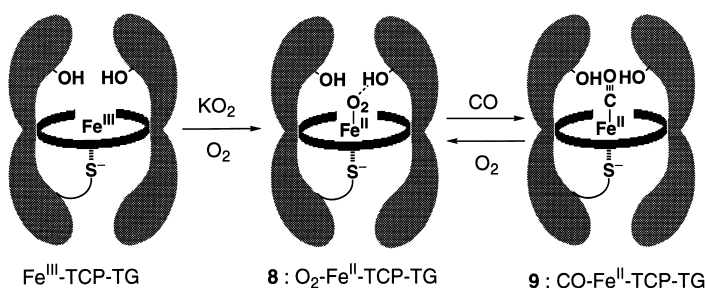
TCP-TG: UV/Vis (CH_2Cl_2): λ_{max} ($\epsilon \cdot 10^{-3}$) = 325 (18.2), 339 (18.7), 363 (17.1), 418 (31.6), 513 (5.8), 575 (2.7), 658 nm ($1.7 \text{ M}^{-1} \text{ cm}^{-1}$); IR (neat): $\tilde{\nu}$ = 3453, 3062, 2957, 2927, 2873, 1746, 1585, 1456, 1367, 1260, 1229, 1111, 1085, 997, 788, 747, 719 cm^{-1} ; ESR (CH_2Cl_2 , 7 K): g = 5.73, 2.37, 2.26, 2.00, 1.94; HR-MS ($\text{C}_{154}\text{H}_{118}\text{O}_{21}\text{N}_4\text{SFe}$): calcd: 2446.7359; found: 2446.7417; elemental analysis ($\text{C}_{154}\text{H}_{117}\text{FeN}_4\text{O}_{21}\text{S} \cdot 8\text{H}_2\text{O}$): calcd: C 71.37, H 5.17, N 2.16; found: C 71.66, H 5.49, N 1.94.

8: UV/Vis (CH_2Cl_2): λ_{max} (%) = 339 (37), 421 (100), 544 (6), 629 (4).

9: UV/Vis (CH_2Cl_2): λ_{max} (%) = 366 (28), 444 (100), 537 (5).

iron *meso*-tetramesitylporphyrin chloride (–0.64 V), the strongly negatively shifted potential of **TCP-TG** indicates the strong electron donation from the thiolate ligand to the iron atom.

The dioxygen adduct **8** was obtained in the reaction of Fe^{III} –**TCP-TG** with an equimolar amount of KO_2 [10] under an oxygen atmosphere (Scheme 2). Addition of KO_2 to the THF



Scheme 2. The formation of the hydrogen bonded dioxygen adduct **8** and CO adduct **9**.

solution of Fe^{III} –**TCP-TG** at -80°C induced immediately a drastic change in the UV/Vis spectrum. The Soret band (λ_{max} = 424 nm) decreased in intensity and blue shifted slightly to 421 nm. The Q band absorption (λ_{max} = 530 nm) greatly diminished. The oxy form transformed into the corresponding CO adduct **9** on exposure to CO, which exhibited a typical hyperporphyrin spectrum with split Soret bands at 366 and 444 nm. This process was reversible. Thus, the Fe^{II} state and

the axial coordination of the thiolate are maintained under these conditions without any autoxidation or other oxidative decomposition. The resonance Raman spectrum of the dioxygen adduct exhibited a strong band at 1137 cm^{-1} . By replacing K^{16}O_2 and $^{16}\text{O}_2$ with K^{18}O_2 and $^{18}\text{O}_2$, this band shifted to 1073 cm^{-1} (Figure 1). The observed isotopic effect

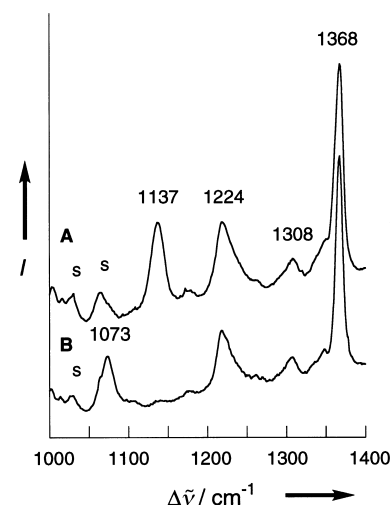


Figure 1. High-frequency region of the resonance Raman spectra of the dioxygen adduct (THF/ CH_3CN , -80°C , λ_{ex} = 413.1 nm, 20 mW). A: $\text{K}^{16}\text{O}_2 + ^{16}\text{O}_2$; B: $\text{K}^{18}\text{O}_2 + ^{18}\text{O}_2$.

(64 cm^{-1}) upon $^{18}\text{O}_2$ substitution is in good agreement with the value (65 cm^{-1}) calculated from the harmonic oscillator approximation of the O–O stretching vibration. The 1137 cm^{-1} band is therefore assigned to this $\nu(\text{O-O})$ mode. The observed frequency of $\tilde{\nu}(\text{O-O})$ is very close to those of oxy-cytochrome P450_{cam} (1140 cm^{-1}) [11] and model oxygen adducts ($1140, 1147 \text{ cm}^{-1}$). [12] No specific band involving the oxygen ligand was detectable in the low-frequency region with Soret excitation (λ_{ex} = 413.1, 427.0, 441.6 nm). The Raman active $\nu(\text{O-O})$ stretch mode has been known to be characteristic of the dioxygen adduct of thiolate-coordinated heme. In contrast, the $\nu(\text{O-O})$ stretch mode of oxy-heme with the nitrogen base as an axial ligand is Raman inactive. [13] A thiolate ligand increases the electron density of the porphyrin π^* orbital through the p_π and d_π orbitals to induce the charge-transfer transition from the π^* to the empty Fe–O σ^* orbital in the oxy form. This leads to the enhancement of $\nu(\text{O-O})$ band. [14] Therefore, the present results in the resonance Raman spectra reveal the successful formation of the desired dioxygen adduct of the thiolate-coordinated heme.

This dioxygen adduct was stable even at 0°C . We assumed that an intramolecular hydrogen bond between the bound oxygen and the hydroxyl groups of the binaphthyl moieties was mainly responsible for its higher stability. To verify this prediction, we investigated the frequency of the O–O stretching mode of the dioxygen complex upon deuterium substitution of the exchangeable protons. The Fe^{III} –**TCP-TG** remained in $\text{CH}_3\text{OH}/\text{CH}_3\text{CN}$ or $\text{CD}_3\text{OD}/\text{CH}_3\text{CN}$ for several hours and then dried in vacuo overnight, then the resonance Raman spectra were measured. Only the $\tilde{\nu}(\text{O-O})$ mode shifted by 2 cm^{-1} to a higher frequency on exchange of CH_3OH by CD_3OD (Figure 2). No other vibrations exhibited

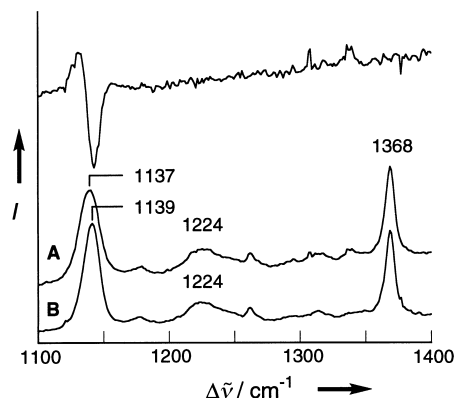


Figure 2. A selected region of the resonance Raman spectra of the dioxygen adduct in CH_3OH (Spectrum A) and in CH_3OD (Spectrum B) ($\text{THF}/\text{CH}_3\text{CN}$, -80°C , $\lambda_{\text{ex}} = 413.1\text{ nm}$, 20 mW); the trace above represents the difference spectrum (A – B).

any frequency shifts, which means that there is no perturbation in the porphyrin skeleton on the H/D exchange. Using resonance Raman spectroscopy, Kitagawa and co-workers have already reported evidence for hydrogen bonded dioxygen to the distal histidine in oxycobalt myoglobin and hemoglobin.^[15] Our present results agree closely with their observations: the frequencies of $\nu(\text{O}-\text{O})$, measured in D_2O , were also $2-5\text{ cm}^{-1}$ higher than those measured in H_2O . We therefore conclude that the bound oxygen in **TCP-TG** interacts with the adjacent exchangeable protons and that the dioxygen adduct of **TCP-TG** is stabilized by hydrogen bonds between the dioxygen and the inner hydroxyl groups, as predicted in the molecular design of **TCP-TG** (Scheme 2).

It has been firmly established that hydrogen bonds to bound dioxygen stabilize the oxy-complexes of globins and their model complexes with a nitrogen base as an axial ligand.^[16] An analogous hydrogen bond has never been confirmed in cytochrome P450. To the best of our knowledge, the present complex **TCP-TG** shows the first direct evidence for a hydrogen bond to dioxygen in a thiolate-coordinated oxyheme, which has significance for the dioxygen binding and activation in cytochrome P450.^[17]

Experimental Section

TCP-TG: 7 (5.3 mg , $2.16 \times 10^{-3}\text{ mmol}$) was dissolved in dry toluene (10 mL) and heated at 50°C under N_2 . $[\text{Fe}(\text{CO})_5]$ ($105\text{ }\mu\text{L}$, 0.78 mmol) and a solution of I_2 in toluene (6.8 mg , $26.8 \times 10^{-3}\text{ mmol}$) were added. The mixture was stirred overnight in the dark, quenched with water, and then extracted into CH_2Cl_2 . After removal of the solvent and drying, the residue was purified by flash chromatography (Merck silica gel 60H, CH_2Cl_2). **TCP-TG** was obtained as a brown solid (3.6 mg , $1.47 \times 10^{-3}\text{ mmol}$, 41 %).

Treatment of **TCP-TG** with KO_2 : **TCP-TG** (0.2 mg , $8.2 \times 10^{-5}\text{ mmol}$) was dissolved in THF (0.6 mL , $1.37 \times 10^{-4}\text{ M}$). The solution was cooled to -80°C and a solution of KO_2 ($50\text{ }\mu\text{L}$, CH_3CN , $1.65 \times 10^{-3}\text{ M}$), which was dissolved with [2.2.2]cryptand, was added into the porphyrin solution. The atmosphere was replaced with O_2 . The resulting solution was characterized with UV/Vis absorption and resonance Raman spectroscopy.

Resonance Raman spectra were obtained on a SpectraPro-300i spectrometer (Acton Research Co.) with a 2400-groove grating, a Beamlok 2060 Kr ion laser (Spectra Physics), a holographic supernotch filter (Kaiser Optical), and a LN-1100PB CCD detector (Princeton Instruments) cooled with liquid N_2 . Spectra were collected on solvated samples in spinning cells (2 cm diameter, 1500 rpm) at -80°C at an excitation wavelength $\lambda_{\text{ex}} =$

413.1 nm (20 mW), 90° scattering geometry, and 5 min data accumulation. Peak frequencies were calibrated relative to indene and CCl_4 standards (accurate to $\pm 1\text{ cm}^{-1}$). During each Raman experiment, UV/Vis spectra were simultaneously collected on a PMA-11 CCD spectrophotometer (Hamamatsu) with a Photol MC-2530 (D_2/W_2) light source.

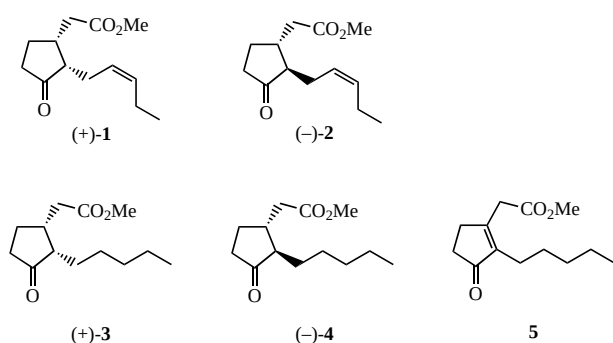
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- [1] a) Y. Watanabe, J. T. Groves, *Enzymes* **1992**, 20, 405; b) P. Ortiz de Montellano, *Cytochrome P-450. Structure, Mechanism, and Biochemistry*, 2nd ed., Plenum Press, New York, **1995**; c) M. Sono, M. P. Roach, E. D. Coulter, J. H. Dawson, *Chem. Rev.* **1996**, 96, 2841; d) H. Shimada, S. G. Sligar, H. Xeom, Y. Ishimura in *Oxygenase and Model Systems* (Ed.: F. Funabiki), Kluwer Academic, Dordrecht, **1997**, pp. 195–221, and references therein.
- [2] T. L. Poulos, B. C. Finzel, I. C. Gunsalus, G. C. Wagner, J. Kraut, *J. Biol. Chem.* **1984**, 260, 16122.
- [3] a) M. Imai, H. Shimada, Y. Watanabe, Y. Matsushima-Hibiya, R. Makino, H. Koga, T. Horiguchi, Y. Ishimura, *Proc. Natl. Acad. Sci. USA* **1989**, 86, 7823; b) N. C. Gerber, S. G. Sligar, *J. Am. Chem. Soc.* **1992**, 114, 8742; c) M. Vidakovic, S. G. Sligar, H. Li, T. L. Poulos, *Biochemistry* **1998**, 37, 9211.
- [4] a) J. H. Dawson, *Science* **1988**, 240, 433; b) T. L. Poulos, *Adv. Inorg. Biochem.* **1988**, 7, 1.
- [5] For typical examples, see: a) H. Ogoshi, H. Sugimoto, Z. Yoshida, *Tetrahedron Lett.* **1975**, 27, 2289; b) J. P. Collman, S. E. Groh, *J. Am. Chem. Soc.* **1982**, 104, 1391; c) H. Patzelt, W.-D. Woggon, *Helv. Chim. Acta* **1992**, 75, 523; d) T. Higuchi, S. Uzu, M. Hirobe, *J. Am. Chem. Soc.* **1990**, 112, 7051; e) N. Ueyama, N. Nishikawa, Y. Yamada, T. Okamura, A. Nakamura, *J. Am. Chem. Soc.* **1996**, 118, 12826; f) H. Volz, M. Holtzbecher, *Angew. Chem.* **1997**, 109, 1510; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1442.
- [6] a) C. K. Chang, D. Dolphin, *J. Am. Chem. Soc.* **1976**, 98, 1607; b) M. Schappacher, L. Ricard, J. Fisher, R. Weiss, E. Bill, R. Montiel-Montoya, H. Winkler, A. X. Trautwein, *Eur. J. Biochem.* **1987**, 168, 419; c) D. El-Kasmi, C. Tetreau, D. Lavatte, M. Momenteau, *J. Am. Chem. Soc.* **1995**, 117, 6041.
- [7] F. Tani, S. Nakayama, M. Ichimura, N. Nakamura, Y. Naruta, *Chem. Lett.* **1999**, 729.
- [8] a) Y. Naruta, F. Tani, N. Ishihara, K. Maruyama, *J. Am. Chem. Soc.* **1991**, 113, 6865; b) Y. Naruta, F. Tani, K. Maruyama, *Tetrahedron: Asymmetry* **1991**, 2, 533.
- [9] The other isomer,^[8a] in which compound the positions of the binaphthalene bridges are different between the upper and lower sides of the heme plane is omitted in Scheme 1.
- [10] E. McCandlish, A. R. Micsztal, M. Nappa, A. Q. Sprenger, J. S. Valentine, J. D. Stong, T. G. Spiro, *J. Am. Chem. Soc.* **1980**, 102, 4268.
- [11] a) S. Hu, A. J. Schneider, J. R. Kincaid, *J. Am. Chem. Soc.* **1991**, 113, 4815; b) T. Egawa, T. Ogura, R. Makino, Y. Ishimura, T. Kitagawa, *J. Biol. Chem.* **1991**, 266, 10246; c) I. D. G. Macdonald, S. G. Sligar, J. F. Christian, M. Unno, P. M. Champion, *J. Am. Chem. Soc.* **1999**, 121, 376.
- [12] G. Chottard, M. Schappacher, L. Ricard, R. Weiss, *Inorg. Chem.* **1984**, 23, 4557.
- [13] H. Brunner, *Naturwissenschaften* **1974**, 61, 129.
- [14] M. Tsubaki, N.-T. Yu, *Proc. Natl. Acad. Sci. USA* **1981**, 78, 3581.
- [15] T. Kitagawa, M. R. Ondrias, D. L. Rousseau, M. Ikeda-Saito, T. Yonetani, *Nature* **1982**, 298, 869.
- [16] a) M. Momenteau, C. A. Reed, *Chem. Rev.* **1994**, 94, 659; b) G. B. Jameson, J. A. Ibers in *Bioinorganic Chemistry* (Eds.: I. Bertini, H. B. Gray, S. Lippard, J. Valentine), University Science Books, California, **1994**, pp. 167–252, and references therein.
- [17] Note added in proof (April 7, 2000): A crystallographic study of the catalytic intermediates, including the oxy complex of cytochrome P450_{cam} has been recently reported. I. Schlichting, J. Berendzen, K. Chu, A. M. Stock, S. A. Maves, D. E. Benson, R. M. Sweet, D. Ringe, G. A. Petsko, S. G. Sligar, *Science* **2000**, 287, 1615.

Industrial Synthesis of (+)-*cis*-Methyl Dihydrojasmonate by Enantioselective Catalytic Hydrogenation; Identification of the Precatalyst [Ru((–)-Me-DuPHOS)(H)-(η⁶-1,3,5-cyclooctatriene)](BF₄)**

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The jasmonoids, (+)-*cis*-jasmonic acid and its derivatives, have numerous phytochemical activities,^[1] and in particular the methyl ester (+)-*cis*-methyl jasmonate^[2] (+)-**1** also has an odor^[3] that is greatly appreciated in perfumery.^[4] The study of the jasmonoids began in 1962, when a near-equilibrium mixture of (–)-*trans*-methyl jasmonate (–)-**2** (main component, see below) and (+)-**1** was isolated from jasmine oil and



identified.^[5] The connectivities within (+)-**1** and (–)-**2** were established by hydrogenation to the corresponding methyl dihydrojasmonates (+)-**3** and (–)-**4** and by comparison with a synthetic mixture of (±)-**3** and (±)-**4**. Subsequent work established the relative and absolute configurations, the

biosynthesis, the biological activities, and, in particular, that only the unstable (+)-*cis*-jasmonic acid and its derivatives are biologically active.^[1] Acid- and base-catalyzed epimerization at C(2) of the *cis* isomers is facile and the resulting *trans* isomers are thermodynamically favored. The equilibrium between (+)-**1** and (–)-**2** and that between (+)-**3** and (–)-**4** thus favors the *trans* isomers (–)-**2** and (–)-**4** by about 95:5 at ambient temperature.

Numerous syntheses of methyl jasmonates have been reported; early syntheses targeted (±)-**2**^[2, 5c] and later work (±)-**1** and (+)-**1**.^[6] To date, all reported syntheses of (±)-**1** and (+)-**1** are still at the laboratory scale and only the racemic equilibrium mixture is produced industrially but on a relatively small scale.

Since the early 1970s, Firmenich has commercialized the corresponding racemic, near-equilibrium mixture of methyl dihydrojasmonates^[5b] under the trade name Hedione (approximately 10% (±)-**3**, 90% (±)-**4**) and this is now an important, large volume perfumery chemical.^[4] Dihydrojasmonic acids and methyl dihydrojasmonates have since also been identified as natural products that have some biological activities.^[7] These fragmentary data for the dihydro series, plus the possible analogy to the jasmonoids suggested that (+)-*cis*-methyl dihydrojasmonate (+)-**3**, the dihydro derivative of (+)-**1**, might be the only component of Hedione that has an odor. To investigate the olfactory properties, all four stereoisomers were prepared on a small scale.^[8] Evaluation by our perfumer colleagues established that (+)-**3** is indeed the only stereoisomer that has an odor but the presence of the other three modifies the odor of (+)-**3** and also affects the performance of a perfume in which the mixture is used. One is constrained to use equilibrium mixtures in many applications because of rapid epimerization but it was found that one can use (+)-**3** and avoid equilibration in fine perfumery and thereby achieve a striking “radiance” of the perfume. To develop an industrial synthesis of (+)-**3** was therefore a desirable and challenging goal.^[9]

In principle, the most direct route to (+)-**3** was enantioselective catalytic hydrogenation of the vinylogous β-oxoester **5**.^[10] The *syn* addition of H₂ across the C=C bond would generate the required *cis* geometry and neutral conditions might avoid epimerization. As expected, all attempts to use known chiral catalysts to hydrogenate **5** failed. A new, more electrophilic, coordinatively unsaturated catalyst system that would react with the tetrasubstituted C=C bond in **5** was required.

We found that the treatment of equimolar solutions of [Ru(1,2:5,6-η-cod)(η³-methallyl)₂] **6**^[11] (cod = 1,5-cyclooctadiene) and various chiral diphosphane ligands (P–P) in CH₂Cl₂ with one equivalent of HBF₄·Et₂O^[12] and a catalytic amount of BF₃·Et₂O at ambient temperature for about 30 min generates mixtures containing precatalysts that can be used directly for the hydrogenation of **5** (0.01M solutions of **5** in CH₂Cl₂, nominal precatalyst loading 1–3 mol %, ambient temperature, 30–100 bar). This process is derived from procedures that were developed by one of us^[13] and in parallel by Heiser et al.^[14] These groups treated **6** with approximately two equivalents of acids that contain coordinating conjugate bases (HCl, HBr, HI, and CF₃CO₂H, respectively). Both

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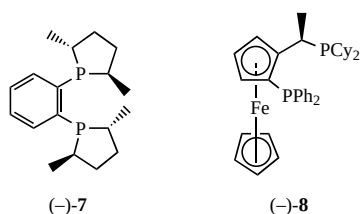
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methallyl groups are abstracted on protonation and added P–P displaces the cod ligand to generate precatalysts “[Ru(P–P)X₂]_n” (*n* = 2, 3) and [Ru(P–P)(CF₃CO₂)₂], respectively.

On attempting to generate cationic, and hence more electrophilic, coordinatively unsaturated catalysts, we instead used both an acid (HBF₄·Et₂O, soluble in many organic solvents) with a weakly coordinating conjugate base (BF₄[−]), and a weakly coordinating, aprotic reaction medium (CH₂Cl₂). Note that the known, direct reaction between **6** and P–P, which leads to the species [Ru(P–P)(η³-methallyl)₂],^[13a,b] is negligibly slow under the conditions of our process and that catalytic amounts of BF₃·Et₂O are required, although the exact role of the BF₃·Et₂O is unclear.

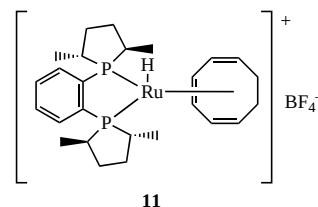
The most active and enantioselective chiral diphospane P–P ligands among the 40 we screened were (−)-Me-DuPHOS (−)-**7**,^[15] (−)-JOSIPHOS (−)-**8**,^[16] (Cy = cyclohexyl) and the JOSIPHOS-derivative (−)-**9** with *p*-CF₃-phenyl groups instead of the phenyl groups in **8**.^[17] Electron-rich



P–P ligands containing dialkylphosphane groups are required for high activity. Use of P–P ligands with two diphenylphosphane groups produced precatalysts with poor activities. Use of (+)-(BINAP)^[18] resulted in low activity and *ee* (enantiomer ratio (e.r.): (+)-**3**:(−)-**3** = 71:29 (GC^[60])). Our best enantioselectivities were modest but acceptable for our purposes (e.r.: (+)-**3**:(−)-**3** = 88:12 with (−)-**9**, 82:18 with (−)-**7**, and 75:25 with (−)-**8**). The *cis* selectivities were mostly very good throughout the series of ligands surveyed and excellent (+)-**3**:(−)-**4** ≥ 99:1 (GC)) with (−)-**7**, (−)-**8**, and (−)-**9**. The traces of the *trans* isomer (−)-**4** we detect are likely due to epimerization.

Optimization of the (−)-Me-DuPHOS (−)-**7** based process revealed that there are no substantial solvent effects and it can be run in almost neat substrate, **5**. In contrast, the (−)-JOSIPHOS (−)-**8** process requires a solvent. Use of a suitable solvent not only speeds up the hydrogenation but also increases the enantioselectivity. For example, the slow reaction without solvent leads to a final e.r. (+)-**3**:(−)-**3** of 79:21 (30 °C, 90 bar) but the reaction rate increases six fold and the e.r. increases to 94:6 when four volumes of *tert*-butyl methyl ether relative to **5** are added. We report final e.r. values for complete conversion because the product (+)-**3** itself gives a solvent effect at the high concentrations we use, so that its e.r. increases in the course of the reaction. For both processes, the kinetics are, up to ≈ 90 % conversion, close to zero order in substrate **5**. Typical, readily obtainable turnover numbers using either ligand are about 2000 at 25 °C and 90 bar, with turnover frequencies between 100 and 200 h^{−1}.

Using (−)-**7** as a model ligand, the reaction generating the precatalysts was monitored by NMR spectroscopy in CD₂Cl₂. The species that forms immediately upon treatment of the mixture of **6** and (−)-**7** with HBF₄·Et₂O and BF₃·Et₂O is the phosphonium salt (−)-**7**·HBF₄. Its identity was confirmed by preparing it separately.^[19] The reaction between **6** and (−)-**7**·HBF₄ in the presence of BF₃·Et₂O first gives an intermediate **10** that transforms slowly into a stable precatalyst **11**.



Compound **11** can be conveniently isolated when the reaction is run in a CH₂Cl₂/MeOAc solvent mixture, from which **11** precipitates as a yellow crystalline solid. It is even more convenient to treat separately prepared crystalline, (−)-**7**·HBF₄^[19] with **6** and BF₃·Et₂O in neat MeOAc (see the Experimental Section), which avoids the need to measure out one equivalent of HBF₄·Et₂O. Both procedures give **11** in about 65 % yield and it remains stable for months at −30 °C under argon. This is the first use of a protiophosphonium salt of a chiral P–P ligand to prepare a precatalyst.

The identity of [Ru((−)-Me-DuPHOS)(H)(η⁶-cot)](BF₄) **11** (cot = 1,3,5-cyclooctatriene) was ascertained from multinuclear one- and two-dimensional NMR spectroscopy and mass spectrometry (see the Experimental Section) and the solid-state structure was later determined by X-ray diffraction.^[20] A second, minor product that formed is a [Ru((−)-Me-DuPHOS)(H)(η⁴-cod′)](BF₄) **12** (cod′ = 1,3-cyclooctadiene), which was identified later.^[20] This minor product can be separated by recrystallization but that is normally not necessary as it is just as good a precatalyst as **11**. By analogy to work reported by one of us,^[21] we suggest that **10**, which is only observed in solution,^[22] is [Ru((−)-Me-DuPHOS)-(1-3:5,6-η-C₈H₁₁)](sol)](BF₄) (C₈H₁₁ = 2,5-cyclooctadienyl; sol = Et₂O, CH₂Cl₂). If so, **10** could be generated through protolytic cleavage of a methallyl group from **6** (to generate isobutene), coordination of (−)-**7**, allylic C–H activation at the cod ligand, and release of a second isobutene moiety. Loss of a solvent molecule from **10** with allylic activation from 1-3:5,6-η-C₈H₁₁ would generate **11**.

Crystalline **11** and **10**, generated in solution, can both be used for the hydrogenation of **5**, which shows that they have similar reactivities to H₂ to generate the same active catalyst. Exposure to H₂ brings about the hydrogenation of the cot and C₈H₁₁ ligands (to generate cyclooctane); the nature of the resulting, extremely electrophilic catalyst “[Ru((−)-Me-DuPHOS)(H)(sol)](BF₄)” is explored in detail in another paper.^[20]

Monitoring the reaction by NMR spectroscopy shows, that when the same kind of process is applied to (−)-JOSIPHOS (−)-**8**, several compounds are formed and only a minor component, a [Ru((−)-JOSIPHOS)(H)(η⁶-cot)](BF₄) **13** is

identified. It seems that all the components of this mixture react with H₂ under catalytic conditions to generate the same catalyst, because the activity of the mixture about equals that of pure **13** generated by another route.^[20]

Experimental Section

All operations were carried out in a glove box (under Ar; <1 ppm O₂ and H₂O) at ambient temperature unless indicated otherwise. NMR tubes were sealed under Ar and spectra were measured in CD₂Cl₂ at 300 K.

(–)-**7**·2 HBF₄: To a stirred solution of (–)-**7** (4.00 g, 13.06 mmol) in CH₂Cl₂ (25 mL) was added HBF₄·Et₂O^[12] (3910 µL, 4.652 g, 28.73 mmol). The reaction mixture was stirred for 10 min and the solvent was removed in vacuo.^[12] Addition of Et₂O (250 mL) precipitated a white powder, which was filtered, washed with Et₂O (5 × 60 mL), and dried in vacuo. Yield: 6.169 g (12.80 mmol, 98 %).

(–)-**7**·HBF₄: (–)-**7** (3.922 g, 12.80 mmol) and (–)-**7**·2 HBF₄ (6.169 g, 12.80 mmol) were dissolved in CH₂Cl₂ (115 mL) and the solution was stirred for 10 min. The solvent was removed in vacuo and addition of Et₂O (750 mL) precipitated a white powder, which was filtered, washed with Et₂O (2 × 200 mL), and dried in vacuo. Yield: 4.793 g (12.16 mmol, 95 %).

11: To a stirred solution of (–)-**7**·HBF₄ (1.182 g, 3.00 mmol) in MeOAc (75 mL) was added first **6** (0.958 g, 3.00 mmol) and, upon its dissolution, BF₃·Et₂O (380 µL, 0.426 g, 3.00 mmol). The reaction mixture was stirred for 24 h and then maintained at –30 °C for 80 h without stirring. A bright yellow, crystalline precipitate formed, which was collected by filtration, rapidly washed with MeOAc (2 × 5 mL), and dried in vacuo. Yield: 1.190 g^[23] (1.979 mmol, 66 %). ¹H NMR (400.1 MHz): δ = –9.97 (1 H, br. t, J_{PH} = 26.0 Hz), 0.70 (3 H, dd, J = 6.9, 15.3 Hz), 0.93 (3 H, dd, J = 7.4, 16.2 Hz), 1.14 (3 H, dd, J = 7.4, 18.2 Hz), 1.33 (3 H, dd, J = 6.9, 17.2 Hz), 1.30 (1 H, m), 1.42–1.86 (5 H, m), 2.13–2.59 (8 H, m), 2.71 (2 H, m), 5.24 (1 H, br. t, J = 7.9 Hz), 5.38 (1 H, br. q, J = 8.4 Hz), 5.62 (2 H, m), 6.30 (1 H, dd, J = 6.4, 8.9 Hz), 6.57 (1 H, t, J = 8.4 Hz), 7.57 (4 H, m); ¹³C{¹H} NMR (100.5 MHz): δ = 12.6, 14.4 (s, CH₃), 16.2, 18.3 (d, J_{PC} = 6.4 Hz, CH₃), 32.0, 34.8, 35.9, 36.4, 37.4, 37.5 (s, CH₂), 39.9 (d, J_{PC} = 25.7 Hz, CH), 40.9 (d, J_{PC} = 33.7 Hz, CH), 44.4 (d, J_{PC} = 16.1 Hz, CH), 44.7 (d, J_{PC} = 6.4 Hz, CH), 94.1, 94.3, 96.1, 99.1, 101.0, 102.3, 131.0, 131.3 (s, CH), 131.4 (d, J_{PC} = 17.7 Hz, CH), 132.0 (d, J_{PC} = 14.5 Hz, CH), the signal for C (m) was too weak; ³¹P NMR (161.9 MHz): δ = 84.9, 87.5 (d, J_{pp} = 20.3 Hz); ¹⁹F NMR (376.4 MHz): δ = –152.4; MS (desorption–CI using NH₃ as the reagent gas): isotopic cluster for [C₂₆H₃₉P₂Ru]⁺ around m/z 515.

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- [1] a) Reviews: T. K. Sarkar, B. K. Ghorai, *J. Indian Chem. Soc.* **1999**, *76*, 693–706; b) F. Schröder, *Angew. Chem.* **1998**, *110*, 1271–1274; *Angew. Chem. Int. Ed.* **1998**, *37*, 1213–1216; c) M. H. Beale, J. L. Ward, *Nat. Prod. Rep.* **1998**, 433–548; d) G. Sembdner, P. Parthier, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1993**, *44*, 569–589; e) M. Hamberg, H. W. Gardner, *Biochem. Biophys. Acta* **1992**, *1165*, 1–18. See also: f) W. Boland, J. Hopke, J. Donath, J. Nüske, F. Bublit, *Angew. Chem.* **1995**, *107*, 1715–1717; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1600–1602.
- [2] Compound (–)-**2** was at first thought to be the primary natural product and responsible for the odor, and therefore named methyl jasmonate. Later, (+)-**1** was found to be both the primary product and the biologically active and odorant compound, and named methyl epijasmonate. For simplicity, we name as follows: (+)-*cis*-methyl jasmonate (+)-**1**, (–)-*trans*-methyl jasmonate (–)-**2**, (+)-*cis*-methyl dihydrojasmonate (+)-**3**, (–)-*trans*-methyl dihydrojasmonate (–)-**4**.
- [3] T. E. Acree, R. Nishida, H. Fukami, *J. Agric. Food Chem.* **1985**, *33*, 425–427; R. Nishida, T. E. Acree, H. Fukami, *Agric. Biol. Chem.* **1985**, *49*, 769–772.
- [4] G. Ohloff, *Scent and Fragrances*, Springer, Berlin, **1994**, pp. 151–154; G. Fráter, J. A. Bajgrowicz, P. Kraft, *Tetrahedron* **1998**, *54*, 7633–7703.
- [5] a) E. Demole, E. Lederer, D. Mercier, *Helv. Chim. Acta* **1962**, *45*, 675–685; b) E. Demole, E. Lederer, D. Mercier, *Helv. Chim. Acta* **1962**, *45*, 685–692; c) E. Demole, M. Stoll, *Helv. Chim. Acta* **1962**, *45*,

- 692–703. For an important early review, see: d) E. Demole in *Fragrance Chemistry* (Ed.: E. T. Theimer), Academic Press, New York, **1982**, pp. 349–396.
- [6] Reviews^[1a, 5d]; (±)-**1**: a) M. Sannigrahi, D. L. Mayhew, D. L. J. Clive, *J. Org. Chem.* **1999**, *64*, 2776–2788; b) T. K. Sarkar, B. K. Ghorai, S. K. Nandy, B. Mukherjee, A. Banerji, *J. Org. Chem.* **1997**, *62*, 6006–6011; c) T. K. Sarkar, B. K. Ghorai, A. Banerji, *Tetrahedron Lett.* **1994**, *35*, 6907–6909; d) G. Stork, O. Ouerfelli, *New J. Chem.* **1992**, *16*, 95–98; e) T. Kitahara, Y. Warita, M. Abe, M. Seya, Y. Takagi, K. Mori, *Agric. Biol. Chem.* **1991**, *55*, 1013–1017; f) L. Crombie, K. M. Mistry, *J. Chem. Soc. Perkin I* **1991**, 1981–1991; g) H. Seto, H. Yoshioka, *Chem. Lett.* **1990**, 1797–1800; h) L. Crombie, K. M. Mistry, *J. Chem. Soc. Chem. Commun.* **1988**, 539–540; i) T. Kitahara, K. Miura, Y. Warita, Y. Takagi, K. Mori, *Agric. Biol. Chem.* **1987**, *51*, 1129–1133; j) R. V. Stevens, N. Hrib, *Tetrahedron Lett.* **1981**, *22*, 4791–4794; k) H. Tanaka, S. Torii, *J. Org. Chem.* **1975**, *40*, 462–465. (+)-**1**: l) C. Fehr, J. Galindo, *Angew. Chem.* **2000**, *112*, 581–585; *Angew. Chem. Int. Ed.* **2000**, *39*, 569–573; m) T. K. Sarkar, B. Mukherjee, S. K. Gosh, *Tetrahedron* **1998**, *54*, 3243–3254; n) G. J. Roth, S. Kirschbaum, H. J. Bestmann, *Synlett* **1997**, 618–620; o) H. Stadtmüller, A. Vaupel, C. E. Tucker, T. Stüdemann, P. Knochel, *Chem. Eur. J.* **1996**, *2*, 1204–1220; p) H. Stadtmüller, P. Knochel, *Synlett* **1995**, 463–464; q) T. Kitahara, T. Nishi, K. Mori, *Tetrahedron* **1991**, *47*, 6999–7006; r) G. Helmchen, A. Goeke, G. Lauer, M. Urmann, J. Fries, *Angew. Chem.* **1990**, *102*, 1079–1080; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1024–1025; s) K. Weinges, U. Lernhardt, *Liebigs Ann. Chem.* **1990**, 751–754.
- [7] O. Miersch, R. Kramell, B. Parthier, C. Wasternack, *Phytochemistry* **1999**, *50*, 353–361; M. C. S. Cano, E. G. Plaza, R. G. Muñoz, A. G. Benavente, *J. Essent. Oil Res.* **1998**, *10*, 67–69; A. S. C. Sing, J. Barbier, J. Smadja, *J. Nat.* **1997**, *9*, 8–16; G. Buchbauer, L. Jirovetz, V. K. Kaul, *J. Essent. Oil Res.* **1995**, *7*, 5–9; O. Miersch, G. Sembdner, K. Schreiber, *Phytochemistry* **1989**, *28*, 339–340; O. Miersch, A. Preiss, G. Sembdner, K. Schreiber, *Phytochemistry* **1987**, *26*, 1037–1039; W. Renold, R. Näf-Müller, U. Keller, B. Willhalm, G. Ohloff, *Helv. Chim. Acta* **1974**, *57*, 1301–1308.
- [8] V. Rautenstrauch, J.-J. Riedhauser (Firmenich SA), WO 9600206, **1996** [*Chem. Abstr.* **1996**, *124*, 232126c]. Reduction of (±)-**3** with L-selectride and saponification gives (±)-all-*cis*-dihydrocucurbitic acid (R. Kaiser, D. Lamparsky, *Tetrahedron Lett.* **1974**, *38*, 3413–3416). Resolution with (+)- and (–)-1-phenylethylamine, oxidation with pyridinium chlorochromate/NaOAc, and esterification with CH₂N₂ gives (+)-**3** and (–)-**3**. Epimerization with a catalytic amount of KNH₂ in liquid NH₃ at –75 °C gives (–)-**4** and (+)-**4**.
- [9] The following patents cover the work described herein: V. Rautenstrauch, J.-P. Genêt, J.-Y. Lenoir (Firmenich SA), WO 9718894, **1997** [*Chem. Abstr.* **1997**, *127*, 83071p]; D. A. Dobbs, K. P. M. Vanhessche, V. Rautenstrauch (Firmenich SA), WO 9852687, **1998** [*Chem. Abstr.* **1999**, *130*, 40076p]. An alternative approach to (+)-**3** (and (+)-**1**) that does not involve catalytic hydrogenation was also developed at Firmenich.^[6f]
- [10] P. Oberhänsli (Givaudan SA), DO 2008833, **1969** [*Chem. Abstr.* **1970**, *73*, 109364e].
- [11] M. O. Albers, E. Singleton, J. E. Yates, *Inorg. Synth.* **1989**, *26*, 249–258.
- [12] HBF₄·Et₂O, prepared by adding one equivalent of anhydrous HF to BF₃·Et₂O (K. Hafner, A. Stephan, C. Bernhard, *Liebigs Ann. Chem.* **1961**, *650*, 42–62), is often designated as a 54 wt % solution of HBF₄ in Et₂O and is volatile (the components HF and BF₃·Et₂O probably codistill).
- [13] a) J.-P. Genêt, S. Mallart, C. Pinel, S. Juge, J. A. Laffitte, *Tetrahedron: Asymmetry* **1991**, *2*, 43–46; b) J.-P. Genêt, C. Pinel, V. Ratovelomanana-Vidal, S. Mallart, X. Pfister, M. C. Caño De Andrade, J. A. Laffitte, *Tetrahedron: Asymmetry* **1994**, *5*, 665–674; c) J.-P. Genêt, C. Pinel, V. Ratovelomanana-Vidal, S. Mallart, X. Pfister, L. Bischoff, M. C. Caño De Andrade, S. Darses, C. Galopin, J. A. Laffitte, *Tetrahedron: Asymmetry* **1994**, *5*, 675–690; d) J.-P. Genêt, *Acros Chimica Acta* **1994**, *1*, 1–8; e) J.-P. Genêt, V. Ratovelomanana-Vidal, M. C. Caño De Andrade, X. Pfister, P. Guerreiro, J.-Y. Lenoir, *Tetrahedron Lett.* **1995**, *36*, 4801–4804; f) C. Girard, J.-P. Genêt, M. Bulliard, *Eur. J. Org. Chem.* **1999**, 2937–2942.
- [14] B. Heiser, E. A. Broger, Y. Cramer, *Tetrahedron: Asymmetry* **1991**, *2*, 51–62.

- [15] (–)-Me-DuPHOS (–)-**7** = (–)-1,2-bis((2*R*,5*R*)2,5-dimethylphosphoranyl)benzene; M. J. Burk, J. E. Feaster, W. A. Nugent, R. L. Harlow, *J. Am. Chem. Soc.* **1993**, *115*, 10125–10138.
- [16] (–)-JOSIPHOS (–)-**8** = (–)-(R)-(1)-[(S)-2-(diphenylphosphanyl)ferrocenyl]ethyldicyclohexylphosphane; A. Togni, C. Breutel, A. Schnyder, F. Spindler, H. Landert, A. Tijani, *J. Am. Chem. Soc.* **1994**, *116*, 4062–4066.
- [17] (–)-**9** = (–)-(R)-(1)-[(S)-2-(di(4-trifluoromethylphenyl)phosphanyl)ferrocenyl]ethyldicyclohexylphosphane; F. Spindler (Ciba-Geigy AG), EP 646 590, **1995** [*Chem. Abstr.* **1995**, *123*, 112416e].
- [18] (+)-(R)-BINAP = (+)-(R)-2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl; A. Myashita, A. Yasuda, H. Takaya, K. Toriumi, T. Ito, T. Souchi, R. Noyori, *J. Am. Chem. Soc.* **1980**, *102*, 7932–7934.
- [19] Treatment of (–)-**7** in Et₂O with 1 equiv. HBF₄·Et₂O precipitates (–)-**7**·HBF₄ but generation and isolation of (–)-**7**·2HBF₄ by reaction with excess HBF₄·Et₂O, and comproportionation with 1 equiv. (–)-**7** (see Experimental Section) is somewhat more convenient. Both salts can be isolated as analytically pure, crystalline solids. The solid-state structure of (–)-**7**·HBF₄ was determined by X-ray diffraction. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-138771. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [20] K. P. M. Vanhessche, D. A. Dobbs, V. Rautenstrauch, J. Wiles, S. H. Bergens, unpublished results.
- [21] J. A. Wiles, C. E. Lee, R. McDonald, S. H. Bergens, *Organometallics* **1996**, *15*, 3782–3784.
- [22] ³¹P NMR (161.9 MHz, CD₂Cl₂, 300 K): δ = 79.2, 86.2 (d, *J*_{PP} = 30.5 Hz).
- [23] This quality still contains ~5% of **12**, as determined by ³¹P NMR.

Constant Selectivity Relationships of Addition Reactions of Carbanions**

Roland Lucius and Herbert Mayr*

*Dedicated to Professor Rolf Saalfrank
on the occasion of his 60th birthday*

Reactions of carbocations and related electrophiles with uncharged nucleophiles obey the linear free-energy relationship given in [Eq. (1)], where *E* = electrophilicity parameter, *N* = nucleophilicity parameter, and *s* = nucleophile-specific slope parameter.^[1]

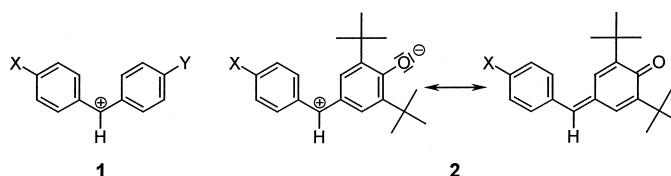
$$\log k (20^\circ\text{C}) = s(N + E) \quad (1)$$

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Since the slope parameter *s* is usually close to unity, it may be neglected for qualitative considerations, so that in practice reactions will be sufficiently fast at 20 °C if (*N* + *E*) > –5. Since the change of polarity is small in the rate-determining step of these ion–molecule reactions, the solvent effects on reaction rates are also small and can, to a first approximation, be neglected.^[1c]

For the development of reactivity scales for uncharged nucleophiles, the benzhydryl cations **1** proved to be extraordinarily suitable reference electrophiles since their electrophilicities can be altered by almost 20 orders of magnitude by varying the substituents X and Y but the steric situation at the reactive site remains constant. Benzhydryl cations with amino groups in the *p*-position are the weakest reference electro-



philes used so far to characterize the nucleophilicities of silyl enol ethers,^[2] silyl ketene acetals,^[2] and enamines.^[3] In order to perform kinetic investigations with still stronger nucleophiles, a further reduction of the electrophilicity of benzhydryl cations is necessary, which may be achieved by employing the strong electron donor O[–] at the position X or Y of compound **1**. Thus, the quinone methides **2** represent uncharged analogues of the benzhydryl cations **1**, which again allows a variation of electrophilicity under a constant steric situation. Richard et al.^[4] have already reported that quinone methides behave as highly resonance-stabilized carbocations.

The quinone methides **2a–d** are accessible through a Mannich-type reaction from 2,6-di-*tert*-butylphenol in a one-pot procedure.^[5] For the determination of the reaction rates, the potassium^[6] or tetra-*n*-butylammonium^[7] salts of the carbanions **3** were dissolved in DMSO. After addition of 0.02 to 0.2 equivalents of **2**, the change in their UV/Vis absorbance between λ = 200–600 nm was monitored with a diode-array spectrometer,^[8a] and featuring a fiber-optic immersion probe.^[8b] The pseudo first-order rate constants, *k*_{1ψ}, determined from the exponential decay of the absorbance at the absorption maximum, were divided by the carbanion concentration to yield the concentration-independent rate constant *k* [Eq. (2)], to prove that second-order kinetics are present.

$$-d[\mathbf{2}]/dt = k[\mathbf{2}][\mathbf{3}] = k_{1\psi}[\mathbf{2}] \quad (2)$$

$$k = k_{1\psi}/[\mathbf{3}]$$

The observation of isosbestic points (Figure 1) excludes long-lived intermediates, and one can assume the simple mechanism outlined in Scheme 1.

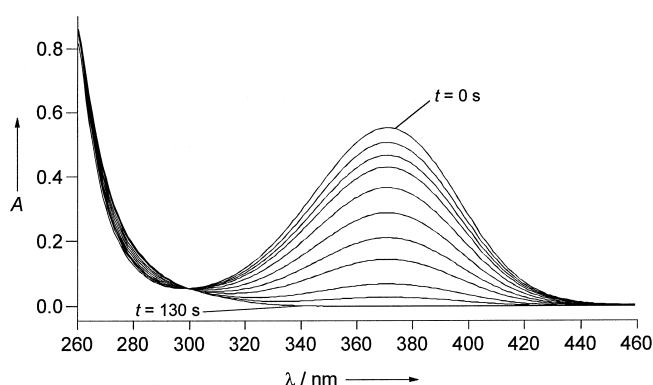
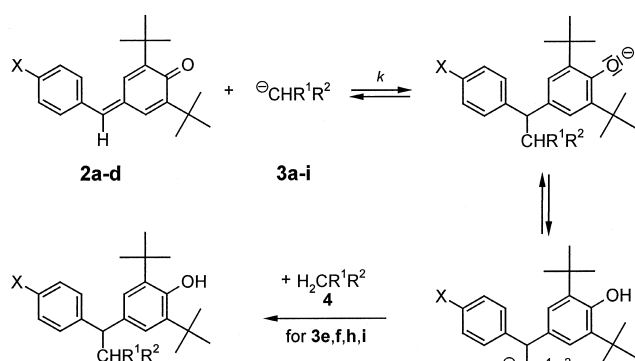


Figure 1. UV spectra during the reaction of the quinone methide **2d** with the tetra-*n*-butylammonium salt of ethyl cyanoacetate **3c** (DMSO, 20 °C).



Scheme 1. Reactions of the quinone methides **2a–d** with the carbanions **3a–i**.

Table 1 shows that under these reaction conditions, at carbanion concentrations 10^{-4} – 10^{-3} mol L $^{-1}$, equal reaction rates are found for the tetra-*n*-butylammonium and potassium salts in presence or absence of crown ether or cryptand. The addition reactions of the carbanions **3e**, **f**, **h**, **i** do not proceed quantitatively. In the presence of an excess of **4**, the corresponding acids of the carbanions **3**, the equilibrium is shifted so far towards the products that the final concentrations of the quinone methides **2** are negligibly small.

When the rate constants of the addition reactions of the carbanions **3a–i** to the quinone methides **2a–d** (Table 2) are plotted against the rate constants of the reference reactions of

Table 1. Influence of the counterion on the rate constants of the addition reactions of **2** with the anions **3** in DMSO at 20 °C.

Electrophile	$\ominus\text{CHR}^1\text{R}^2$	Counterion	$k^{[d]}$ [L mol $^{-1}$ s $^{-1}$]
2c	3b	K $^+$ /[18]crown-6 ^[a]	$(5.28 \pm 0.04) \times 10^2$
		N(<i>n</i> Bu) $_4^+$ ^[b]	$(5.17 \pm 0.07) \times 10^2$
2d	3c	K $^+$	$(3.35 \pm 0.08) \times 10^2$
		K $^+$ /[18]crown-6 ^[c]	$(3.25 \pm 0.09) \times 10^2$
2d	3g	K $^+$ /Cryptofix 222 ^[c]	$(3.29 \pm 0.07) \times 10^2$
		N(<i>n</i> Bu) $_4^+$ ^[b]	$(3.49 \pm 0.05) \times 10^2$
		K $^+$	3.90
		K $^+$ /[18]crown-6 ^[c]	3.81 ± 0.03

[a] 1.05 equivalents. [b] The existence of dimers, derived by Reetz et al. (ref. [7a–d]) from cryoscopic measurements in benzene, does not affect the rate constant under the conditions employed in these experiments. [c] 1.1 equivalents. [d] The listed rate constants have been averaged from two to five experiments with different anion concentrations.

Table 2. Rate constants for the reactions of the quinone methides **2a–d** with the carbanions **3a–i** in DMSO at 20 °C.

$\ominus\text{CHR}^1\text{R}^2$	M ^[a]	Electrophile	$k^{[c]}$ [L mol $^{-1}$ s $^{-1}$]
3a	N(<i>n</i> Bu) $_4^+$	2a	$(2.00 \pm 0.05) \times 10^2$
		2b	$(4.12 \pm 0.10) \times 10^2$
		2c	$(2.06 \pm 0.05) \times 10^3$
		2d	$(5.17 \pm 0.07) \times 10^2$
3b	N(<i>n</i> Bu) $_4^+$	2a	$(2.89 \pm 0.02) \times 10^1$
		2b	$(6.99 \pm 0.13) \times 10^2$
		2c	$(5.17 \pm 0.07) \times 10^2$
		2d	$(8.65 \pm 0.07) \times 10^2$
3c	N(<i>n</i> Bu) $_4^+$	2a	$(1.43 \pm 0.02) \times 10^1$
		2b	$(3.39 \pm 0.04) \times 10^1$
		2c	$(2.13 \pm 0.04) \times 10^2$
		2d	$(3.49 \pm 0.05) \times 10^2$
3d	K $^+$ /[18]crown-6	2a	9.90 ± 0.05
		2b	$(2.55 \pm 0.05) \times 10^1$
		2c	$(1.39 \pm 0.01) \times 10^2$
		2d	$(2.16 \pm 0.01) \times 10^2$
3e	K $^+$ /[18]crown-6	2a ^[b]	3.52 ± 0.06
		2b ^[b]	$(1.07 \pm 0.05) \times 10^1$
		2c ^[b]	$(7.98 \pm 0.21) \times 10^1$
		2d ^[b]	$(1.28 \pm 0.03) \times 10^2$
3f	K $^+$ /[18]crown-6	2a ^[b]	$(5.57 \pm 0.04) \times 10^{-1}$
		2b ^[b]	1.97 ± 0.02
		2c ^[b]	$(1.31 \pm 0.02) \times 10^1$
		2d ^[b]	$(2.15 \pm 0.06) \times 10^1$
3g	K $^+$ /[18]crown-6	2b	$(4.28 \pm 0.03) \times 10^{-1}$
		2c	2.69
		2d	3.81 ± 0.03
3h	K $^+$ /[18]crown-6	2a ^[b]	$(8.15 \pm 0.17) \times 10^{-2}$
		2b ^[b]	$(1.87 \pm 0.08) \times 10^{-1}$
		2c ^[b]	1.26 ± 0.01
		2d ^[b]	1.96 ± 0.03
3i	K $^+$ /[18]crown-6	2c ^[b]	$(1.38 \pm 0.01) \times 10^{-2}$
		2d ^[b]	$(2.06 \pm 0.01) \times 10^{-2}$

[a] The potassium salts were combined with 1.01 to 1.10 equivalents of [18]crown-6. [b] With addition of 0.5 to 2.5 equivalents of the corresponding acid **4**. [c] The listed rate constants have been averaged from two to five experiments with different anion concentrations.

3c with **2a–d**, linear correlations are observed (Figure 2). The parallel correlation lines imply that the relative reactivities of the carbanions **3a–i** are independent of the electrophilicity of the reaction partners, that is, the vertical arrangement of the carbanions **3a–i** corresponds to a nucleophilicity scale. Vice versa, the relative reactivities of the quinone methides **2a–d** are independent of the nature of the carbanions and an electrophilicity scale of the quinone methides **2a–d** can be derived from their positions on the abscissa of Figure 2. Like the reactions of carbocations with nucleophiles,^[1, 9, 10] these reactions are controlled by constant selectivity relationships which appear to be more general than the inverse relationships between reactivity and selectivity postulated by the reactivity–selectivity principle.^[11, 12]

The reactivities of the carbanions **3a–i** towards the quinone methides **2** correlate only moderately ($r = 0.88$, nine points) with the corresponding $\text{p}K_{\text{a}}$ values^[13] (Figure 3). The correlation between the oxidation potentials of the carbanions **3b–f**^[14] and **3h**^[14a] and their nucleophilic reactivities is even weaker ($r = -0.69$, six points). In an analogous case, Bordwell et al. reported a considerable scatter in the correlation of $\text{p}K_{\text{a}}$ values with oxidation potentials of these carbanions.^[14a]

Having demonstrated that the reactions of carbanions with uncharged electrophiles follow analogous relationships as the reactions of carbocations with uncharged nucleophiles, we will now attempt to connect the two reactivity scales.

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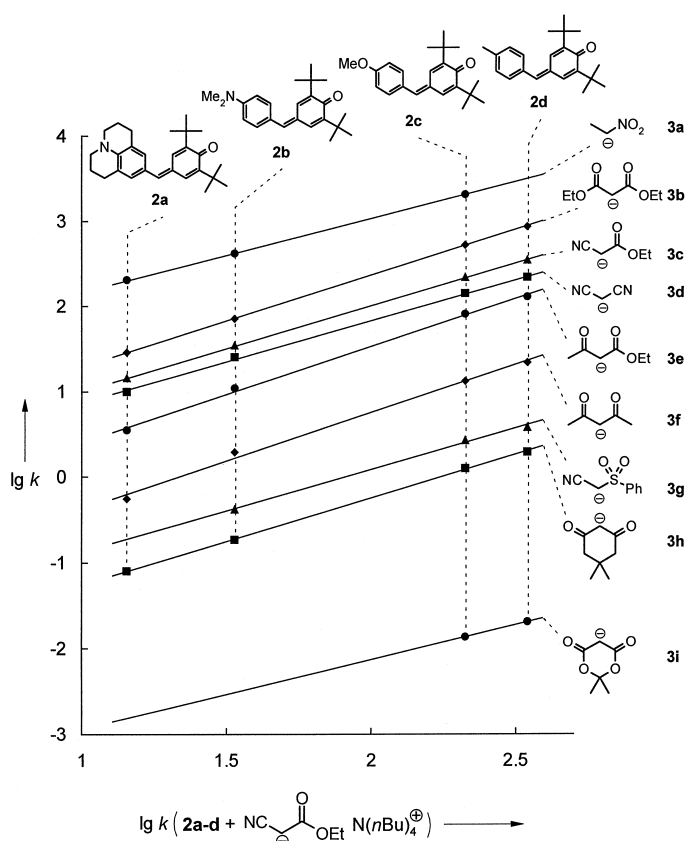


Figure 2. Constant selectivity relationships for the reactions of the quinone methides **2a–d** with the carbanions **3a–i** (DMSO, 20 °C).

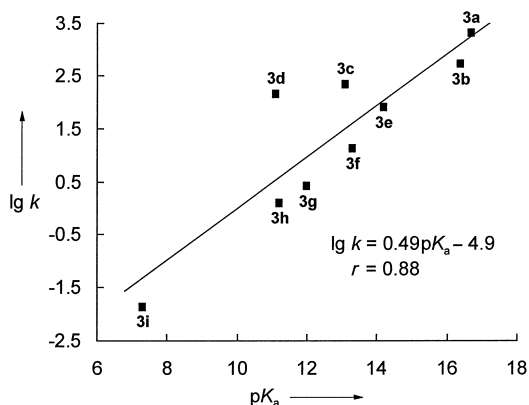


Figure 3. Linear correlation between the rate constants $\lg k$ for the reactions of the quinone methide **2c** with the carbanions **3a–i** (DMSO, 20 °C) and the pK_a values (DMSO)^[13] of the corresponding acids.

Constant selectivity relationships have also been reported for S_N2 reactions of phenylsulfonyl-substituted benzyl anions with 1-chlorobutane and 1-bromobutane,^[15] as well as for the reactions of 9-substituted fluorenyl anions with eight different benzyl chlorides.^[16] Only few kinetic data on addition reactions of carbanions to electron-deficient π -electron systems have been reported.^[17–20] While some of these data^[20] can be interpreted in terms of constant selectivity relationships, the paucity of electrophiles investigated prevents definite conclusions.

- Reviews: a) H. Mayr, M. Patz, M. F. Gotta, A. R. Ofial, *Pure Appl. Chem.* **1998**, *70*, 1993–2000; b) H. Mayr, O. Kuhn, M. F. Gotta, M. Patz, *J. Phys. Org. Chem.* **1998**, *11*, 642–654; c) H. Mayr, M. Patz, *Angew. Chem.* **1994**, *106*, 990–1010; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 938–957.
- J. Burfeindt, M. Patz, M. Müller, H. Mayr, *J. Am. Chem. Soc.* **1998**, *120*, 3629–3634.
- N. Hering, Diploma thesis, Technische Hochschule Darmstadt, **1996**.
- a) J. P. Richard, M. M. Toteva, J. Crueiras, *J. Am. Chem. Soc.* **2000**, *122*, 1664–1674; b) J. P. Richard, *J. Am. Chem. Soc.* **1991**, *113*, 4588–4595; c) J. P. Richard, T. L. Amyes, L. Bei, V. Stubblefield, *J. Am. Chem. Soc.* **1990**, *112*, 9513–9519.
- S. Evans, P. Nesvadba, S. Allenbach (Ciba-Geigy AG), EP-B 744 392, **1996** [*Chem. Abstr.* **1997**, *126*, 46968v].
- Prepared analogously to E. M. Arnett, S. G. Maroldo, S. L. Schilling, A. Harrelson, *J. Am. Chem. Soc.* **1984**, *106*, 6759–6767.
- Prepared analogously to a) M. T. Reetz, S. Hütte, R. Goddard, *Eur. J. Org. Chem.* **1999**, 2475–2478; b) M. T. Reetz, S. Hütte, R. Goddard, *Z. Naturforsch. B* **1995**, *50*, 415–422; c) S. Hütte, PhD thesis, Philipps-Universität Marburg, **1993**; d) M. T. Reetz, S. Hütte, R. Goddard, *J. Am. Chem. Soc.* **1993**, *115*, 9339–9340.
- a) Diode-array spectrometer: TIDAS DAD 2062 (J&M); b) Probe: 661.502-UV (Hellma).
- C. D. Ritchie, *Acc. Chem. Res.* **1972**, *5*, 348–354.
- L. A. P. Kane-Maguire, E. D. Honig, D. A. Sweigart, *Chem. Rev.* **1984**, *84*, 525–543.
- a) J. E. Leffler, E. Grunwald, *Rates and Equilibria of Organic Reactions*, Wiley, New York, **1963**, pp. 162–168; b) O. Exner, *J. Chem. Soc. Perkin Trans. 2* **1993**, 973–979; c) S. J. Formosinho, *J. Chem. Soc. Perkin Trans. 2* **1988**, 839–846; d) A. Pross, *Adv. Phys. Org. Chem.* **1977**, *14*, 69–132; e) C. D. Johnson, B. Stratton, *J. Chem. Soc. Perkin Trans. 2* **1988**, 1903–1907; f) B. Giese, *Angew. Chem.* **1977**, *89*, 162–173; *Angew. Chem. Int. Ed. Engl.* **1977**, *16*, 125–136; g) E. Bunzel, H. Wilson, *J. Chem. Educ.* **1987**, *64*, 475–480.
- M. Roth, H. Mayr, *Angew. Chem.* **1995**, *107*, 2428–2430; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2250–2252.
- a) F. G. Bordwell, *Acc. Chem. Res.* **1988**, *21*, 456–463; b) W. S. Matthews, J. E. Bares, J. E. Bartmess, F. G. Bordwell, F. J. Cornforth, G. E. Drucker, Z. Margolin, R. J. McCallum, G. J. McCallum, N. R. Vanier, *J. Am. Chem. Soc.* **1975**, *97*, 7006–7014; c) F. G. Bordwell, H. E. Fried, *J. Org. Chem.* **1981**, *46*, 4327–4331.
- a) F. G. Bordwell, J. A. Harrelson, Jr., A. V. Satish, *J. Org. Chem.* **1989**, *54*, 3101–3105; b) F. G. Bordwell, J. A. Harrelson, Jr., X. Zhang, *J. Org. Chem.* **1991**, *56*, 4448–4450.
- F. G. Bordwell, J. C. Branca, T. A. Cripe, *Isr. J. Chem.* **1985**, *26*, 357–366.
- F. G. Bordwell, D. L. Hughes, *J. Org. Chem.* **1980**, *45*, 3320–3325.
- a) C. F. Bernasconi, *Tetrahedron* **1989**, *45*, 4017–4090; b) C. F. Bernasconi, J. L. Zitomer, D. F. Schuck, *J. Org. Chem.* **1992**, *57*, 1132–1139.
- I. Kolb, V. Macháček, V. Štěrbá, *Collect. Czech. Chem. Commun.* **1976**, *41*, 1914–1925.
- F. Terrier, R. Goumont, M.-J. Pouet, J.-C. Hallé, *J. Chem. Soc. Perkin Trans. 2* **1995**, 1629–1637.
- a) M. R. Crampton, J. A. Stevens, *J. Chem. Soc. Perkin Trans. 2* **1991**, 1715–1720; b) J. P. L. Cox, M. R. Crampton, P. Wight, *J. Chem. Soc. Perkin Trans. 2* **1988**, 25–29.

[PtIn₆]¹⁰⁺ Octahedra in PtIn₇F₁₃: The First Compound of a New Class of Metal-Cluster Fluorides

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Fluoride compounds containing low valent indium are largely unknown and all experiments to reduce InF₃ with H₂ or metallic In, which date back to the 1930s,^[1] have failed. Our own experiments for the reduction of InF₃ with elemental In in Pt ampoules often resulted in a pale yellow products whose X-ray powder patterns could not be assigned to any known phase. Since the hypothetical "InF" is isoelectronic with SnO and the recently discovered Ru₃Sn₁₅O₁₄,^[2] which can formally be considered as "Ru₃Sn · 14SnO", it seemed likely that the corresponding low valent indium fluorides exist. Through addition of Pt powder to the well known In–InF₃ system, we succeeded in the synthesis of the new fluoride PtIn₇F₁₃ as pure powder samples and single crystals (see Experimental Section), from which the crystal structure was solved and the structural parameters refined.^[3, 4]

The crystal structure of PtIn₇F₁₃ is shown in Figure 1. Characteristic building units are the [PtIn₆] octahedra, which are observed for the first time. These units are stacked in an

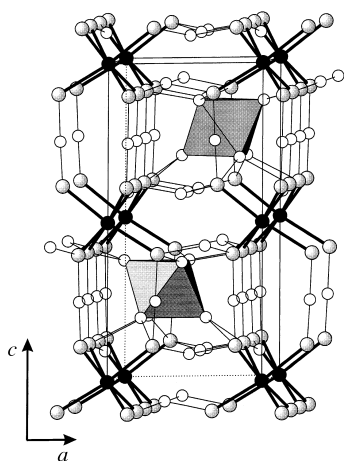


Figure 1. Projection of the crystal structure of PtIn₇F₁₃ with the unit cell. Small black circles represent Pt, grey circles In, and open circles F. Short Pt–In distances (< 260 pm) within the [PtIn₆] octahedra are represented by thick lines and the [In(1)F₆] octahedra are represented as polyhedra.

alternating fashion together with [InF₆] octahedra along [001]. Platinum centers are octahedrally coordinated by six In atoms with Pt–In bond lengths ranging from 253.0 to 254.7 pm (Figure 2). These distances are significantly smaller than in intermetallic phases with six-fold coordinated Pt atoms, for example, LaPtIn₃^[5] with *d*_{Pt–In} = 269 pm or Sr₂Pt₃In₄^[6] with *d*_{Pt–In} = 265 pm. The In–In distances within the [PtIn₆] cluster of PtIn₇F₁₃ are, at 344 pm to 378 pm, only slightly longer than

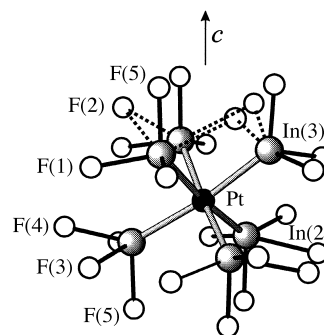


Figure 2. Projection of a [PtIn₆F₂₄] cluster with threefold symmetry in PtIn₇F₁₃ together with the numbering of the atoms. Selected distances [pm]: Pt–In(2) 254.7(1), Pt–In(3) 253.0(1), In(2)–In(2) 378.0(1), In(2)–In(3) 360.5(1), In(3)–In(3) 344.2(1), In(2)–F(5) 207.9(12), In(2)–F(4) 209.4(2), In(2)–F(3) 237.5(6), In(3)–F(5) 198.0(12), In(3)–F(1) 206.5(3), In(3)–F(2) 254.6(10).

in elemental In (*d*_{In–In} = 325 pm (× 4) or in molecular In clusters (338 pm (× 8)) such as In₈(2,6-Mes₂C₆H₃)₄ (Mes = 2,4,6-Me₃C₆H₂).^[7] For a further comparison, [In(2)In(1)₄]⁷⁺ tetrahedra in Na₂₃In₅O₁₅^[8] are present with short In(1)–In(2) distances of 275 pm, whereas the In(1)–In(1) edges are relatively long (445 pm).

The [PtIn₆] octahedra are surrounded by a total of 24 F ligands, which lie from 198 to 255 pm distant. Such a [PtIn₆F₂₄] cluster is unique as no fluorides are known which contain comparable units. Only within the higher homologues of the halides are there compounds with discrete octahedral clusters which are centered by transition metals, for example, Cs₄Pr₆OsI₁₃^[9] and Bi₃₄Ir₃Br₃₇.^[10] The In(2) atoms are terminally coordinated by four F atoms, whereas the In(3) atoms have three nearest terminal F atoms and, in addition, two further F(2) atoms coordinate and these lie above the In(3)–In(3) edges. The In(3)–F(2) distances are, at 255 pm, relatively long and it is remarkable that μ_2 -bridging F ligands in corresponding molecular metal clusters are unknown. Analogous to the highly ionic transition metal compounds containing M₆X₁₂X₆^a clusters (i = inner (German: innere), a = outer (äußere))^[11] with only 18 surrounding anions and significantly shorter M–M distances (for example, low valent oxoniobates^[12, 13] or oxomolybdates^[14]) the F(2) atoms can be denoted as inner F ligands (Fⁱ). However, in Nb₆F₁₅^[15] or Na₂Nb₇F_{21–y}Cl_y (*y* ≥ 8),^[16] the hitherto example of a metal cluster fluoride complex (Nb₆F₁₂F₆^a), the Nb–Fⁱ distances are shorter than those of the Nb–F^a. The different coordination spheres of the In(3) and In(2) atoms in PtIn₇F₁₃ are reflected in different distances from the [In(1)F₆] octahedra to the three In(3) and three In(2) of neighboring [PtIn₆] octahedra (Figure 1) and, as a result, the space group is acentric (*P6₃mc* instead of *P6₃/mmc*).

The In–F distances within the [In(1)F₆] octahedra are between 207 and 217 pm as found in complex fluorindates(III), for example, K₂NaInF₆,^[17] and it seems reasonable to assume a +3 oxidation state for the In(1) atom. For a deeper analysis of the bonding situation in PtIn₇F₁₃, we have calculated bond-order sums according to $\Sigma s_i = \Sigma \exp[(r_0 - r_i)/B]$ (*B* = 37 pm) with *r*₀ = 180 pm for the In–F distances.^[18] As expected, one obtains $\Sigma s_i \approx 1$ for the single F ligands and $\Sigma s_i =$

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2.55 for the octahedrally coordinated In(1) center. For the In(2) and In(3) atoms one obtains significantly smaller values, $\Sigma s_i \approx 1.35$ and $\Sigma s_i \approx 1.85$, respectively, and therefore their oxidation states should lie between +1 and +2. According to the representation of the ionic limit, $\text{PtIn}_7\text{F}_{13}$ is therefore $[\text{PtIn}_6]^{10+}(\text{F}^-)_7[\text{In}^{3+}\text{F}_6]^{3-}$, a mixed-valent Pt–In fluoride–fluorooxide(III).

There are two possibilities for the charge distribution within the $[\text{PtIn}_6]^{10+}$ octahedra resulting in integer oxidation states for the In(2), In(3), and Pt atoms and which are all in agreement with an octahedral coordination sphere for Pt with respect to the approach of the ligand field theory [Eq. (1)].



In both cases, platinum achieves a stable 18 electron configuration on assuming that In^+ is a two-electron and In^{2+} is a one-electron σ -donor, respectively. This is in agreement with the diamagnetism of $\text{PtIn}_7\text{F}_{13}$ (see Experimental Section) and it therefore has to be considered as a valence compound with only paired electrons.

Band-structure calculations, on the basis of the Extended-Hückel method,^[19, 20] indicate that $\text{PtIn}_7\text{F}_{13}$ is an insulator and both Pt–In as well as In–In interactions dominate below the Fermi level (Figure 3). It is clear from the COOP

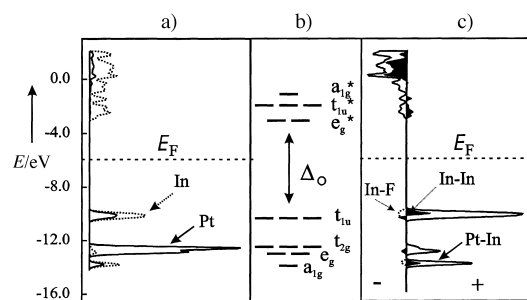


Figure 3. a) Projected density of states (DOS) for In and Pt, b) MO scheme for the metal-centered orbitals, and c) overlap population (COOP curve) for the highest occupied bands in $\text{PtIn}_7\text{F}_{13}$.

diagram (Figure 3c) that these interactions have bonding character and are, as expected, antibonding with respect to In–F interactions. The interstitial Pt atom provides electrons and orbitals to the strong bonding with the s orbitals of the surrounding In atoms. In contrast to “classical” complexes such as $[\text{PtCl}_6]^{2-}$, the highest occupied orbital (HOMO) of the $[\text{PtIn}_6]^{10+}$ cluster is not a t_{2g} state but rather a t_{1u} state because of the energetically high In 4s orbitals. The calculated band gap, Δ_0 , is defined by the energy difference between this state and the lowest unoccupied orbital (LUMO), e_g^* . Band gaps obtained from Extended-Hückel calculations are normally too large and, therefore, it is not unusual that the calculated value of $\Delta_0 = 3.3$ eV for $\text{PtIn}_7\text{F}_{13}$ lies slightly above the value obtained by UV absorption spectroscopy. This value corresponds to the pale yellow color of $\text{PtIn}_7\text{F}_{13}$ crystals.

The question arises as to which oxidation states could be assigned to the atoms of the $[\text{PtIn}_6]^{10+}$ octahedra. For such a discussion, the bonding situation of the $[\text{PtIn}_6]$ octahedra in $\text{PtIn}_7\text{F}_{13}$ is compared with the $[\text{RuSn}_6]$ octahedra in $\text{Ru}_3\text{Sn}_{15}\text{O}_{14}$,

in which the octahedra are connected through common corners to triple chains (Figure 4). In this oxide, it seems reasonable to assign the +1 oxidation state to the four connected Sn atoms and the +2 oxidation state to the two peripheral Sn atoms. This assignment allows 18 valence

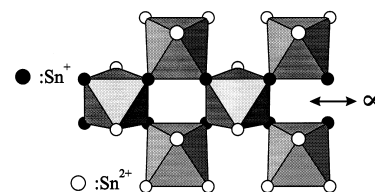


Figure 4. Projection of a fragment of the chain of corner-sharing $[\text{RuSn}_6]$ octahedra in $\text{Ru}_3\text{Sn}_{15}\text{O}_{14}$.

electrons for each of the single Ru atoms and additionally clarifies the condensation of the $[\text{RuSn}_6]$ octahedra.^[2] As the discrete $[\text{PtIn}_6]$ octahedra contain only peripheral In atoms, In should correspondingly have the +1 oxidation state and the Pt atoms the +4 oxidation state. Then however, one would oxidize metallic Pt with InF_3 during the synthesis of $\text{PtIn}_7\text{F}_{13}$, a situation which seems less plausible. However, by band structure calculations, $\text{Ru}_3\text{Sn}_{15}\text{O}_{14}$ cannot directly be compared with $\text{PtIn}_7\text{F}_{13}$ as there is non-negligible In–In bonding within the PtIn_6 octahedra, whereas no Sn–Sn bonding has been observed in $\text{Ru}_3\text{Sn}_{15}\text{O}_{14}$. Alternatively, one could assume the 0 oxidation state for Pt and distribute the remaining valence electrons to In(2) and In(3).

It seems, at first, daring to assign the –2 oxidation state to the Pt atoms within the $[\text{PtIn}_6]^{10+}$ octahedra. However, Pt is more electronegative than In and, moreover, the electron affinity of Pt (205 kJ mol^{-1}) is larger than that of S (200 kJ mol^{-1}).^[21] Of course, the second electron affinity of Pt might be positive but this holds also for O in the rich chemistry of oxides. A Pt^{2-} ion would be isoelectronic with the well known Au^- ion in Cs_3AuO ,^[23] and therefore does not appear too unusual.^[22] Furthermore, there are some hints from band structure calculations for a negatively charged Pt as shown for CaPtIn_2 .^[24] Investigation of the valence of Pt centers is necessary, yet they cannot be obtained easily as ^{196}Pt Mössbauer spectroscopy is, unfortunately, not a routine method and X-ray adsorption near-edge structure (XANES) measurements are relatively complex.

We have now succeeded in the preparation of further fluorides and oxides with main group element clusters with interstitial transition metal atoms, especially with $[\text{IrIn}_6]^{9+}$ octahedra. By such structures, the question of oxidation states will be easier to answer with studies of the corresponding Mössbauer spectra.

Experimental Section

Carefully dried InF_3 (Merck, p.a.) was mixed with Pt powder (Merck, p.a.) and In powder (Alfa, 99.99%, 325 mesh) in the ratio 13:3:8, then ground in an achate mortar to a grey powder under an Ar atmosphere. The powder was pressed to a pellet, sealed under Ar in a Pt tube, and maintained at 750°C for 14 d. On cooling to room temperature, pale yellow transparent crystals of $\text{PtIn}_7\text{F}_{13}$ with a platelike trigonal habit formed within one day. The crystals are stable in air and soluble in dilute HCl. The elemental

analysis of a powder sample of $\text{PtIn}_7\text{F}_{13}$ with ICP^[25] resulted in 4.5 mol % Pt and 30.9 mol % In (calculated: 4.8 mol % Pt and 33.3 mol % In).

The magnetic susceptibility of $\text{PtIn}_7\text{F}_{13}$ was measured using a Superconducting Quantum Interference Device (SQUID)-V.T.S.-Susceptometer (S.H. Corp., San Diego (USA) and Quantum Design (MPMS: Magnetic Property Measurement System)) at a constant external magnetic field as a function of temperature. The diamagnetic contribution for $\text{PtIn}_7\text{F}_{13}$ of $3.6 \times 10^{-4} \text{ emu mol}^{-1}$ is in approximate agreement with the value calculated from atomic increments^[26] (F^- : 1.1×10^{-5} ; In^{3+} : 1.9×10^{-5} ; Pt^{2+} : $2.0 \times 10^{-5} \text{ emu mol}^{-1}$).

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- [1] O. Hannebohn, W. Klemm, *Z. Anorg. Allg. Chem.* **1936**, 269, 2.
- [2] W. Reichelt, T. Söhnel, O. Rademacher, H. Oppermann, A. Simon, J. Köhler, *Angew. Chem.* **1995**, 107, 2307; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 2113.
- [3] Crystallographic data and crystal structure determination of $\text{PtIn}_7\text{F}_{13}$: $M = 1245.8 \text{ g mol}^{-1}$, pale yellow platelets, $0.05 \times 0.05 \times 0.01 \text{ mm}$, hexagonal, space group $P6_3mc$ (No. 186); $a = 734.2(1)$, $c = 1384.4(2) \text{ pm}$, $Z = 2$, $\text{AgK}\alpha$ radiation ($\lambda = 56.086 \text{ pm}$), graphite monochromator, CAD4 diffractometer, $T = 293 \text{ K}$, structure solution with direct methods (SHELX86), least-squares refinement on F_o^2 values, complete matrix (SHELX93), 3132 reflections, 943 independent, 47 parameters, $R1 = 0.025$ ($896F_o > 4\sigma(F_o)$), $wR2 = 0.057$, $\text{GOF} = 1.27$, max./min. residual electron density $+1.2/-1.3 \text{ e } 10^{-6} \text{ pm}^3$. The acentric space group results from the refinement and an inversion twin (twin factor of 0.45(2)) is present.^[4] Atomic positions [isotropic displacement parameters]: Pt: 0, 0, 0 [0.0080(1)]; In(1): 1/3, 2/3, 0.2770(1) [0.0188(3)]; In(2): 0.1706(1), 0.3411(1), 0.9036(1) [0.0254(2)]; In(3): 0.3096(1), 0.1548(1), 0.1149(8) [0.0277(2)]; F(1): 0.5367(9), 0.4633(9), 0.0887(8) [0.043(2)]; F(2): 0.1947(12), 0.8053(12), 0.1987(12) [0.091(5)]; F(3): 0.4669(7), 0.5331(7), 0.3744(6) [0.030(2)]; F(4): 1/3, 2/3, 0.9263(8) [0.015(2)]; F(5): 0.3595(28), 0.2151(29), 0.2548(8) [0.077(7)]. The position of F(5) is only half occupied. Refinements in a less symmetrical space group did not lead to a better result. Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49)7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-41113.
- [4] H. D. Flack, G. Bernardinelli, *Acta Crystallogr. Sect. A* **1985**, 41, 500.
- [5] Y. V. Galadzhun, R. Pöttgen, *Z. Anorg. Allg. Chem.* **1998**, 625, 481.
- [6] R.-D. Hoffmann, R. Pöttgen, *Z. Anorg. Allg. Chem.* **1998**, 625, 994.
- [7] B. E. Eichler, N. J. Hardman, P. P. Power, *Angew. Chem.* **2000**, 112, 391; *Angew. Chem.* **2000**, 39, 383.
- [8] H. J. Deiseroth, H. Kerber, R. Hoppe, *Z. Anorg. Allg. Chem.* **1998**, 624, 541.
- [9] M. Lulei, J. D. Corbett, *Inorg. Chem.* **1996**, 35, 4084.
- [10] M. Ruck, *Z. Anorg. Allg. Chem.* **1998**, 624, 521.
- [11] H. Schäfer, H. G. Schnering, *Angew. Chem.* **1964**, 76, 833.
- [12] J. Köhler, G. Svensson, A. Simon: *Angew. Chem.* **1992**, 104, 1463; *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 1437.
- [13] G. Svensson, J. Köhler, A. Simon in *Metal Clusters in Chemistry*, Vol. 3 (Eds: P. Braunstein, L. A. Oro, P. R. Raithby), Wiley-VCH, **1999**, p. 1509.
- [14] R. E. McCarley, K.-H. Lii, P. A. Edwards, L. F. Brough, *J. Solid State Chem.* **1985**, 57, 17.
- [15] H. Schäfer, H. G. v. Schnering, K. J. Niehues, H. G. Nieder-Vahrenholz, *J. Less-Common Met.* **1965**, 9, 95.
- [16] S. Cordier, A. Simon, *Solid State Sci.* **1999**, 1, 209.
- [17] S. Schneider, R. Hoppe, *Z. Anorg. Allg. Chem.* **1970**, 376, 268.
- [18] I. D. Brown, D. Altermatt, *Acta Crystallogr. Sect. B* **1985**, 41, 244.
- [19] R. Hoffmann, *J. Chem. Phys.* **1963**, 39, 1397; J. H. Ammeter, H. B. Bürgi, J. C. Thibault, R. Hoffmann, *J. Am. Chem. Soc.* **1978**, 100, 3686; R. H. Summerville, R. Hoffmann, *J. Am. Chem. Soc.* **1976**, 98, 7240; M. H. Whangbo, R. Hoffmann, *J. Am. Chem. Soc.* **1978**, 100, 6093; R. Ramirez, M. C. Böhm, *Int. J. Quantum Chem.* **1986**, 30, 391.
- [20] J. Köhler, P. Wunsch, PC version of the program EHMACC (Extended-Hückel program, written by M. H. Whangbo, R. Hoffmann, modified by M. Evain, J. Mitchel) Stuttgart, **1991**. Parameters

used in the EH calculations: Orbital energies H_{ij} [eV] (coefficient ζ_1): F: 2s -40.185 (2.425), 2p: -18.5 (2.425); In: 5s -12.6 (1.903), 5p -6.19 (1.677); Pt: 6s -9.08 (2.554), 6p -5.47 (2.554), 5d -12.59 (6.013). Double ζ functions have been used for Pt: 0.6334, 2.696, 0.5513.

- [21] <http://webelements.com>
- [22] C. Feldmann, M. Jansen, *Angew. Chem.* **1993**, 105, 1107; *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 1049; C. Feldmann, M. Jansen, *Z. Anorg. Allg. Chem.* **1995**, 621, 201.
- [23] This stimulates for the search of compounds in which one can clearly assign the oxidation state -2 to Pt, for example, in hypothetical " Cs_3PtF " or " CsBa_2PtN ".
- [24] R.-D. Hoffmann, R. Pöttgen, G. A. Landrum, R. Dronskowski, B. Künnen, G. Kotzyba, *Z. Anorg. Allg. Chem.* **1999**, 625, 789.
- [25] O. Buresch, H. G. von Schnering, *Fresenius Z. Anal. Chem.* **1984**, 319, 418.
- [26] P. W. Selwood, *Magnetochemistry*, 2nd ed., Interscience, New York, **1956**.

A Novel Bovine β -1,4-Galactosyltransferase Reaction To Yield β -D-Galactopyranosyl-(1-3)-Linked Disaccharides from L-Sugars**

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Among mammalian glycosyltransferases, bovine β -1,4-galactosyltransferase (β -1,4-GalTase) has been the most extensively studied regarding substrate specificity.^[1] In nature this enzyme catalyzes galactosyl transfer from UDP-galactose to the 4-OH position of *N*-acetyl D-glucosamine (D-GlcNAc) and also of D-glucose in the presence of α -lactalbumin (α -LA). The enzyme is known to catalyze other reactions, for example, the transfer of *N*-acetyl-D-galactosamine to D-GlcNAc at high concentrations of α -LA.^[2] Although the β -1,4-GalTase reaction had long been thought to be regiospecific at the 4-OH position of acceptor sugars, we previously found a new type of reactions that catalyze galactosyl transfer to the β -anomeric position of *N*-acetyl kannosamine (3-acetamido-3-deoxy-D-glucose),^[3] *N*-acetyl gentosamines (3-acetamido-3-deoxy-D-xylose),^[4] and D-xylose.^[5] In this paper, we describe the first β -1,4-GalTase reaction that utilizes L-series sugars as the acceptor substrates. Moreover, the novel

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reaction catalyzes unusual galactosyl transfer to the 3-OH position of the L-sugars, that is, a β -1,3-galactosyl transfer.

Our previous β -1,1-transfer reactions for the 3-acetamido-3-deoxy sugars strongly imply that the 3-NAc group may play a key role in an enzyme–substrate binding event to direct the unusual transfer.^[4] Provided this is true, variable glycosyl linkages can be synthesized by this enzyme with acceptor substrates bearing the NAc group at different positions. In this study, we introduced an NAc group to the β -anomeric position of L-glucose, L-xylose, and their D-isomers and examined the effect of this group on the enzyme reactions.

The NAc group was introduced by the treatment of each reducing sugar with ammonium hydrogen carbonate in water followed by acetic anhydride in methanol.^[6] The 1-NAc derivatives were subjected to an enzyme assay^[7] both in the presence and the absence of α -LA. The reactivity was compared with that of the reducing D- and L-sugars in terms of relative initial reaction rates (r) at substrate concentrations of both 1 and 10 mM (Figure 1).

The enzyme assay has proved that the 1-NAc has a significant effect on the enzyme reactions for both the D- and L-sugars. The opposite effect is observed between D- and L-enantiomers as follows: The NAc group changes L-glucose and L-xylose into reactive substrates, while the same group renders their D-isomers less reactive to a considerable extent. The reactivity of L-sugar-1-NAc is comparable to that of D-xylose in the presence of α -LA. D-Glucose-1-NAc is reactive only in the presence of α -LA, while D-xylose-1-NAc is not reactive even in its presence. These results apparently indicate a novel phenomenon, whereby the anomeric NAc group switches the enantiomer discrimination of this enzyme from a mode for D-sugars into one for L-sugars.

For the reactive substrates, such as L-glucose-1-NAc, L-xylose-1-NAc, and D-glucose-1-NAc, the enzyme reaction was performed on a preparative scale (approximately 30 mg) in a way similar to our previous studies.^[3–5] Each of the disaccharide products was isolated in 15–20% yield, and their ^1H NMR spectra enabled us to determine that every transfer reaction was regiospecific to produce a single disaccharide product (Table 1). For a disaccharide from D-glucose-1-NAc, the ^1H NMR signals of the substrate are deshielded due to a galactosyl linkage at O-4 in the order H-4 > H-5 > H-3 >

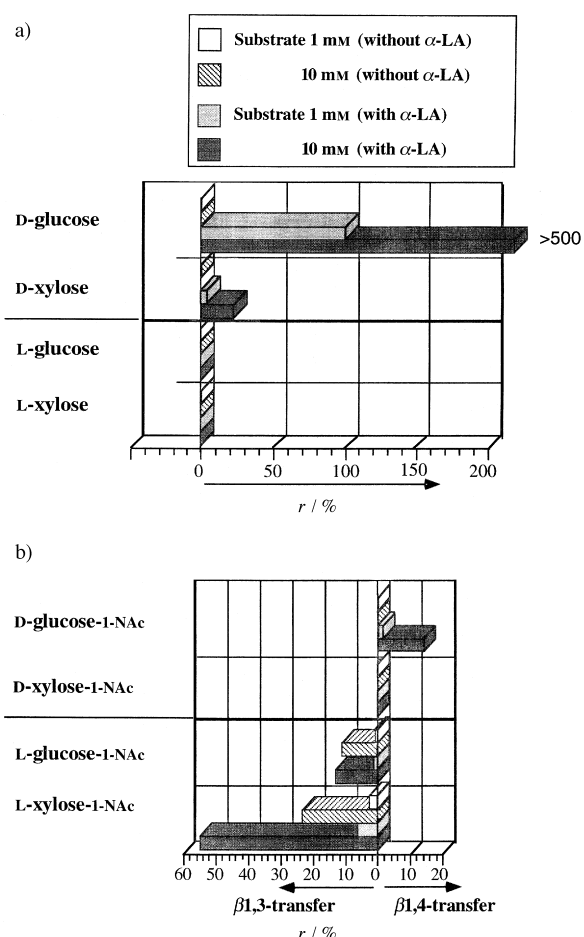


Figure 1. Relative rates (r) for the reaction of bovine β -1,4-galactosyl-transferase with enantiomeric glucoses and xyloses (a) and their 1-NAc derivatives (b). The assays were carried for each acceptor substrate at concentrations of 1 and 10 mM and also in the presence and the absence of bovine α -lactalbumin (α -LA; 0.1 mg mL⁻¹). The type of galactosyl transfer in (b) was determined by ^1H NMR spectroscopy (see text).

H-6 > H-2 > H-1. On the other hand, for disaccharides from L-glucose-1-NAc and L-xylose-1-NAc, the deshielding shift is observed in the order H-3 > H-4 > H-2 > H-1 > H-5 > H-6. This reflects a galactosyl linkage at the position O-3. The per-O-acetylation of a disaccharide from L-xylose-1-NAc caused

Table 1. Chemical shifts (^1H NMR; 500 MHz, D₂O) of the disaccharide products from the β -1,4-galactosyltransferase reactions of *N*-acetyl- β -D- and *N*-acetyl- β -L-glycopyranosyl amines.^[a]

	H-1	H-2	H-3	H-4	H-5 _{ax}	H-5 _{eq}	H-6 _{proR}	H-6 _{proS}	NHAc
β -D-Glc-1-NAc	5.070	3.516	3.678	3.547	3.643		3.846	4.000	2.200
β -L-Glc-1-NAc	5.070	3.516	3.678	3.547	3.643		4.000	3.846	2.200
β -D-Gal-(1-4)- β -D-Glc-1-NAc									
β -D-Glc-1-NAc	5.085	3.552	3.790	3.730	3.790		3.923	4.041	2.200
β -D-Gal	4.572	3.668	3.784	4.041	3.836		3.913	3.869	
β -D-Gal-(1-3)- β -L-Glc-1-NAc									
β -L-Glc-1-NAc	5.117	3.634	3.951	3.772	3.790		4.010	3.865	2.206
β -D-Gal	4.825	3.745	3.819	4.073	3.867		3.932	3.897	
β -L-Xyl-1-NAc	4.989	3.508	3.617	3.746	3.499	4.055			2.190
β -D-Gal-(1-3)- β -L-Xyl-1-NAc									
β -L-Xyl-1-NAc	5.031	3.626	3.891	3.953	3.533	4.070			2.190
β -D-Gal	4.824	3.735	3.815	4.065	3.855		3.928	3.888	

[a] Assignments of all ring protons are based on ^1H - ^1H COSY spectra, while those of C-6 prochiral protons are based on the spectra of the (6S)-(6- $^2\text{H}_1$)-D-glucoside and (6S)-(6- $^2\text{H}_1$)-D-galactoside derivatives reported in ref. [15].

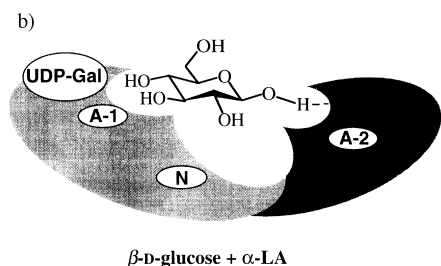
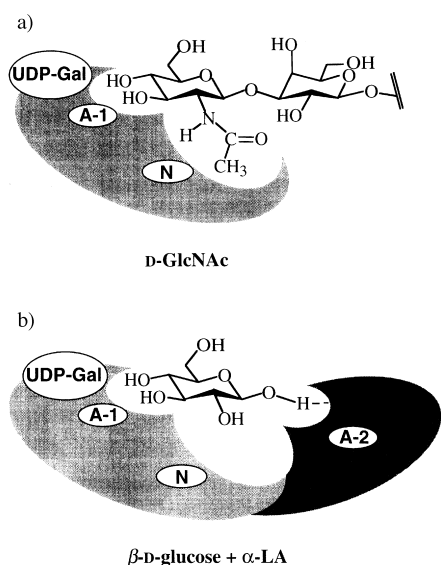


Figure 2. Proposed key enzyme–substrate interactions in reactions of bovine β -1,4-GalTase with natural acceptor substrates. In the absence of α -LA, a catalytic domain (A-1) and an NAc binding locus (N) provide key binding interactions for the terminal D-GlcNAc residue of oligosaccharides (a). α -LA binds to the enzyme adjacent to an acceptor binding site of D-glucose and induces a supplementary interaction at A-2 that is key for the D-glucose reaction to yield lactose (b).

strong deshielding and shifting of the H-2 ($\Delta\delta = 1.14$) and H-4 signals ($\Delta\delta = 0.56$), but not of the H-3 signal ($\Delta\delta = 0.05$) supporting the idea of a galactosyl linkage at O-3.

Thus, a 1-NAc group was proved to change L-glucose and L-xylose into unique β -1,4-GalTase substrates and to permit

galactosyl transfer at the 3-OH position. It is also of significance that both D- and L-enantiomers of glucose-1-NAc can become substrates, to yield β -1,4- and β -1,3-linked disaccharides, respectively. In order to rationalize these findings, we propose three key interactions at the A-1, A-2 and N loci (Figure 2). The A-1 locus provides a key polar interaction^[8] with a reactive hydroxyl group of the acceptor substrate, as suggested by Kajihara et al.^[9] This locus is considered to recognize one of the 1,2-*trans* diols with anticlockwise helicity (such as OH-3/OH-4 of D-GlcNAc and OH-2/OH-3 of L-glucose-1-NAc) in a similar way to the recognition by D-glucose/b-mannose-binding lectins (for example, concanavalin A).^[10] Although the binding at A-1 is essential for the enzymatic reaction, the interaction itself is assumed not to be strong enough for reactions below 10 mM substrate concentration under physiological conditions. The enzymatic reaction, thus, requires a supplementary binding either at the A-2 or the N locus. An NAc group binding site (N locus), as suggested by Berliner et al.,^[11] is considered to provide a key interaction for D-GlcNAc and other substrates carrying an NAc group (Figure 2a). Another important interaction at A-2 is induced by the action of α -LA, which forms a 1:1 enzyme complex adjacent to the substrate binding site.^[11, 12] Judging from the fact that substitution of an anomeric hydroxyl group with NAc and OMe groups^[11] suppresses the reactivity of D-glucose and D-xylose significantly, the A-2 locus is assumed to interact with such a group (Figure 2b).

Although L-glucose and L-xylose do not satisfy dual interactions at A-1 and A-2 per se (Figure 3a), introduction

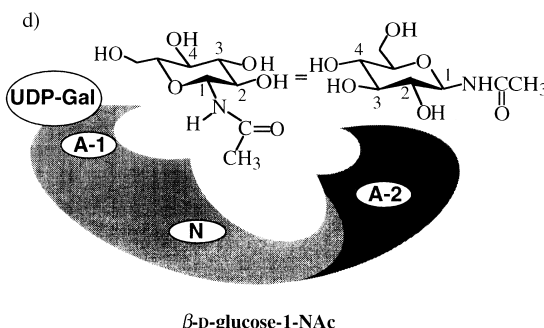
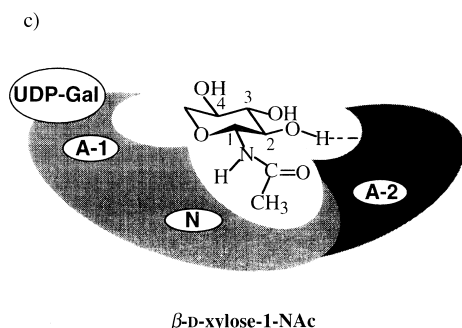
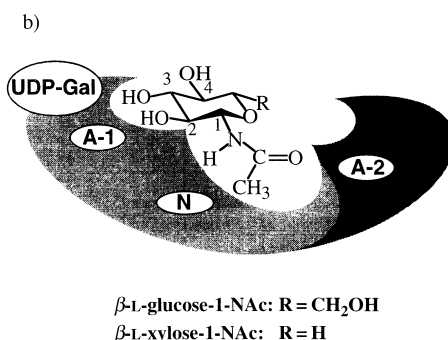
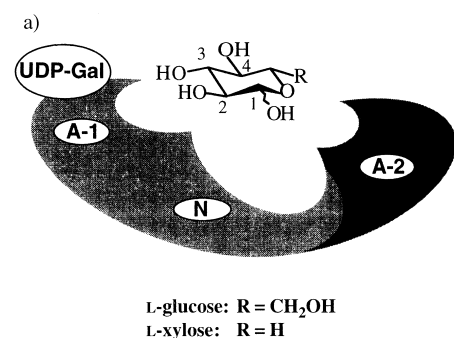


Figure 3. Possible roles for the 1-NAc group of D- and L-sugars in β -1,4-GalTase reactions. L-Glucose and L-xylose are inactive since they cannot locate hydroxyl groups simultaneously at the A-1 and A-2 loci (a). The 1-NAc group allows them to have an interaction at the N locus leading to galactosyl transfer at the 3-OH position (b). D-Glucose-1-NAc and D-xylose-1-NAc cannot adopt the A-1 + N or the A-1 + A-2 interactions. Instead, an alternative binding at the N and A-2 loci may be possible for D-xylose-1-NAc, in which the reaction site A-1 is not occupied (c). The exocyclic 5-CH₂OH group of D-glucose-1-NAc disrupts any binding at N and A-2 and instead, it provides a peripheral binding interaction that allows the natural β -1,4-transfer reaction (d).

of the 1-Nac group allows them to occupy the **A-1** and **N** loci (Figure 3b). In this case, the 3-OH group at **A-1** is galactosylated, as is actually observed. On the contrary, 1-Nac derivatives of D-sugars can not adopt the supplementary interaction at the **N** or **A-2** loci. Instead, D-xylose-1-Nac may adopt an alternative bonding at the **A-2** and **N** loci where no reactive OH is placed at **A-1** (Figure 3c). For D-glucose-1-Nac, which is reactive in the presence of α -LA, the exocyclic C-5 group may disturb this bonding (Figure 3d). Moreover, judging from the higher reactivity of D-glucose than D-xylose (Figure 1 a), a peripheral binding interaction may exist around the C-5 group of D-glucose and contribute to the usual β -1,4-transfer reaction.

In conclusion, we have proved that an anomeric NAc group changes unnatural L-glucose and L-xylose into acceptor substrates for bovine β -1,4-GalTase. The transfer reaction is regiospecific at the 3-OH position and so this is the first β -1,3-transfer reaction for this enzyme. This finding, together with our previous ones, have shown that variable unnatural glycosyl linkages can be produced enzymatically by a single enzyme. This fact may exceed the general concepts of "one glycosyl enzyme for one glycosyl linkage"^[13] and "biological homochirality"^[14] that express the high substrate and enantiospecificity of enzyme reactions in biosynthetic pathways.

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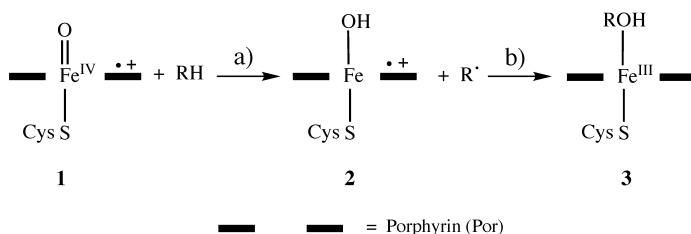
Revised: January 31, 2000

- [1] a) C.-H. Wong, R. L. Halcomb, Y. Ichikawa, T. Kajimoto, *Angew. Chem.* **1995**, 107, 559; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 521–546; b) M. M. Palcic, O. Hindsgaul, *Trends Glycosci. Glycotechnol.* **1996**, 8, 37–49.
- [2] K.-Y. Do, S.-I. Do, R. D. Cummings, *J. Biol. Chem.* **1995**, 270, 18447–18451.
- [3] Y. Nishida, T. Wiemann, V. Sinnwell, J. Thiem, *J. Am. Chem. Soc.* **1993**, 115, 2536–2537.
- [4] a) Y. Nishida, T. Wiemann, J. Thiem, *Tetrahedron Lett.* **1992**, 33, 8043–8046; b) Y. Nishida, T. Wiemann, J. Thiem, *Tetrahedron Lett.* **1993**, 34, 2905–2906.
- [5] T. Wiemann, Y. Nishida, V. Sinnwell, J. Thiem, *J. Org. Chem.* **1994**, 59, 6744–6747.
- [6] L. M. Likhoshershtov, O. S. Novikova, V. A. Derevitskaja, N. K. Kochetkov, *Carbohydr. Res.* **1986**, 146, C1–C5.
- [7] D. K. Fitzgerald, B. Colvin, R. Marval, K. E. Ebner, *Anal. Biochem.* **1970**, 36, 43–61.
- [8] a) R. U. Lemieux, *Chem. Soc. Rev.* **1989**, 18, 347–374; b) M.-H. Du, U. Spohr, R. U. Lemieux, *Glycoconjugate J.* **1994**, 11, 443–461.
- [9] Y. Kajihara, H. Kodama, T. Endo, H. Hashimoto, *Carbohydr. Res.* **1998**, 306, 361–378.
- [10] a) K. Drickamer, *Nature* **1992**, 360, 183–186; b) K. Drickamer, *J. Biol. Chem.* **1988**, 263, 9557–9560.
- [11] L. J. Berliner, M. E. Davies, K. E. Ebner, T. A. Bayer, J. E. Bell, *Mol. Cell. Biochem.* **1984**, 62, 37–42.
- [12] J. A. Grobler, M. Wang, A. C. W. Pike, K. Brew, *J. Biol. Chem.* **1994**, 269, 5106–5114.
- [13] S. Natsuka, J. B. Lowe, *Curr. Opin. Struct. Biol.* **1994**, 4, 683–691.
- [14] a) V. V. Avetisov, V. I. Goldanskii, *BioSystems* **1991**, 25, 141–150; b) J. M. Bailey, *FASEB J.* **1998**, 12, 503–507.
- [15] H. Ohru, Y. Nishida, H. Itoh, H. Hori, H. Meguro, *J. Org. Chem.* **1991**, 56, 1726–1731.

Two-State Reactivity in the Rebound Step of Alkane Hydroxylation by Cytochrome P-450: Origins of Free Radicals with Finite Lifetimes**

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Alkane hydroxylation^[1] by cytochrome P-450 poses tantalizing mechanistic questions. The consensus rebound mechanism, Scheme 1,^[2] involves hydrogen abstraction from the



Scheme 1. Schematic representation of the rebound mechanism.

alkane (RH) in step (a), followed by hydroxyl rebound onto the radical to generate the ferric–alcohol complex, in step (b). The rebound mechanism has gained support from findings of rearranged alcohol products, which indicate the presence of a free radical with a finite lifetime.^[3] Further support has recently been provided by the kinetic isotope effect (KIE) measurements of Dinnocenzo, Jones et al.^[4a] They found that for a few substituted alkanes, the KIE's of cytochrome P-450 hydroxylation are virtually identical to those of a corresponding hydrogen abstraction reaction by *tert*-butoxyl radical, thus implying isostructural transition states for the two processes.^[4] In an apparent contrast, mechanistic studies by Newcomb and co-workers,^[5] designed to probe the radical using alkane substrates that would yield ultrafast radical clocks, do not concur with a free-radical intermediacy; apparent lifetimes are too fast to correspond to a real free-radical intermediate (for example, $\tau \leq 70$ fs),^[5b] and have no correlation with independently clocked rearrangement lifetimes of the free radicals.^[5a] Thus, while the evidence for incursion of radicals is strong, their presence is nevertheless controversial and merits elucidation.

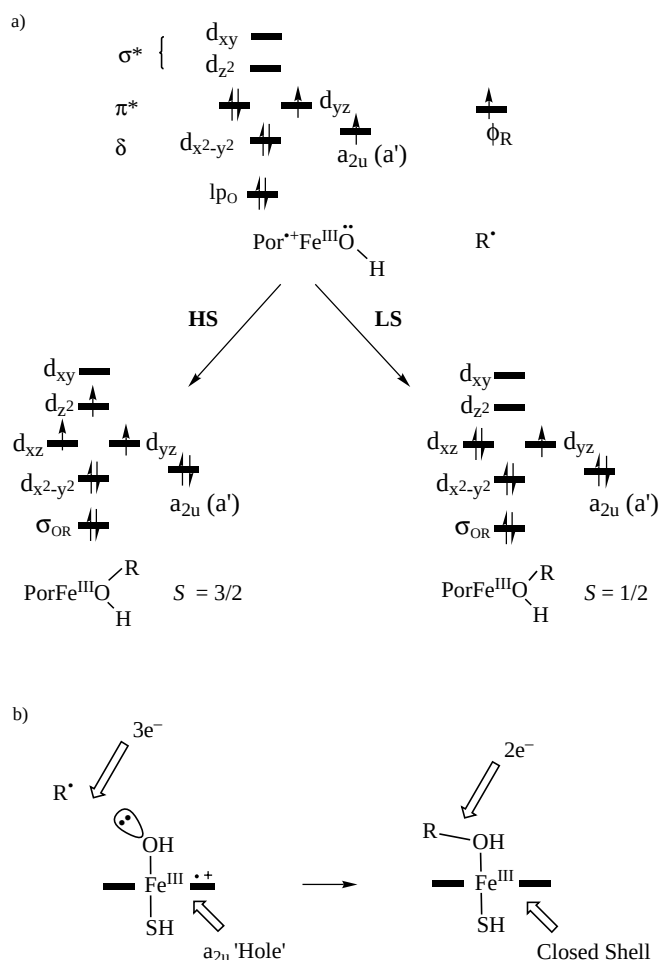
The origin of the radicals in the rebound process is the key issue addressed in this communication. Our approach to tackle this question, is to use density functional calculations to ascertain whether the hypothetical rebound process in step (b) of Scheme 1, involves a barrier which would endow the

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radical with a finite lifetime, and if so what is the precise nature of the iron–porphyrin state that originates the barrier? Thus, our study focuses on modeling of the rebound process itself, and is not meant to be a study of the full hydroxylation mechanism of a particular alkane.

In a previous paper,^[6] we showed by means of density functional calculations, that in a dielectric environment (such as in a solvent or in a protein cavity) the iron–hydroxo intermediate **2** in Scheme 1 involves an Fe^{III} (d⁵ configuration) ion and a cation radical situated in the porphyrin. The ground state of the iron–hydroxo complex was found to be the triplet state ($S=1$) with single electrons occupying the $d_{yz}(\pi^*)$ orbital of the iron and the a_{2u} type orbital of the porphyrin, which is delocalized toward the sulfur.^[6b] As shown in part (a) of Scheme 2, this iron–hydroxo complex with two unpaired



Scheme 2. a) Orbital diagrams showing the low-spin and high-spin coupling modes of the triplet iron–hydroxo and alkyl radical species during the rebound step. b) An electron-counting diagram illustrating the filling of the porphyrin “hole”.

electrons in a triplet situation ($S=1$) can couple with the odd electron of the radical ($S=1/2$) in low-spin (LS) and high-spin (HS) manners, resulting in doublet ($S=1/2$) and quartet ($S=3/2$) states of the ferric–alcohol products, respectively. These two processes are studied here and, as will be shown, the radical has a finite lifetime due to a barrier on the high-spin

surface, while the low-spin coupling proceeds to the ferric–alcohol complex with no significant barrier. This and the spin-state branching ratio^[7] may well be the roots of the apparent controversy in the experimental data.

Hybrid (Hartree–Fock/DFT) density functional B3LYP calculations^[8] are performed with the JAGUAR 3.5^[9] and GAUSSIAN98^[10] packages, and used for geometry optimization of the species in Scheme 1 with $\text{CH}_3^\bullet$ as a model radical and HS^- as an axial ligand. Following the previous study^[6], we use the Los Alamos effective core potential coupled with the double zeta LACVP basis set^[11] for iron, and an all-electron 6-31G basis set for the rest of the atoms. Molecular geometries of all species are fully optimized without any symmetry constraints.

For the ferric–methanol complex **3** ($\text{R}=\text{Me}$ in Scheme 1), the ground state is found to be the doublet state ($S=1/2$) with the quartet state ($S=3/2$) lying $7.5 \text{ kcal mol}^{-1}$ higher.^[12] While transient alcohol complexes have not as yet been detected in a mechanistic study of the hydroxylation process, ferric–alcohol complexes of cytochrome P-450 are nevertheless known. Our computational result is in accord with the experimental assignment of the 5-hydroxycamphor complex (a ferric–alcohol complex) of cytochrome P-450_{cam} as a doublet-spin complex ($S=1/2$).^[13] The strong binding, indicated by the short Fe–O bond in the computed ferric–methanol complex (see Figure 1), suggests that an alcohol transient is reasonable,

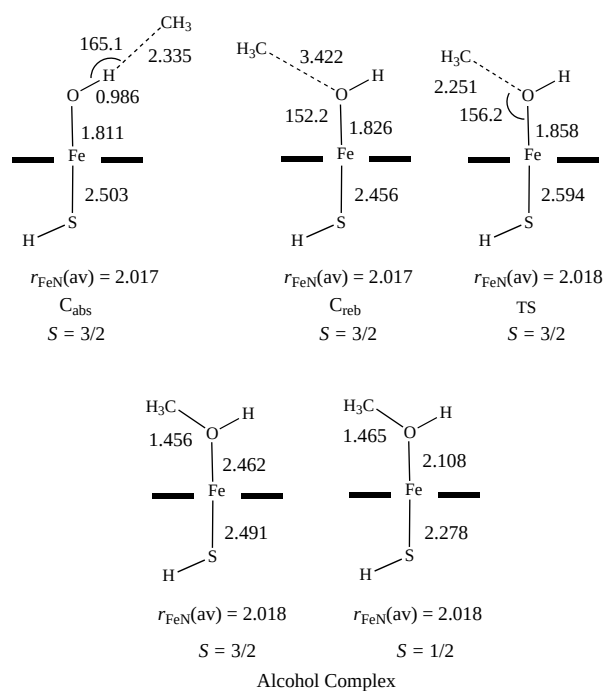


Figure 1. Stationary species for the rebound process.

while the alternative direct OH transfer to the alkyl radical to form a free alcohol may be energetically more demanding.^[14]

From this point on, the term low spin (LS) will denote the doublet state while the term high spin (HS) will denote the quartet state.^[12a] The HS rebound step involves a cluster that precedes a transition state (TS) en route to the HS ferric–methanol complex. These stationary species are depicted

schematically in Figure 1 which shows only key geometric parameters. The cluster C_{abs} is seen to correspond to the product of the hydrogen-abstraction step. Rotation of the methyl group to the other side of the oxygen occurs at the expense of a small energy increase ($2.1 \text{ kcal mol}^{-1}$) and generates a second cluster, C_{reb} , which is not a true minimum but is a species en route to the rebound. In contrast, the only stationary point found in the LS rebound is the LS ferric-methanol product.

Location of the transition states on the HS and LS surfaces was achieved in two steps. First, the minimum energy HS and LS paths were scanned along the $r_{C\cdots O}$ coordinate (approach of the CH_3 radical to the oxygen of the iron-hydroxo complex) with C_s symmetry constraints. Figure 2 shows the energy

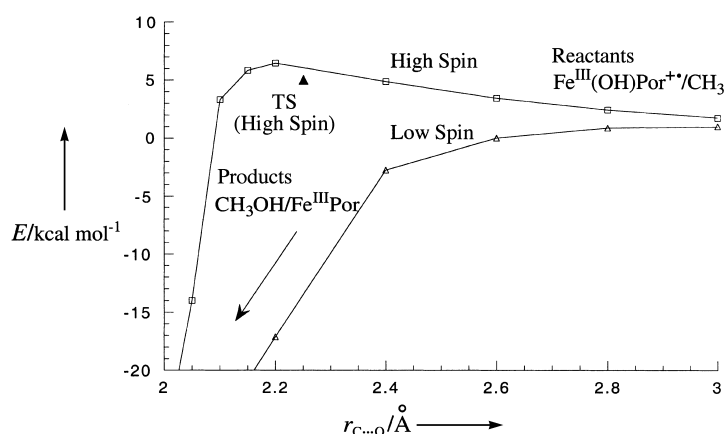


Figure 2. Potential energy curves of the high-spin (HS) and low-spin (LS) rebound processes along the $CH_3 \cdots O$ coordinate ($r_{C\cdots O}$).

profiles obtained and it is apparent that, while the LS profile descends smoothly^[15] toward the LS ferric-methanol complex, the HS surface exhibits a barrier near $r_{C\cdots O} = 2.2 \text{ Å}$. The true transition state (TS) on the HS surface (shown with a filled triangle in Figure 2) was then located by saddle-point optimization of all geometric variables with no symmetry constraints. This was followed by a complete vibrational analysis which revealed that there is only one mode with an imaginary frequency of $i447.6 \text{ cm}^{-1}$ which corresponds to the expected mode along the $r_{C\cdots O}$ reaction coordinate that connects reactants to products.

Table 1 displays key features of the stationary structures along the path. The barrier relative to the separated fragments is $5.0 \text{ kcal mol}^{-1}$, and relative to the reactant cluster it is $7.1 \text{ kcal mol}^{-1}$. Considering the known tendency of density functionals to underestimate barriers,^[16] the HS barrier feature found in this way is not likely to be an artifact. The energy stabilization of the LS surface in the region of the HS transition state (approximately 17 kcal mol^{-1} relative to the onset at 3 Å) is significantly larger than the expected B3LYP error.^[16d] Thus, even if B3LYP

overestimates the LS state somewhat, a significant barrier is still unlikely on the LS surface. We cannot however, rule out a tiny barrier at a much "earlier" position along the $r_{C\cdots O}$ coordinate.

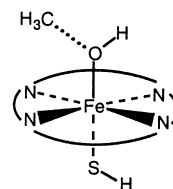
Further inspection of Table 1 reveals that the HS TS possesses geometric parameters between those of the ferryl-hydroxo reactant complex, $[Por^{+}Fe^{III}OH]$, and those of the HS ferric-methanol complex, $[PorFe^{III}(CH_3OH)]$. The spin density data in Table 1 further show that the vicinity of the TS is characterized by an extensive electronic reorganization. We shall now analyze this electronic reorganization, and thereby show that there is a simple reason for the HS barrier and the lack of an LS barrier.

Table 1 shows that the attacking $CH_3\cdot$ radical and the porphyrin ring lose their initial spins in both the HS and LS rebounds. What makes the difference is the spin density of the iron, which rises steeply on the HS path (0.85 – 2.42), but remains essentially constant (0.84 – 0.86) along the LS path. The orbital diagrams, part (a) of Scheme 2, show the origins of this reorganization. Thus, initially in the ferryl-hydroxo reactant complex the $a_{2u}(a')$ porphyrin orbital^[6b] is singly occupied, and so is the orbital of the alkyl group ($1p_O$ is an oxygen lone-pair orbital). During the rebound, the $a_{2u}(a')$ orbital is filled by an electron formally provided by the alkyl radical $R\cdot$ which now participates in the newly formed σ_{OR} orbital. However, while in the LS process this is the primary change, during the HS process the filling of the porphyrin shell must be attended by excitation of an electron from the d_{xz} orbital to the higher energy (σ^* type) d_{z^2} orbital. Thus, whereas in the iron-hydroxo complex/ $CH_3\cdot$ cluster all the unpaired electrons reside in relatively low-lying orbitals, in

Table 1. Selected geometric parameters r [Å], spin densities ρ , and relative energies E_{rel} [kcal mol^{-1}] of the critical species along the rebound pathway.

parameter	[Por ⁺ Fe ^{III} OH]/CH ₃ [·]		TS HS	[PorFe ^{III} (CH ₃ OH)]	
	reactants	HS cluster ^[a]		HS	LS
$r_{C\cdots O}$	∞	3.298	2.251	1.456	1.465 ^[b]
$r_{Fe\cdots O}$	1.824 (1.829) ^[d]	1.811	1.858	2.462	2.108 ^[c]
$r_{Fe\cdots S}$	2.490 (2.458) ^[d]	2.503	2.594	2.491	2.278
$r_{Fe-N(av)}$ ^[e]	2.017 (2.017) ^[d]	2.017	2.018	2.018	2.018
$r_{Fe-ring}$	+0.09	+0.09	+0.08	−0.14	−0.09
E_{rel}	0	−2.1	+5.0 ^[f] −17.1 ^[g]	−61.2	−68.7
ρ_{CH_3}	0.99	0.96	0.66–0.33 ^[h]	0.003	0.000
ρ_{ring}	0.67	0.52	0.66–0.13 ^[h]	0.18	0.032
ρ_{Fe}	0.85	0.84	1.05–2.26 ^[h]	2.42	0.86

[a] The C_{abs} cluster shown in Figure 1. [b] C–O distance. [c] Fe $\cdots$ O distance. [d] Data in parentheses are for the C_s structure^[6] which is $0.88 \text{ kcal mol}^{-1}$ higher than the C_1 structure (data not in parentheses). [e] The average measurements of four Fe–N distances. [f] The barrier relative to the optimized reactant at C_1 symmetry. [g] Energy of the corresponding LS structure at 2.2 Å . [h] Range of spin density at $r_{C\cdots O} = 2.2$ – 2.05 Å .



the HS TS and in the corresponding HS ferric–alcohol complex, one electron is excited to a higher level $\sigma^*(d_z)$ orbital in order to conserve the total quartet spin. This additional excitation is accompanied by the aforementioned increase of the spin density on the iron, as well as by a considerable elongation of the Fe–O and Fe–S bond lengths in the TS relative to the iron–hydroxo complex, and the loss of bonding is manifested as a barrier for the HS rebound process. Thus, the barrier on the HS path has a straightforward electronic origin.

Since the LS path is barrier-free, or very flat, it cannot endow a radical intermediate with a significant lifetime, if at all. It follows that LS hydroxylation will proceed in an effectively concerted manner,^[5b, 7, 17] even if the trajectory of approach of the ferryl–oxene species to the R–H alkane will resemble a hydrogen abstraction process. Along the same trajectory, an alkyl radical will be produced on the HS path and will face a rebound barrier. Thus, assuming that the rate of the rebound step obeys the Arrhenius equation with the same preexponential factor for the HS and LS pathways, the potential barrier of 5 to 7 kcal mol^{−1} on the HS profile will slow down the HS rebound by four to six orders of magnitude, and will thereby endow the radical with a significant lifetime, during which radical rearrangement can occur. In this dual pathway scenario, where rearranged alcohol is produced through only one state (HS), while unrearranged alcohol is produced from two states (LS and HS), apparent lifetimes—calculated from percentages of rearrangement—may occasionally appear unrealistically short and will bear no correlation to clocked lifetimes of the free radicals, as observed by Newcomb et al.^[5] At the same time, the measured KIE will reflect a “hydrogen abstraction trajectory” (common to the two states), as deduced in the study by Dinnocenzo, Jones et al.^[4] Thus, the proposed two-state scenario provides a reasonable reconciliation of the apparent contradiction in the two sets of data.

The percentage of rearrangement will depend on the initial concentration of the radical, as determined by the branching ratio of the reaction complexes between the HS and LS processes,^[7] as well as on the HS rebound rate, which is sized by the HS barrier. Two factors will influence the height of the barrier in the HS rebound, and can be deduced from Scheme 2. The first factor is associated with the electron excitation from the lower level d_{xz} orbital to the higher energy $d_z(\sigma^*)$ orbital which is antibonding across the axial O–Fe–S axis. The stronger the S-binding ability of the thiolate ligand, the higher the $d_z(\sigma^*)$ orbital is, and a higher HS rebound barrier is the result. Since in the natural enzyme the thiolate ligand is a side group of the encapsulating protein, the protein is expected to affect the binding ability of the thiolate through variation in the polarity and acidity of its pocket as well as through steric constraints for Fe–S bonding.

The second factor of the rebound barrier is associated with the properties of the attacking radical and can be deduced from part (b) of Scheme 2. A simple electron count reveals that, initially, the R–O linkage involves three electrons and the porphyrin has a “hole”,^[6b] and later the R–O linkage becomes a two-electron R–O bond and the porphyrin attains a closed shell. Thus, the electron that fills the porphyrin “hole” originates in the extra electron of the R–O linkage and

is formally transferred from the electropositive alkyl radical. Consequently, the HS rebound process involves concomitantly an R–O bond formation and an electron shift (from R[•] to Por⁺). It follows therefore that the percentage of radical rearrangement will depend on the oxidation potential of the radical. All other factors being equal, the lower the oxidation potential, the shorter the true lifetime of the radical on the HS surface and the less rearranged alcohol can be expected. This trend is in agreement with recent observations by Newcomb et al., who found that as the alkyl radical moiety of the substrates (2-aryl-cyclopropyl alkanes) becomes a better electron donor, the amount of rearranged alcohol decreases from 26 % (for a *p*-CF₃ aryl substituent) down to less than 1 % for the best donor situation (for 2-phenyl-cyclopropyl isopropane).^[5c] Nevertheless, the experimental results are more complicated due to rearrangement of cationic intermediates and the presence of more than one oxidizing species.^[5]

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- [1] M. Sono, M. P. Roach, E. D. Coulter, J. H. Dawson, *Chem. Rev.* **1996**, 96, 2841.
- [2] a) J. T. Groves, *J. Chem. Educ.* **1985**, 62, 928; b) J. T. Groves, G. A. McClusky, *J. Am. Chem. Soc.* **1976**, 98, 859.
- [3] a) J. T. Groves, Y.-Z. Hang in *Cytochrome P450: Structure, Mechanisms and Biochemistry*, 2nd ed. (Ed.: P. R. Ortiz de Montellano), Plenum, New York, **1995**, chap. 1; b) B. Meunier, *Chem. Rev.* **1992**, 92, 1411; c) W. D. Woggon, *Top. Curr. Chem.* **1996**, 184, 40.
- [4] a) J. I. Manchester, J. P. Dinnocenzo, L. A. Higgins, J. P. Jones, *J. Am. Chem. Soc.* **1997**, 119, 5069; b) The dimethyl aniline substrates in ref. [4a] were suggested later to undergo an initial electron transfer by the ferryl–oxene species of horseradish peroxidase and by a synthetic ferryl–oxene model (Y. Goto, Y. Watanabe, S. Fukuzumi, J. P. Jones, J. P. Dinnocenzo, *J. Am. Chem. Soc.* **1998**, 120, 10762). This conclusion does not, however, pertain to the toluene, *p*-xylene, and benzyl alcohol species in ref. [4a]. Furthermore, all the model ferryl–oxene systems, used in the study, are better electron acceptors than the cytochrome P-450 ferryl–oxene complex so that the results may not pertain at all to oxidation by cytochrome P-450.
- [5] a) M. Newcomb, M.-H. Le Tadic, D. A. Putt, P. F. Hollenberg, *J. Am. Chem. Soc.* **1995**, 117, 3312; b) M. Newcomb, M.-H. Le Tadic-Beadatti, D. L. Chestney, E. S. Roberts, P. F. Hollenberg, *J. Am. Chem. Soc.* **1995**, 117, 12085; c) P. H. Toy, M. Newcomb, P. F. Hollenberg, *J. Am. Chem. Soc.* **1998**, 120, 7719.
- [6] a) M. Filatov, N. Harris, S. Shaik, *Angew. Chem.* **1999**, 111, 3730; *Angew. Chem. Int. Ed.* **1999**, 38, 3512. The paper shows that in the gas phase the electromeric forms, [PorFe^{IV}OH] (*S* = 1) and [Por⁺Fe^{III}OH] (*S* = 1) are almost degenerate, but a dielectric environment makes [Por⁺Fe^{III}OH] (*S* = 1) the ground state. This is in accord with electrochemical results in: J. T. Groves, Z. Gross, M. K. Stern, *Inorg. Chem.* **1994**, 33, 5065. b) The spin density is 67 % in the porphyrin and 30 % on the thiolate ligand for the model thiolate used here. This model is closer to the full cysteinato ligand than, for example, the CH₃S[−] model.
- [7] S. Shaik, M. Filatov, D. Schroder, H. Schwarz, *Chem. Eur. J.* **1998**, 4, 193.
- [8] P. J. Stevens, F. J. Devlin, C. F. Chabrowski, M. J. Frisch, *J. Phys. Chem.* **1994**, 98, 11623.
- [9] JAGUAR 3.5, Schrödinger, Inc., Portland, OR, **1998**. The program employs the restricted open-shell B3LYP method.
- [10] GAUSSIAN98, Gaussian, Inc., Pittsburgh, PA, **1998**.
- [11] J. P. Hay, W. R. Wadt, *J. Chem. Phys.* **1985**, 82, 299.
- [12] a) The state with maximum spin (*S* = 3/2) did not converge in our calculations. However, it must be kept in mind that whether the ground state of the product ferric–alcohol complex is *S* = 3/2 or *S* = 1/2 does not have bearing on the conclusions of this study, as summarized

- in Figure 2. What matters is the behavior of states obtained by the two spin coupling modes in Scheme 2. b) Density functional calculations of corresponding ferric–water complexes give $S = \frac{1}{2}$ ground state and a higher energy $S = \frac{3}{2}$ state. See: M. Filatov, N. Harris, S. Shaik, *J. Chem. Soc. Perkin Trans. 2* **1999**, 399; M. T. Green, *J. Am. Chem. Soc.* **1998**, 120, 10772. This is supported by experimental assignment. See: H. Thomann, M. Bernardo, D. Goldfrab, P. M. H. Kroneck, V. Ulrich, *J. Am. Chem. Soc.* **1995**, 117, 8243. However, a recent experimental investigation of a model compound with a spatially fixed thiophenoxy ligand shows an $S = \frac{3}{2}$ ground state and indicates the role of the protein in stabilizing the $S = \frac{1}{2}$ ground state, as predicted initially by Harris and Loew. See: H. Aissaoui, R. Bachmann, A. Schweiger, W.-D. Woggon, *Angew. Chem.* **1998**, 110, 3191; *Angew. Chem. Int. Ed.* **1998**, 37, 2998; D. Harris, G. H. Loew, *J. Am. Chem. Soc.* **1993**, 115, 8775.
- [13] H. Li, S. Narashmhulu, L. M. Havran, J. D. Winkler, T. L. Poulos, *J. Am. Chem. Soc.* **1995**, 117, 6297.
- [14] A direct OH transfer mechanism would entail spin-state crossing to the $S = \frac{3}{2}$ state of the five-coordinate ferric complex (for example, see: ref. [1]).
- [15] All HS data are obtained with B3LYP using JAGUAR 3.5 (ref. [9]). The LS profile is calculated with the unrestricted B3LYP method in GAUSSIAN 98 (ref. [10]). For convenience, the zero of the energy scale in Figure 2 is common to the LS and HS curves.
- [16] a) M. Filatov, W. Thiel, *Chem. Phys. Lett.* **1998**, 295, 467; b) H. Basch, S. Hoz, *J. Phys. Chem. A* **1997**, 101, 4416; c) S. Skokov, R. A. Wheeler, *Chem. Phys. Lett.* **1997**, 271, 251; d) The average underestimation error of B3LYP (in ref. [16a–c]) for related reactions involving radicals is $\leq 6 \text{ kcal mol}^{-1}$, while for related reactions involving OH and CH_3 groups, the error is $0.5 \text{ kcal mol}^{-1}$.^[16c] For reactions involving Fe, O, and H atoms, the underestimation error of the B3LYP barrier is approximately 1 kcal mol^{-1} relative to experimental data and $5.4 \text{ kcal mol}^{-1}$ relative to the CASPT2 result which seems to overestimate the barrier.^[17]
- [17] M. Filatov, S. Shaik, *J. Phys. Chem. A* **1998**, 102, 3835.

Redirecting Secondary Bonds To Control Molecular and Crystal Properties of an Iodosyl- and an Iodylbenzene**

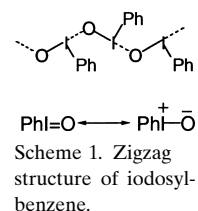
Dainius Macikenas, Ewa Skrzypczak-Jankun, and John D. Protasiewicz*

Noncovalent, attractive interactions are extremely important forces that can influence molecular, solution, and solid-state properties. For example, hydrogen bonding is unarguably the most important directing intermolecular force and

structure generating motif in many chemical and biological systems. Much less appreciated (and utilized) are the related forces that exist between heavier atoms. A class of such attractive interactions have often been termed secondary bonds.^[1] Like hydrogen bonds, these bonds have strong electrostatic components and show directional preferences.^[2] From the similarity to hydrogen bonds, it has also been suggested that secondary bonds might have potential for the rational design of supramolecular structures.^[3]

Secondary bonds, particularly $\text{I} \cdots \text{O}$ bonds, are a pervasive feature of structural organoiodine(III) chemistry.^[4] Recently, such secondary bond interactions have been exploited to introduce chiral environments about iodine(III) centers for enantioselective oxidation reactions.^[5] In other cases, secondary bonds can be counterproductive. In iodosylbenzene (PhIO)_n, $\text{I} \cdots \text{O}$ bonds dominate the structure to such an extent that this extremely potent and important reagent is essentially insoluble in all nonreactive media.^[6] Nonetheless, this veritable “oxygen atom warehouse” has found widespread use in catalytic oxygenation reactions after the discoveries of its efficacy as a source of oxygen atoms for oxidations catalyzed by cytochrome P-450^[7] and by discrete transition metal complexes.^[8]

Though known for over 100 years,^[9] structural details for iodosylbenzene—or any members of this class of materials—are still limited. Various spectroscopic studies have suggested that iodosylbenzene adopts the form of a zigzag polymer (Scheme 1),^[10] whereby monomeric units of PhIO are linked



by intermolecular $\text{I} \cdots \text{O}$ secondary bonds. Within PhIO monomers, $\text{I}-\text{O}$ single bonds ($2.04 \text{ \AA}$) and a $\text{C}-\text{I}-\text{O}$ bond angle near 90° have been deduced from extended X-ray absorption fine-structure (EXAFS) analysis.^[10g] The tight, solid-state aggregation of the PhIO monomers is undoubtedly caused by the strong electrostatic attractions between the oppositely charged iodine and oxygen atoms (right resonance structure, Scheme 1). Polymeric iodosylbenzene is also likely to be terminated by addition of water and thus iodosylbenzene can be further formulated as $\text{HO}(\text{PhIO})_n\text{H}$.^[11] We have recently discovered that soluble derivatives of (tosyliminoiodo)benzene and iodosylbenzene are realized if strong internal dipoles capable of introducing intramolecular $\text{I} \cdots \text{O}$ secondary bonds, for the purpose of replacing intermolecular $\text{I} \cdots \text{N}$ and $\text{I} \cdots \text{O}$ secondary bonds, are incorporated into these materials.^[12] Indeed, a single-crystal X-ray structure analysis of the (tosyliminoiodo)benzene derivative showed the presence of significant intramolecular $\text{I} \cdots \text{O}$ secondary bonding and thus confirmed our expectations. We now have succeeded in obtaining single crystals suitable for X-ray diffraction studies of the corresponding soluble iodosylbenzene and herein report the first crystal structure of an iodosylbenzene derivative ArIO (**1**; $\text{Ar} = 2\text{-}t\text{BuSO}_2\text{C}_6\text{H}_4$). Furthermore, the crystal structure of the corresponding iodylbenzene ArIO_2 (**2**) has also been obtained. Comparisons between these two structures reveal interesting aspects of the nature of iodosyl and iodoxy bonds.

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Single crystals of **1**·CDCl₃ for X-ray structural analysis were grown from a [D₁]chloroform/toluene mixture; Figure 1 presents the local crystalline environment of **1**.^[13] Immediately apparent is a close contact (2.707(5) Å) of one of the sulfone oxygen atoms to the hypervalent iodine center.^[14] The

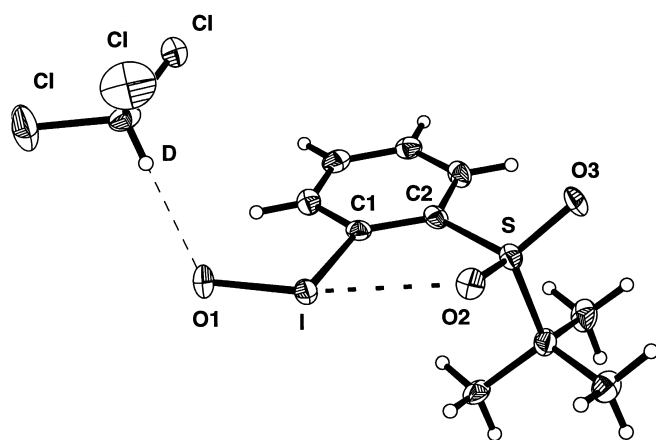
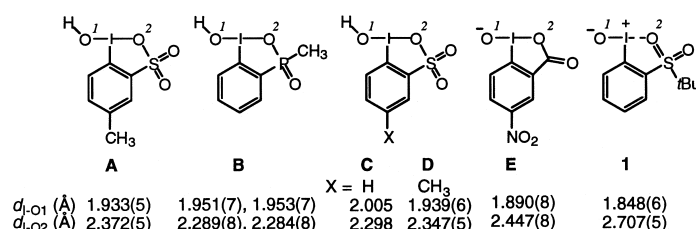


Figure 1. Structure of **1**·CDCl₃. Selected distances [Å] and angles [°]: I–O1 1.848(6), I···O2 2.707(5), I–C 2.128(7), S–O2 1.441(5), S–O3 1.425(5), C–D 0.81(7), D···O1 2.24(7); C1–I–O1 94.8(3), O2···I–O1 167.3(2), I–O2···S 113.3(3), D···O1–I 117(2), C–D···O1 174(6).

presence of this intramolecular I···O bond within the monomers of **1** enforces a planar array for the I, C1, C2, S, and O2 atoms (root-mean-square deviation from planarity is 0.08 Å) and results in a structure that resembles benziodoxoles.^[15] Some structurally characterized representatives are depicted in Scheme 2.^[16] Such species display an inverse



Scheme 2. Iodine–oxygen interatomic distances in **1** and in selected iodine–oxygen heterocyclic species.

relationship between the exocyclic I–O1 and the I–O2 bond lengths. The longer I–O2 bonds within the heterocycles **A**–**E** can be rationalized by the different basicities of the two iodine-bound oxygen atoms and their corresponding competition for engaging in the three-center four-electron bond.^[6g] Compound **E** (as the [Na(OH₂)₃]⁺ salt) displays the most extreme I–O bond lengths of of the group **A**–**E**, probably as a result of the increased charge and basicity of the exocyclic oxygen atom. Compared to **E**, **1** displays an I–O bond length that is only 0.04 Å shorter. This marginal decrease is most likely a result of the increased positive charge located on the iodine atom and corresponding increased electrostatic attractions between the iodine and oxygen atoms (ylide-type resonance structure), rather than the occurrence of any significant I–O multiple bonding.

The above trends also suggest that a further lengthening of the secondary contact between the I and O2 atoms would only result in a marginal decrease of the I–O1 bond length. The high basicity of O2 manifests itself by the establishment of weak hydrogen bonding to chloroform in the crystal structure of **1**. A Cl₃CD···O distance of 2.24(7) Å in **1** is slightly shorter than the mean value 2.31(1) Å found for hydrogen bonds between oxygen acceptors and chloroform.^[17] A highly basic oxygen atom in PhIO is also indicated by the fact that Lewis acids, such as BF₃, can be used to form soluble adducts.^[6c]

Figure 2 portrays the fashion in which molecules of **1** aggregate in the solid state. Each molecule of **1** achieves a pseudo square-planar geometry by the formation of an I···O

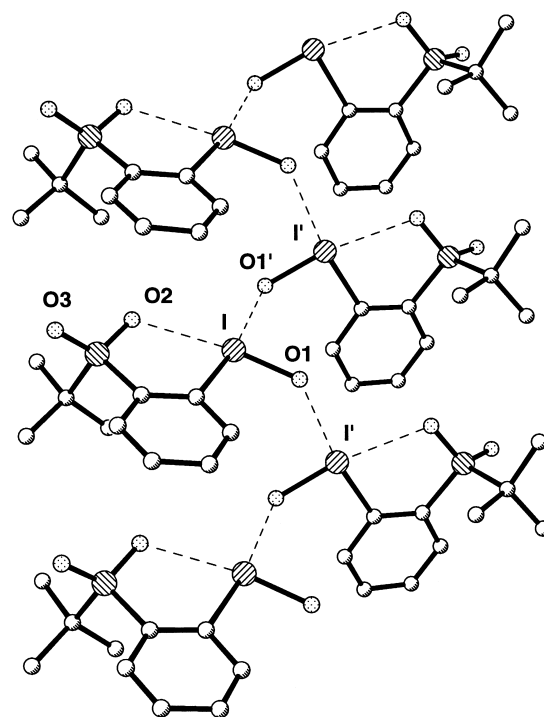


Figure 2. Structure of **1** showing the polymeric connectivity (chloroform and hydrogen atoms omitted for clarity). Selected distances [Å] and angles [°]: I···O1' 2.665(6); O1–I···O1' 93.7(1), I–O1···I' 128.4(3), O2···I···O1' 98.6(2).

secondary bond ($d_{I···O} = 2.665(6)$ Å) to a neighboring iodosyl oxygen atom. The strength of this intermolecular interaction may be inferred to be weaker than analogous secondary bonds in iodosylbenzene ($d_{I···O} = 2.37(1)$ Å). Remarkably, if one ignores the *ortho*-sulfonyl group in **1**, the structure depicted in Scheme 1 resembles that in Figure 2, except that the primary intermolecular I···O bond is turned through 90°, the O–I···O ensemble is now bent rather than linear.

Solutions of compound **1** slowly produce a colorless precipitate of the iodylbenzene **2**, which results from the disproportionation of **1** into **2** and ArI. The decreased solubility of **2** relative to **1** suggests that in **2** there is a greater number of intermolecular I···O secondary bonds in the solid-state structure and/or the intermolecular I···O secondary bonds are stronger. Indeed, an X-ray structural determination of crystalline **2**·CH₂Cl₂ confirmed that both of these factors

play a role.^[13] Each iodine atom of **2** uses both intramolecular (2.693(3) Å) and intermolecular I⋯O bonds (2.777(3) and 2.566(2) Å) to adopt a pseudo octahedral geometry and a chainlike structure (Figure 3). Within the monomers of **2**, I–O bond distances of 1.796(2) and 1.822(2) Å are found (Figure 4). These distances are notably shorter than the

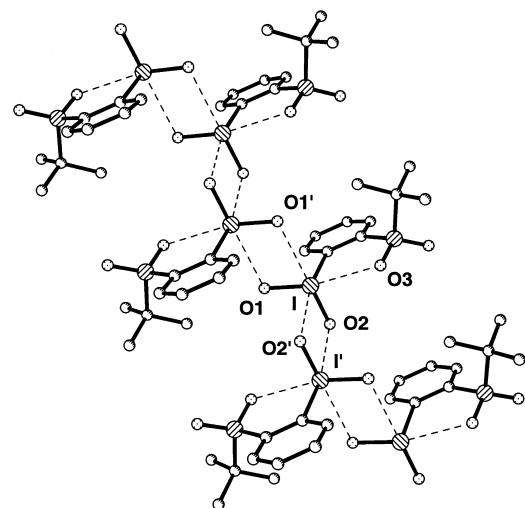


Figure 3. Structure of **2** showing the polymeric connectivity (dichloromethane and hydrogen atoms omitted for clarity). Selected distances [Å] and angles [°]: I⋯O1' 2.777(3), I⋯O2' 2.566(2); O1–I⋯O1' 74.7(1), O1–I⋯O2' 90.7(1), O2–I⋯O2' 74.5(1), I–O2⋯I' 105.5(1), I–O1⋯I' 105.3(1).

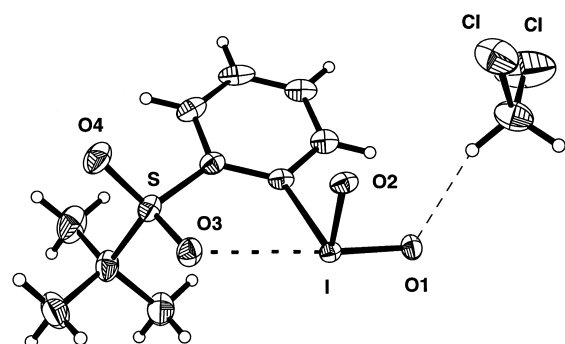


Figure 4. Structure of **2**·CH₂Cl₂. Selected distances [Å] and angles [°]: I–O1 1.796(2), I–O2 1.822(2), I⋯O3 2.693(2), S–O3 1.455(3), S–O4 1.439(3), I–Cl 2.140(4); Cl–I–O1 95.1(1), O3⋯I–O1 166.1(1), I⋯O3–S 114.4(1), I–O1⋯H(CHCl₂) 99.0.

corresponding values reported for either iodylbenzene (**F**; Scheme 3)^[16, 18] or **1**. Again, the pseudo cyclic structure of **2** and I–O bond lengths are actually more comparable to cyclic iodyl analogues **G**–**I** (Scheme 3). A bond 0.026 Å longer for I–O2 compared to I–O1 is found in **2**; this reflects the fact that a shorter secondary interaction contact (intermolecular, 2.566(2) Å) is located *trans* to O2 rather than to O1 (intramolecular, 2.693(3) Å), which correlates well with the stronger interactions of the lone pairs from the oxygen atom with the corresponding σ* orbitals of the I–O bond. Compound **2** shows evidence for weak hydrogen bonding to dichloromethane: The 2.55 Å CH⋯O bond length is slightly longer

	F	G	H	I	2
		racemic	chiral		
d _{I–O1} (Å)	2.021(9)	1.893(3), 1.895(3)	1.784(2)	1.798(2)	1.796(2)
d _{I–O2} (Å)	1.924(9)	1.769(3), 1.784(3)	1.925(2)	1.810(3)	1.822(2)
d _{I–O3} (Å)		2.310(4), 2.324(3)	2.263(2)	2.481(3)	2.693(3)

Scheme 3. Iodine–oxygen interatomic distances in **2**, iodylbenzene, and in selected iodine–oxygen heterocyclic species.

than the mean Cl₂HC–H⋯O hydrogen bond lengths (2.492(8) Å).^[17] The presence of the iodosyl and iodyl units in the plane of the aromatic rings of **1** and **2** also results in short C_{Ar}–H⋯O contacts of 2.38 and 2.39 Å, respectively.

While the impact of the intramolecular I⋯O secondary bonding on the structural properties of **1** is significant, it is also interesting to note that introduction of similar *ortho*-substituted groups bearing a partial negative charge into iodonium ylides of the form ArICR₂ can give rise to antimicrobial activity.^[19] Furthermore, other derivatives of iodosylbenzene, such as 2-iodosylbenzoic acid (**C**; Scheme 1) have been examined as catalysts for the hydrolysis of toxic phosphonates.^[20]

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- [1] N. W. Alcock, *Adv. Inorg. Chem. Radiochem.* **1972**, *15*, 1–58; The secondary I⋯O bonds discussed here are different from the I⋯O charge-transfer bonds discussed in O. Hassel, C. Rommøng, *Quart. Rev. Chem. Soc.* **1962**, *16*, 1–18 and O. Hassel, *Science* **1970**, *170*, 497–502. In reality, a continuum of intermolecular bonding types and interactions exist. See also P. Pyykkö, *Chem. Rev.* **1997**, *97*, 597–636.
- [2] a) G. A. Landrum, R. Hoffmann, *Angew. Chemie* **1998**, *110*, 1989–1992; *Angew. Chem. Int. Ed.* **1998**, *37*, 1887–1890; b) G. A. Landrum, N. Goldberg, R. Hoffmann, R. M. Minyaev, *New J. Chem.* **1998**, *22*, 883–890.
- [3] J. Starbuck, N. C. Norman, A. G. Orpen, *New J. Chem.* **1999**, *23*, 969–972.
- [4] R. J. Batchelor, T. Birchall, J. F. Sawyer, *Inorg. Chem.* **1986**, *25*, 1415–1420.
- [5] a) T. Wirth, U. H. Hirt, *Tetrahedron: Asymmetry* **1997**, *8*, 23–26; b) U. H. Hirt, B. Spingler, T. Wirth, *J. Org. Chem.* **1998**, *63*, 7674–7679.
- [6] Some reviews: a) T. Kitamura, Y. Fujiwara, *Org. Prep. Proced. Int.* **1997**, *29*, 409–458; b) A. Varvoglis, *Tetrahedron* **1997**, *53*, 1179–1255; c) P. J. Stang, V. V. Zhdankin, *Chem. Rev.* **1996**, *96*, 1123–1178; d) A. Varvoglis, *The Organic Chemistry of Polycordinated Iodine*, VCH, New York, **1992**; e) R. M. Moriarty, O. Prakash, *Acc. Chem. Res.* **1986**, *19*, 244–250; f) A. Varvoglis, *Synthesis* **1984**, 709–726; g) G. F. Koser in *The Chemistry of the Functional Groups, Supplement D* (Eds.: S. Patai, Z. Rappoport), Wiley, New York, **1983**, pp. 721–811.
- [7] a) F. Lichtenberger, W. Nastainczyk, V. Ullrich, *Biochem. Biophys. Res. Commun.* **1976**, *70*, 939–946; b) F. Lichtenberger, V. Ullrich, *Microsomes and Drug Oxidations* (Ed.: V. Ullrich), Pergamon, New York, **1977**, pp. 218–223; c) J. T. Groves, T. E. Nemo, R. S. Myers, *J. Am. Chem. Soc.* **1979**, *101*, 1032–1033.
- [8] Some recent reviews: a) Y. Moro-oka, *Catal. Today* **1998**, *45*, 3–12; b) Y. Moro-oka, M. Akita, *Catal. Today* **1998**, *41*, 327–338; c) M. Lautens, W. Klute, W. Tam, *Chem. Rev.* **1996**, *96*, 49–92; d) J. T. Groves, Y.-Z. Han in *Models and Mechanisms of Cytochrome P-450 Action* (Ed.: P. O. D. Montellano), Plenum Press, New York, **1995**, pp. 1–45; e) R. Noyori, *Asymmetric Catalysis in Organic Synthesis*,

- Wiley, New York, **1994**; f) D. H. R. Barton, A. E. Martell, D. T. Sawyer, *The Activation of Dioxygen and Homogeneous Catalytic Oxidation*, Plenum Press, New York, **1993**; g) I. Ojima, *Catalytic Asymmetric Synthesis*, VCH, New York, **1993**; h) R. A. Aitken, S. N. Kilényi, *Asymmetric Synthesis*, Blackie, New York, **1992**; i) D. Ostovic, T. C. Bruice, *Acc. Chem. Res.* **1992**, *25*, 314–320; j) R. H. Holm, J. P. Donahue, *Polyhedron* **1993**, *12*, 571–589; k) R. H. Holm, *Chem. Rev.* **1987**, *87*, 1401–1449.
- [9] C. Willgerodt, *Chem. Ber.* **1892**, *25*, 3494–3502.
- [10] a) W. E. Dasent, T. C. Waddington, *J. Chem. Soc.* **1960**, 2429–2432; b) W. E. Dasent, T. C. Waddington, *J. Chem. Soc.* **1960**, 3350–3356; c) R. Bell, K. J. Morgan, *J. Chem. Soc.* **1960**, 1209–1214; d) H. Siebert, M. Handrich, *Z. Anorg. Allg. Chem.* **1976**, *426*, 173–183; e) B. S. Ehrlich, M. Kaplan, *J. Chem. Phys.* **1971**, *54*, 612–620; f) J. V. Carey, P. A. Chaloner, K. R. Seddon, M. Taylor, *J. Chem. Res. (S)* **1996**, 156–157; g) C. J. Carmalt, J. G. Crossley, J. G. Knight, P. Lightfoot, A. Martin, M. P. Muldowney, N. C. Norman, A. G. Orpen, *J. Chem. Soc. Chem. Commun.* **1994**, 2367–2368.
- [11] H. W. Richter, B. R. Cherry, T. D. Zook, G. F. Koser, *J. Am. Chem. Soc.* **1997**, *119*, 9614–9623.
- [12] D. Macikenas, E. Skrzypczak-Jankun, J. D. Protasiewicz, *J. Am. Chem. Soc.* **1999**, *121*, 7164–7165.
- [13] Crystals of **1** were grown from [D₁]chloroform/toluene at –35 °C. C₁₀H₁₃IO₄S·CDCl₃, *M_r* = 460.54, monoclinic, space group *P*2₁/*c*, *a* = 11.379(5) Å, *b* = 5.674(2) Å, *c* = 25.220(11) Å, *b* = 94.089(8)°, *V* = 1624.0(11) Å³, *Z* = 4, *ρ_{calcd}* = 1.884 g cm^{–3}, *T* = 150(2) K, yellow block 0.22 × 0.34 × 0.40 mm³, 2 θ _{max} = 59.82°, 10382 measured reflections, 4297 unique, *R* values (*I* > 2 σ (*I*)): *R*1 = 0.0655, *wR*2 = 0.1314. Crystals of **2** were grown from dichloromethane at ambient temperature. C₁₀H₁₃IO₄S·CH₂Cl₂, *M_r* = 441.09, monoclinic, space group *C*2/*c*, *a* = 19.784(2) Å, *b* = 6.5461(6) Å, *c* = 24.647(2) Å, *b* = 96.697(2)°, *V* = 3170.2(5) Å³, *Z* = 8, *ρ_{calcd}* = 1.848 g cm^{–3}, *T* = 150(2) K, 2 θ _{max} = 60.02°, 17723 measured reflections, 4322 unique, *R* values (*I* > 2 σ (*I*)): *R*1 = 0.0345, *wR*2 = 0.0703. Bruker SMART Platform with CCD detector, Mo radiation (λ = 0.71073 Å) with graphite monochromator, omega scans with 0.3° increments for the frames. Absorption corrections made with SADABS (G. M. Sheldrick, University of Göttingen, **1998**); structure solution and refinement with SHELXTL 5.10 (Bruker AXS, Inc., Madison, WI, **1997**) against *F*² (177 parameters for **1**, 173 parameters for **2**). All nonhydrogen atoms were refined with anisotropic temperature parameters, all hydrogen atoms (except CDCl₃ deuterium atom in **1**) were placed in calculated positions and refined isotropically. The CDCl₃ deuterium atom was located from the Fourier map and refined isotropically. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC–137694 and CCDC–137693. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). CCDC
- [14] For recent discussion of cyclic intramolecular O⋯X secondary bonds (X = chalcogen) see: R. M. Minyaev, V. I. Minkin, *Can. J. Chem.* **1998**, *76*, 776–788, and references therein.
- [15] V. V. Zhdankin, *Rev. Heteroatom Chem.* **1997**, *17*, 133–151.
- [16] Compound **A**: G. F. Koser, G. Sun, C. W. Porter, W. J. Youngs, *J. Org. Chem.* **1993**, *58*, 7310–7312; Compound **B**: T. M. Balthazor, J. A. Miles, B. R. Stults, *J. Org. Chem.* **1978**, *43*, 4538–4540; Compound **C**: E. Shefter, W. Wolf, *J. Pharm. Sci.* **1965**, *54*, 104–107; Compounds **D**, **E**, and **I**: ref. [20e]; Compound **F**: N. W. Alcock, J. F. Sawyer, *J. Chem. Soc. Dalton Trans.* **1980**, 115–120; Compound **G**: P. J. Stevenson, A. B. Treacy, M. Nieuwenhuyzen, *J. Chem. Soc. Perkin Trans. 2* **1997**, 589–591; Compound **H**: J. Z. Gougoutas, *Cryst. Struct. Commun.* **1981**, *10*, 489–494.
- [17] a) T. Steiner, *New J. Chem.* **1998**, 1099–1103; b) T. Steiner, *Chem. Commun.* **1997**, 727–734; c) G. R. Desiraju, *Acc. Chem. Res.* **1991**, *24*, 290–296.
- [18] Reported I–O bond lengths of 1.60 and 1.65 Å for *para*-chloriodylbenzene in E. M. Archer, *Acta Cryst.* **1948**, *1*, 64–69 are probably not reliable.
- [19] “Iodonium ylide compositions and method for antimicrobial use”: a) A. G. Relenyi, R. W. Walter, G. F. Koser (Dow Chemical Co., USA), EP160322B1, **1989**; “Preparation of iodonium ylide antimicrobial compounds”: b) A. G. Relenyi, W. J. Kruper, Jr., R. W. Walter, R. B. Shankar, A. P. Zelinko, G. F. Koser (Dow Chemical Co., USA), EP225478B1, **1990**; “Iodones and methods for antimicrobial use”: A. G. Relenyi, G. F. Koser, R. W. Walter, Jr., W. S. Kruper, Jr., R. B. Shankar, A. P. Zelinko (Dow Chemical Co., USA), US5106407, **1992**.
- [20] Some recent work: a) R. A. Moss, H. Morales-Rojas, H. Zhang, B.-D. Park, *Langmuir* **1999**, *15*, 2738–2744; b) A. R. Katritzky, G. P. Savage, J. K. Gallos, H. D. Durst, *J. Chem. Soc. Perkin Trans. 2* **1990**, 1515–1518; c) D. R. Leslie, S. Pantelidis, *Aust. J. Chem.* **1990**, *43*, 937–943; d) C. A. Panetta, S. M. Garlick, H. D. Durst, F. R. Longo, J. R. Ward, *J. Org. Chem.* **1990**, *55*, 5202–5205; e) A. R. Katritzky, G. P. Savage, G. J. Palenik, K. Qian, Z. Zhang, H. D. Durst, *J. Chem. Soc. Perkin Trans. 2* **1990**, 1657–1661; f) R. A. Moss, K. Bracken, T. J. Emge, *J. Org. Chem.* **1995**, *60*, 7739–7746; g) R. A. Moss, S. Bose, K. Krogh-Jespersen, *J. Phys. Org. Chem.* **1997**, *10*, 27–32; h) R. A. Moss, *Chem. Commun.* **1998**, 1559–1560.

A General Entry into Glycopeptide “Dendrons”**

Kashinath Sadalapure and Thisbe K. Lindhorst*

*Dedicated to Professor Günter Wulff
on the occasion of his 65th birthday*

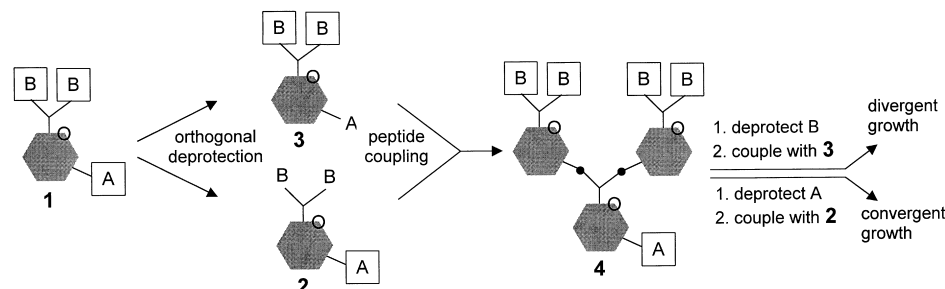
In order to study and manipulate carbohydrate–protein interactions, carbohydrate and dendrimer chemistries have been combined in order to design multivalent glycoconjugate mimetics.^[1] Such carbohydrate dendrimers potentially influence carbohydrate–protein interactions strongly due to the multivalency or cluster effects.^[2,3] Moreover, they may combine the properties of drug binding and transport with other, still unknown, qualities of synthetic biomaterials. Two main entries into the synthesis of carbohydrate dendrimers have been utilized so far: by the glyco-coating of noncarbohydrate dendrimers to form glycodendrimers^[4] or by the convergent combination of molecular wedges derived from carbohydrate clusters^[5] to yield carbohydrate dendrimers of different architectures.^[6] Both synthetic approaches do not allow enlargement of the carbohydrate-containing molecules by the formation of successive generations,^[7] although some reported carbohydrate dendrimers possess a relatively high sugar density.

We now report on the synthesis of glycopeptide dendrons in which the peptide coupling of orthogonally protected AB₂-type carbohydrate units form the basis for an iterative

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reaction sequence to lead to the various generations of carbohydrate-containing dendrons in either a divergent or convergent manner (Scheme 1). The use of peptide chemistry in the linking step avoids sophisticated glycosylation techni-



Scheme 1. The general approach to glycopeptide dendrons from orthogonally protected carbohydrate AB₂-type building blocks such as **1** (see Scheme 2). B is a carboxy group and A is an amino moiety, and the respective protected groups are boxed. This design allows an iterative synthetic sequence of deprotection and peptide-coupling reactions leading to successive generations of dendritic growth. The first-generation glycopeptide dendron **4** may be extended according to a divergent or convergent approach. Convergent growth led to second- and third-generation dendrons **7** and **8** (see Schemes 3 and 4). A gray-shaded hexagon represents a monosaccharide ring and a black circle represents an amide linkage.

ques for each coupling step. Furthermore, this synthetic protocol simplifies adaptation to solid-phase chemistry, which can be used for automated synthesis of glycopeptide dendrons as well as for combinatorial approaches of various kinds.

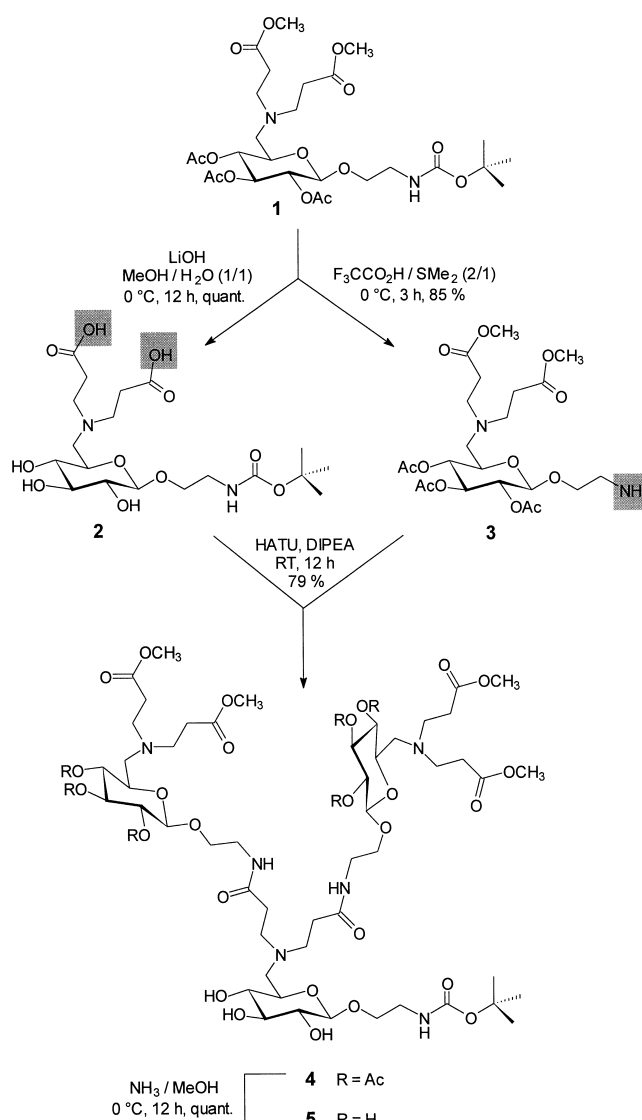
The AB₂-type glucoside building block **1**^[8] forms the basis for the preparation of glycopeptide dendrons **5**, **7**, and **8**. Mildly alkaline hydrolysis of the methyl ester groups in **1** with LiOH·H₂O in MeOH/H₂O (1/1) leads to the unprotected bis-carboxylic acid **2**, while removal of the *tert*-butoxycarbonyl (Boc) protecting group in **1** by a mixture of trifluoroacetic acid and dimethyl sulfide^[9] gave the amino-functionalized glucoside **3** (Scheme 2). Thus, glucoside **1** gives both the complementary building blocks **2** and **3**, which were used in a peptide-coupling reaction.

The uronium-based salt HATU,^[10] together with DIPEA,^[10, 11] proved to be a suitable promoter for the peptide coupling which gave the first generation glycopeptide dendron **4** in 79% yield. Dendron **4** was purified by gel-permeation chromatography (GPC) using a Sephadex LH-20 column and methanol as the eluent. The NMR spectra of **4** showed one set of signals for the acetyl-protected glucose moiety and another set for the core glucose moiety in both the ¹H and ¹³C NMR spectra (Table 1). Deacetylation of **4** with saturated methanolic ammonia gave the unprotected tetramethyl ester **5** in quantitative yield. Compound **5** was purified by GPC using a bio-gel P-6 packed column and an ammonium hydrogen carbonate buffer as the eluent.

Using the Boc-deprotection and peptide-coupling reaction sequence, the synthesis of higher generations of glycopeptide dendrons was carried out in a convergent manner. Thus, the Boc-protected first generation dendron **4** was converted to the respective amine, which coupled with **2** to lead to the protected second generation glycopeptide dendron **6** in 60% yield (Scheme 3). Compound **6** was purified by GPC as per compound **4**. The NMR spectra of **6** displayed two sets of signals for the carbohydrate moieties (Table 1). The deacetylation of **6** could not, unlike **4**, be fully completed

with saturated methanolic ammonia due to solubility problems. However, when aqueous ammonia was used for the deprotection step, saponification of the methyl esters occurred with concomitant removal of the acetate protective groups. In this way the octacarboxylic acid **7** was obtained and purified as per **5**.

Analogous to the preparation of **6**, the third-generation dendron **8**, containing 15 sugar moieties, was obtained after Boc deprotection of **6** and peptide coupling of the resulting amine to **2** (Scheme 4). However, as the high molecular mass glycopeptide mimetic obtained was poorly soluble in methanol, an aqueous carbonate buffer was used as the eluent during gel filtration through bio-gel P-6. The ¹H NMR of the product fractions

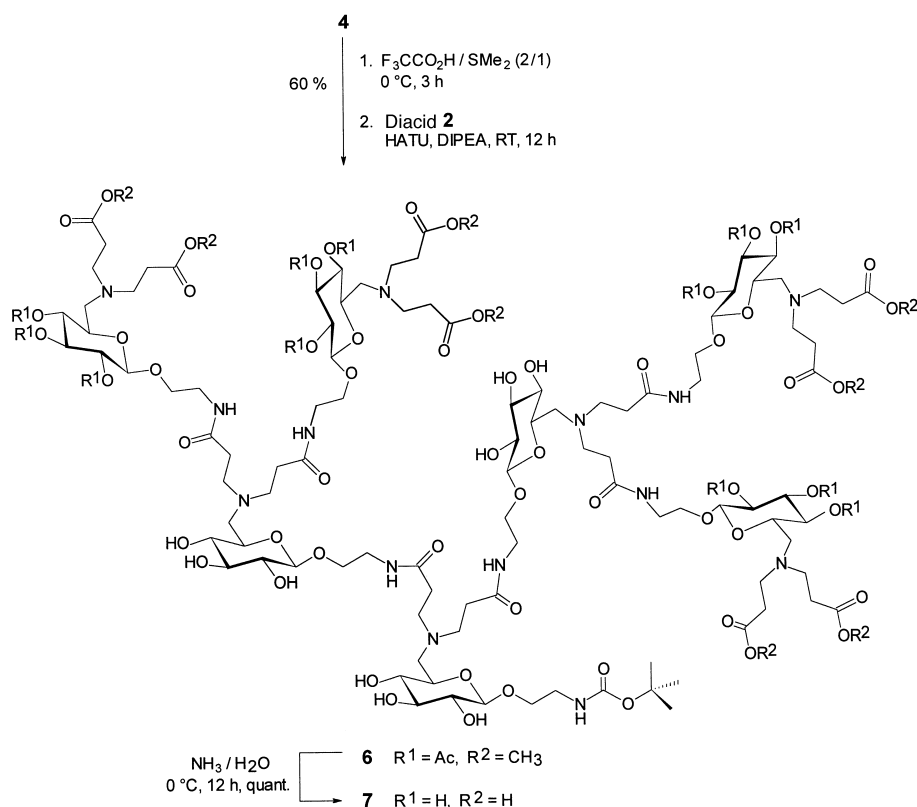


Scheme 2. Synthesis of first-generation glycopeptide dendron.

Table 1. Selected ^1H (500 MHz) and ^{13}C (125.75 MHz) NMR data for compounds **4**–**8**.

	$\delta(\mathbf{4})$ in CD_3OD	$\delta(\mathbf{5})$ in D_2O	$\delta(\mathbf{6})$ in CD_3OD	$\delta(\mathbf{7})$ in D_2O	$\delta(\mathbf{8})$ in D_2O
^1H NMR:					
H-1 ^[a]	4.40 (d, 1H)	4.44 (m _c , 3H)	4.21 (m _c , 3H)	4.51–4.38 (m, 7H)	4.41 (m _c , 15H)
H-1 ^[b]	4.65 (d, 2H)	–	4.56 (d, 4H)	–	–
NCH ₂	2.89 (m _c , 12H)	3.43 (m _c , 2H), 3.28 (m _c , 2H), 3.17–2.96 (m, 8H)	2.80 (m _c , 12H), 2.76 (m, 16H)	3.48 (m _c , 6H), 3.27 (m _c , 6H), 3.16–2.92 (m, 16H)	3.53–3.10 (m, 60H)
CH ₂ C(O)	2.48 (m, 12H)	2.64–2.46 (m, 12H)	2.37 (m, 16H), 2.35 (m, 12H)	2.67–2.45 (m, 28H)	2.76–2.44 (m, 60H)
H ₃ COC(O)	3.68 (s, 6H)	3.29 (s, 12H)	3.56 (s, 24H)	–	–
H ₃ CC(O)O	2.05 (s, 6H), 2.03 (s, 6H), 1.95 (s, 6H)	–	1.95 (s, 12H), 1.93 (s, 12H), 1.86 (s, 12H)	–	–
<i>t</i> Bu	1.44 (s, 9H)	1.38 (s, 9H)	1.34 (s, 9H)	1.37 (s, 9H)	1.31 (s, 9H)
^{13}C NMR:					
C-1	104.35, 101.94	102.94, 102.84	104.54, 101.94	102.39, 102.30	102.41, 102.27

[a] H-1 of OH-free glucose moieties. [b] H-1 of acetylated glucose moieties.



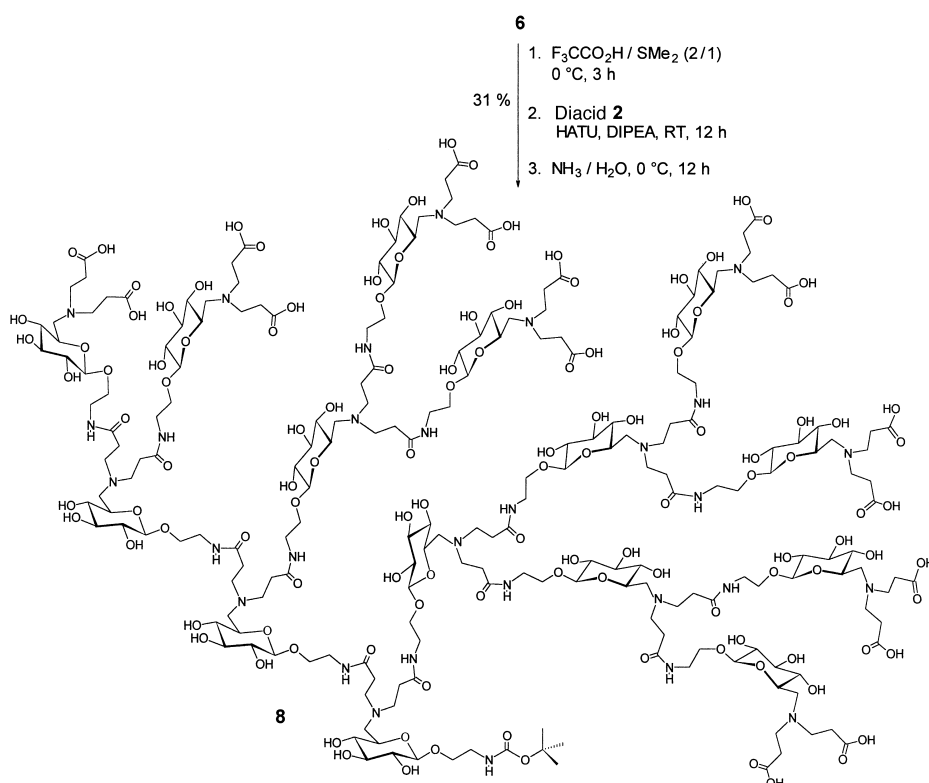
Scheme 3. Synthesis of second-generation glycopeptide dendron.

indicated partial deprotection of the *O*-acetate groups and, therefore, deprotection with saturated aqueous ammonia was performed subsequent to peptide coupling and prior to purification. As in the case of the deprotection of **6**, aqueous ammonia effected deacetylation as well as saponification of the methyl ester groups and consequently the hexadecaacid **8** was isolated as its ammonium salt.

In addition to the NMR analysis, the structures of all of the described compounds were confirmed by MALDI-TOF mass spectrometry (Table 2) and, in most cases, reasonable ele-

mental analyses were obtained. Thus, all the glycopeptide dendrons proved to be monodisperse compounds without structural defects. The described route was superior over alternative strategies for the assembly of the described dendrons, including the divergent approach.

Stability against glycosidases of this new type of multivalent neoglycoconjugates was tested with glycopeptide dendron **5**. No degradation, according to HPLC analysis, was observed when **5** was incubated with β -glucosidase from almonds over several hours.



Scheme 4. Synthesis of third-generation glycopeptide dendron.

Table 2. Peaks obtained in MALDI-TOF mass spectrometry^[a] of glycopeptide dendrons **4–8**.

Compound	Molecular formula	Calcd. mol. mass ^[b] [g mol ⁻¹]	Observed mass peaks
4	C ₆₃ H ₁₀₂ N ₆ O ₃₃	1470.65	1471.58 [M+H] ⁺ , 1494.60 [M+Na] ⁺ , 1509.53 [M+K] ⁺
5	C ₅₁ H ₉₀ N ₆ O ₂₇	1218.58	1219.63 [M+H] ⁺ , 1243.61 [M+Na] ⁺
6	C ₁₃₅ H ₂₁₈ N ₁₄ O ₇₁	3171.38	3172.14 [M+H] ⁺ , 3195.11 [M+Na] ⁺ , 3208.10 [M+K] ⁺
7	C ₁₀₃ H ₁₇₈ N ₁₄ O ₅₉	2555.13	2556.45 [M+H] ⁺ , 2670.08 [M+6NH ₃ +3H] ⁺
8	C ₂₁₅ H ₃₇₀ N ₃₀ O ₁₂₃	5340.36	5341.24 [M+H] ⁺ , 5606.15 [M+16NH ₃ -6H] ⁺

[a] Dihydroxybenzoic acid (DHB) was employed as matrix in acetonitrile/water/TFA (2/1/0.1) for the co-crystallization of compounds **4–8**. The calibration of the mass peaks in a linear mode was made with 4-cyanocinnamic acid (190.05 [M+H]⁺), angiotensin II ([M+H]⁺ 1046.54), bombesin ([M+H]⁺ 1619.82), and bovine insulin (5734.56 [M+H]⁺). [b] Main isotopes were used for the calculation of the molecular masses.

In conclusion, we have described a novel approach to the synthesis of hyperbranched carbohydrate-containing molecules, which can be grown in successive generations, in the form of glycopeptide dendrons. The three-dimensional structure of the compounds may represent elements present in natural glycopeptides and may be developed to a new class of glycocalyx mimetics by further enlargement. Currently we are extending the presented strategy with the aim to prepare hyperbranched molecules with antiadhesive properties.

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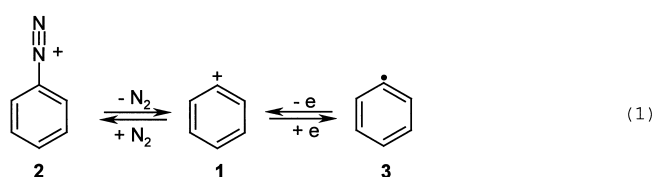
- [1] N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1997**, *3*, 1193–1199.
 [2] a) L. L. Kiessling, N. L. Pohl, *Chem. Biol.* **1996**, *3*, 71–77; b) R. Roy, *Curr. Opin. Struct. Biol.* **1996**, *6*, 692–702.

- [3] a) Y. C. Lee, *Acc. Chem. Res.* **1995**, *28*, 321–327; b) M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794.
 [4] a) T. K. Lindhorst, *Nachr. Chem. Techn. Lab.* **1996**, *44*, 1073–1079; b) R. Roy, D. Zanini, J. Meunier, A. Romanowska, *J. Chem. Soc. Chem. Commun.* **1993**, 1869–1890; c) K. Aoi, K. Itoh, M. Okada, *Macromolecules* **1995**, *28*, 5391–5393; d) T. K. Lindhorst, C. Kieburg, *Angew. Chem.* **1996**, *108*, 2083–2086; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953–1956; e) P. R. Ashton, S. E. Boyd, C. L. Brown, S. A. Nepogodiev, E. W. Meijer, H. W. Peerlings, J. F. Stoddart, *Chem. Eur. J.* **1997**, *3*, 974–984.
 [5] a) Y. C. Lee, *Carbohydr. Res.* **1978**, *67*, 509–514; b) L. A. J. M. Slie-dregt, P. C. N. Rensen, E. T. Rump, P. J. van Santbrink, M. K. Bijsterbosch, A. R. P. M. Valentijn, G. A. van der Marel, J. H. van Boom, T. J. C. van Berkel, E. A. L. Biessen, *J. Med. Chem.* **1999**, *42*, 609–618.
 [6] a) P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1996**, *2*, 1115–1128; b) P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, J. F. Stoddart, *Angew. Chem.* **1997**, *109*, 756–759; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 732–735; c) P. R. Ashton, E. F. Hounsell, N. Jayaraman, T. M. Nilsen, N. Spencer, J. F. Stoddart, M. Young, *J. Org. Chem.* **1998**, *63*, 3429–3437; d) B. Colonna, V. D. Harding, S. A. Nepogodiev, F. M. Raymo, N. Spencer, J. F. Stoddart, *Chem. Eur. J.* **1998**, *4*, 1244–1254.
 [7] a) M. Fischer, F. Vögtle, *Angew. Chem.* **1999**, *111*, 934–955; *Angew. Chem. Int. Ed.* **1999**, *38*, 884–905; b) O. A. Matthews, A. N. Shipway, J. F. Stoddart, *Prog. Polym. Sci.* **1998**, *23*, 1–56.
 [8] C. Kieburg, K. Sadalapure, T. K. Lindhorst, *Eur. J. Org. Chem.*, in press.
 [9] K. Katano, H. A. Y. Aoyagi, M. Overhand, S. J. Sucheck, W. C. Stevens, Jr., C. D. Hess, X. Zhou, S. M. Hecht, *J. Am. Chem. Soc.* **1998**, *120*, 11285–11296.
 [10] Abbreviations used: HATU = *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluroniumhexafluorophosphate; DIPEA = diisopropylethylamine.
 [11] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.

Isolation of the Phenyl Cation in a Solid Argon Matrix**

Michael Winkler and Wolfram Sander*

The phenyl cation (**1**) is of fundamental importance to organic chemistry and one of the most reactive molecules known.^[1] Its existence as a reaction intermediate was postulated more than fifty years ago by Waters.^[2] Since that time, a large number of reports dealing with the chemistry of **1** in solution,^[3] the physical properties of **1** in the gas phase,^[4] and theoretical descriptions of the electronic structure^[5–7] of **1** have appeared in the literature. Evidence that **1** is indeed formed as a transient species in solution stems from ¹⁴N₂/¹⁵N₂ exchange experiments with the benzene diazonium ion (**2**) and from kinetic measurements of the dediazonation of **2** [Eq. (1)].^[8]

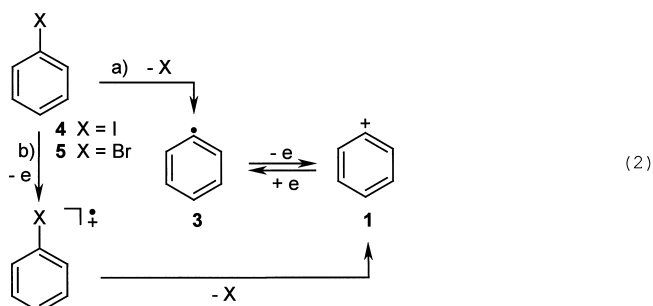


Owing to its extreme electrophilicity and reactivity, **1** is very short-lived in the condensed phase, and, in contrast to the phenyl radical (**3**),^[9] has never been isolated or characterized spectroscopically. Neither NMR investigations in magic acid^[10] nor time-resolved spectroscopy^[11] made a direct observation of the phenyl cation possible. Although mass spectrometry and ICR spectroscopy provided some insight into the gas-phase reactivity of **1**,^[12] no detailed conclusions concerning structural features and electronic properties can be drawn from these experiments. Especially the ground-state multiplicity of **1** and the gap between the lowest singlet (¹A₁) and triplet state (³B₁) has been subject to some debate.^[1, 5–7] From the analysis of the photoelectron spectrum of **3** it was concluded that the singlet state is 31 kJ mol⁻¹ lower in energy than the triplet state.^[13] Recent ab initio calculations predict a larger singlet–triplet gap of 75–80 kJ mol⁻¹^[6] or even 103 kJ mol⁻¹.^[7]

The technique of isolation in low-temperature noble gas matrices has proven to be a viable method for the characterization of highly reactive neutral species such as carbenes and radicals. In a series of papers Andrews et al. reported on the matrix isolation of cations produced by photoionization of neutral precursors with the short-wavelength light from a noble gas discharge source.^[14, 15] In these experiments the major tool for the identification of the cations was UV/Vis spectroscopy. Here we describe the matrix isolation and first

IR spectroscopic characterization of the elusive phenyl cation (**1**), generated by vacuum UV photolysis of iodobenzene (**4**) and bromobenzene (**5**).

The C–I bond dissociation energy for **4** is 2.8 eV (274 kJ mol⁻¹),^[16] and irradiation at 254 nm (corresponding to 473 kJ mol⁻¹) of **4**, matrix isolated in argon at 8 K, readily produces the phenyl radical (**3**) in high yield [Eq. (2)]. The



product was identified by comparison with literature data.^[9] The vertical ionization potential of **3** is 9.0 ± 0.2 eV^[6, 7] and that of **4** is 8.7 eV.^[17] Thus the light emitted from an argon resonance source (105–107 nm ≅ 11.6–11.8 eV)^[15] should be sufficiently energetic to ionize both species and in addition cause the C–I bond in **4** to break.^[18]

In a typical experiment, **4** was slowly sublimed with a large excess of argon and deposited on a cold spectroscopic window (CsI) at 8 K. During the deposition (2–3 h) the gas phase and the matrix formed on top of the CsI window were irradiated with the intense light of an argon discharge lamp. The IR spectrum of the matrix (Figure 1a) reveals that despite the intense irradiation a substantial amount of unchanged starting material **4** is still present. Newly formed products are the phenyl radical (**3**), acyclic photoproducts of **4** along with small

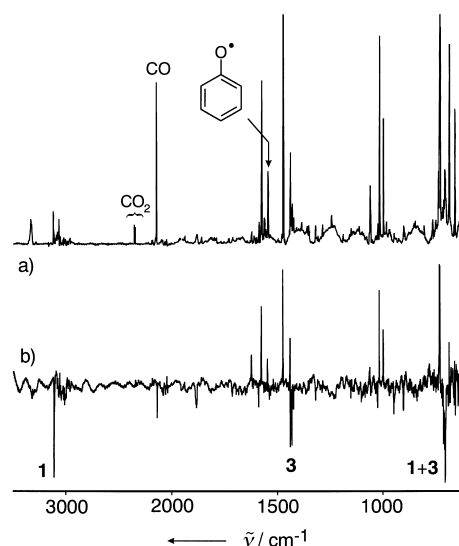


Figure 1. IR detection of the phenyl cation (**1**). a) IR spectrum of iodobenzene (**4**) after argon resonance irradiation; b) difference spectrum of the same matrix showing the bleaching of **1** and **3**; the bands pointing downwards disappear during irradiation with $\lambda > 400$ nm, whereas absorptions of **4** and the phenoxyl radical increase in intensity.

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amounts of CO, CO₂, and the phenoxyl radical. CO₂ is always a contaminant, formed by the oxidation of traces of organic compounds in the microwave discharge, and the intense vacuum UV irradiation leads to the cleavage of CO₂ into CO and oxygen atoms. Reaction of the oxygen atoms with **3** subsequently results in the formation of the phenoxyl radical.

Besides these products, a novel compound with an intense IR absorption at 3110 cm⁻¹ is observed. Neither **4** nor **3** nor any of their products formed on conventional matrix photolysis exhibit IR absorptions between 3100 and 3200 cm⁻¹. On irradiation with visible light ($\lambda > 400$ nm), all absorptions of **3** as well as the 3110 cm⁻¹ absorption are rapidly bleached (Figure 1 b). The presence of an intense band at 3110 cm⁻¹ is in good agreement with calculations of the phenyl cation (**1**) at various levels of theory, which predict a high-frequency absorption, the asymmetric CH stretching vibration of the *ortho*-hydrogen atoms, to be the most intense absorption in the IR spectrum (Table 1).

Table 1. Comparison of calculated (B3LYP/cc-pVDZ) with experimental IR data of **1** and **3**. The frequencies are unscaled, and only bands with more than 10 % of the intensity of the strongest absorption are listed.

Species	Mode	Sym.	B3LYP/cc-pVDZ		Ar, 8 K
			$\tilde{\nu}$ [cm ⁻¹]	I [km mol ⁻¹]	
1 (¹ A ₁)	3	b ₂	412	22	
	7	b ₁	699 ^[a]	136	713
	15	b ₂	1098	43	
	19	a ₁	1350	37	
	22	b ₂	1802	33	
	23	a ₁	3182	26	
	24	b ₂	3183	24	
	25	a ₁	3222	28	
	26	b ₂	3236	204	3110
	27	a ₁	3240	47	
1 (³ B ₁)	4	b ₁	591	18	
	6	b ₁	739	57	
	19	a ₁	1405	105	
	20	b ₂	1409	42	
	21	b ₂	1486	52	
	25	a ₁	3206	21	
3 (² A ₁)	26	b ₂	3215	38	
	5	b ₁	673	14	655
	6	b ₁	721	52	703
	13	a ₁	1048	9	1027
	19	b ₂	1457	5	1432
	20	a ₁	1466	7	1438
	25	a ₁	3183	5	3071
	26	b ₂	3186	22	3073
	27	a ₁	3198	10	3085

[a] This band is considerably blue-shifted (20–30 cm⁻¹) if an argon “solvation shell” is modeled by adding several argon atoms around **1**. In contrast, the solvation effects on the CH stretching vibration at 3110 cm⁻¹ cancel out almost completely if a larger number of argon atoms is considered.

Argon resonance irradiation of bromobenzene (**5**) during deposition of a matrix produces a similar product mixture, and in particular the strong, photolabile 3110 cm⁻¹ absorption is also observed (Figure 2). The major difference is the very low yield of phenyl radicals, which allows observation of the second strongest IR absorption of **1** at 713 cm⁻¹. This band is assigned to the in-phase, out-of-plane CH deformation mode. With **4** as precursor this signal is hidden under the strong

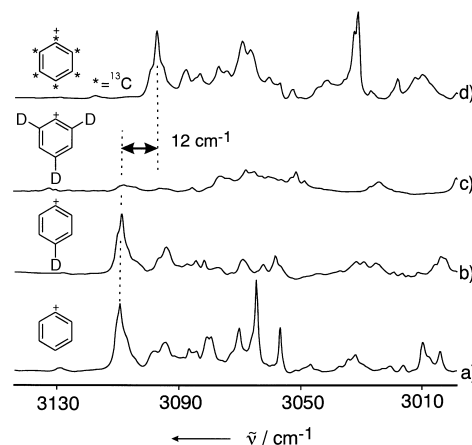


Figure 2. IR spectra obtained after argon resonance irradiation of bromobenzene (**5**) and several of its isotopomers. a) **1**; b) [4-D₁]-**1**; c) [2,4,6-D₃]-**1**; d) [¹³C₆]-**1**. All spectra were recorded under identical experimental conditions. The highest frequency absorptions disappear during irradiation with $\lambda > 400$ nm.

absorptions of **3** in the same region of the spectrum. The phenyl cation (**1**) is thus produced by two independent precursors. The ground state of **1** is the ¹A₁ singlet state, while a triplet ground state (³B₁) is not compatible with the experimental findings (Table 1).

To support the assignment of the 3110 and 713 cm⁻¹ bands, several isotopomers of **1** were generated from the corresponding labeled halobenzenes [¹³C₆]-**5**, [D₃]-**5**, [2,4,6-D₃]-**5**, and [4-D₁]-**5**. In the completely ¹³C-labeled isotopomer [¹³C₆]-**1** the absorptions at 3110 and 713 cm⁻¹ are red-shifted by 12 and 5 cm⁻¹, respectively, in excellent agreement with the predictions of 11 and 5 cm⁻¹ at the B3LYP/cc-pVDZ level of theory.^[19] Selective deuteration of the *para* position in [4-D₁]-**1** hardly effects the 3110 cm⁻¹ absorption, while this band completely disappears for both of the *ortho*-deuterated isotopomers [2,4,6-D₃]-**1** and [D₃]-**1** (Figure 2). Thus, the selective deuteration confirms the assignment of the 3110 cm⁻¹ absorption to the asymmetric CH stretching vibration of the *ortho*-hydrogen atoms. According to the DFT calculations, the CD stretching vibrations are much lower in intensity than the corresponding CH stretching vibrations, and therefore could not be assigned unequivocally in the product mixture. The selective deuteration also reveals that under the reaction conditions no hydrogen migration occurs, as no isotopic scrambling is observed.^[20] The barrier for the [1,2]-hydrogen migration was calculated to be 197 kJ mol⁻¹ (2.04 eV) at the B3LYP/cc-pVTZ level of theory and is thus much higher than for aliphatic carbocations.

Two mechanisms for the formation of **1** by argon resonance irradiation of halobenzenes have to be considered [Eq. (2)]: a) photochemical dissociation of the C–X bond to give **3** and X atoms and subsequent ionization of **3**, or b) photoionization of the halobenzene to give the corresponding radical cation, which in a second step yields **1** under loss of X atoms. Both processes are energetically feasible, but they cannot be discriminated by our experiments. The electrons released upon photoionization are efficiently trapped by the X atoms or by traces of impurities (e.g. O atoms). Recombination of **1** and X⁻ or of **3** and X during the formation of the matrix yields

4 or 5, respectively, which explains the high amount of unchanged starting material.

Chemical evidence for the matrix isolation of the phenyl cation (**1**) stems from trapping experiments with molecular nitrogen. If the argon is doped with 5–10% N₂, the intensity of the 3110 cm⁻¹ absorption decreases and a very strong absorption at 2327 cm⁻¹ is observed (Figure 3). Higher con-

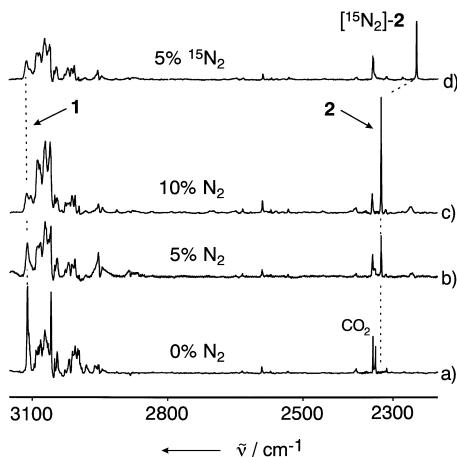


Figure 3. a)–c) “Titration” of **1** with ¹⁴N₂ and d) trapping of **1** with ¹⁵N₂. Spectra a)–c) are recorded under identical experimental conditions. The splitting of the CO₂ band in pure argon (spectrum a) is attributed to matrix site effects^[23] and disappears when the argon lattice is disturbed by larger amounts of N₂. The 2327 cm⁻¹ band of **2** is well separated from these absorptions. In ¹⁵N₂-**2** the N≡N stretching vibration is red-shifted by 78 cm⁻¹ (spectrum d). The N₂ concentrations given are estimated values.

centrations of N₂ result in weaker 3110 and stronger 2327 cm⁻¹ bands, which indicates that **1** can be “titrated” with N₂. With ¹⁵N₂ as trapping reagent a red shift of 78 cm⁻¹ is observed for the 2327 cm⁻¹ band, and thus this absorption can unambiguously be assigned to the N≡N stretching vibration of the benzene diazonium ion (**2**). At the B3LYP/cc-pVDZ level of theory this vibrational mode of **2** corresponds to the most intense absorption at 2350 cm⁻¹ (unscaled), and a red shift of 79 cm⁻¹ is expected upon ¹⁵N₂ labeling.^[21] In solid benzene diazonium chloride this absorption was observed at 2302 cm⁻¹, and the ¹⁵N₂ isotopomer exhibits a similar red shift of 75 cm⁻¹.^[22] The N≡N stretching vibration in matrix-isolated **2** is unaffected by H/D exchange within the phenyl moiety, again in perfect agreement with theoretical predictions.

Our results clearly indicate that the phenyl cation (**1**) has been isolated and spectroscopically characterized in the condensed phase for the first time. The IR data of **1** are in good agreement with DFT-calculated spectra for the ¹A₁ symmetrical singlet state, while predictions for the lowest triplet state (³B₁) are not supported by the experimental finding. Thus, the long-standing question of the ground-state multiplicity of **1** can now be experimentally answered.

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[1] a) H. Zollinger, *Diazochemistry I*, VCH, New York, **1995**; b) *Dicoordinated Carbocations* (Eds.: Z. Rappoport, P. J. Stang), Wiley, Chichester, **1997**.

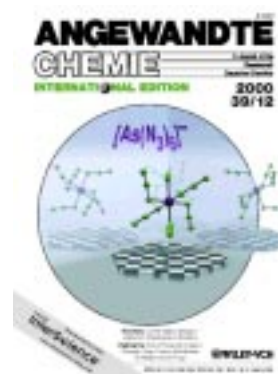
[2] W. A. Waters, *J. Chem. Soc.* **1942**, 266–270.

- [3] a) M. Hanack, W. Holweger, *J. Chem. Soc. Chem. Commun.* **1981**, 713–714; b) H. B. Ambroz, J. T. Kemp, *Chem. Soc. Rev.* **1979**, 8, 353–365 and ref. [1].
- [4] a) M. Speranza, Y. Keheyen, G. Angelini, *J. Am. Chem. Soc.* **1983**, *105*, 6377–6380; b) M. Speranza, *Chem. Rev.* **1993**, *93*, 2933–2980 and ref. [1].
- [5] a) J. D. Dill, P. von R. Schleyer, J. S. Binkley, R. Seeger, J. A. Pople, E. Haselbach, *J. Am. Chem. Soc.* **1976**, *98*, 5428–5431; b) R. Gleiter, R. Hoffmann, W.-D. Stohrer, *Chem. Ber.* **1972**, *105*, 8–23 and ref. [1].
- [6] a) J. Hrusak, D. Schröder, S. Iwata, *J. Chem. Phys.* **1997**, *106*, 7541–7549; b) M. Krauss, S. Roszak, *J. Mol. Struct. (THEOCHEM)* **1994**, *310*, 155–160.
- [7] A. Nicolaides, D. M. Smith, F. Jensen, L. Radom, *J. Am. Chem. Soc.* **1997**, *119*, 8083–8088.
- [8] a) C. G. Swain, J. E. Sheats, K. G. Harbison, *J. Am. Chem. Soc.* **1975**, *97*, 783–795; W. Maurer, I. Szele, H. Zollinger, *Helv. Chim. Acta* **1979**, *62*, 1079–1088; M. D. Ravenscroft, H. Zollinger, *Helv. Chim. Acta* **1988**, *71*, 507–514; M. D. Ravenscroft, P. Skrabal, B. Weiss, H. Zollinger, *Helv. Chim. Acta* **1988**, *71*, 515–520.
- [9] J. G. Radziszewski, M. R. Nimlos, P. R. Winter, G. B. Ellison, *J. Am. Chem. Soc.* **1996**, *118*, 7400–7401.
- [10] K. Laali, I. Szele, H. Zollinger, *Helv. Chim. Acta* **1983**, *66*, 1737–1747.
- [11] S. Steenken, M. Ashokkumar, P. Maruthamuthu, R. A. McClelland, *J. Am. Chem. Soc.* **1998**, *120*, 11925–11931.
- [12] M. Speranza, M. D. Sefcik, J. M. S. Henis, P. G. Gaspar, *J. Am. Chem. Soc.* **1977**, *99*, 5583–5589.
- [13] V. Butcher, M. L. Costa, J. M. Dyke, A. R. Ellis, A. Morris, *Chem. Phys.* **1987**, *115*, 261–267.
- [14] a) C. A. Wight, B. S. Ault, L. Andrews, *J. Chem. Phys.* **1976**, *65*, 1244–1249; b) L. Andrews, B. W. Keelan, *J. Am. Chem. Soc.* **1981**, *103*, 99–103; c) B. J. Kelsall, L. Andrews, *J. Am. Chem. Soc.* **1983**, *105*, 1413–1419; for a review of earlier work, see d) L. Andrews, *Ann. Rev. Phys. Chem.* **1979**, *30*, 79–101.
- [15] Spectral properties of the argon resonance radiation are described in L. Andrews, D. E. Tevault, R. R. Smardzewski, *Appl. Spectrosc.* **1978**, *32*, 157–160.
- [16] D. F. McMillen, D. M. Golden, *Ann. Rev. Phys. Chem.* **1982**, *33*, 493–532.
- [17] “Gas-Phase Ion and Neutral Thermochemistry”: S. G. Lias, J. E. Bartmess, J. F. Liebman, J. L. Holmes, R. D. Levin, W. G. Mallard, *J. Phys. Chem. Ref. Data* **1988**, *17* (Suppl. No. 1).
- [18] The energy considerations given are rough estimates for the gas phase. In the presence of argon the ionization energies should be lowered significantly due to stabilizing interactions of ions with the polarizable medium.
- [19] DFT calculations were carried out using the Gaussian 98 program suite: M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, *Gaussian 98*, Gaussian Inc., Pittsburgh, PA, **1998**.
- [20] There has been some controversy about this reaction; a short overview is given in P. von R. Schleyer, A. J. Kos, K. Raghavachari, *J. Chem. Soc. Chem. Commun.* **1983**, 1296–1299.
- [21] The frequency of the N≡N stretching vibration in **2** is relatively high compared, for example, to that of the isoelectronic borabenzene–N₂ adduct (2198 cm⁻¹): G. Maier, H. P. Reisenauer, J. Henkelmann, C. Kliche, *Angew. Chem.* **1988**, *100*, 303; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 295–296. A detailed discussion of the bonding properties in **2** is given in R. Glaser, C. J. Horan, *J. Org. Chem.* **1995**, *60*, 7518–7525.
- [22] L. S. Gray, Jr., V. A. Fassel, R. N. Kniseley, *Spectrochim. Acta* **1960**, *16*, 514–517.
- [23] R. G. V. Schettino, N. Brigot, *Chem. Phys.* **1978**, *34*, 391–398.

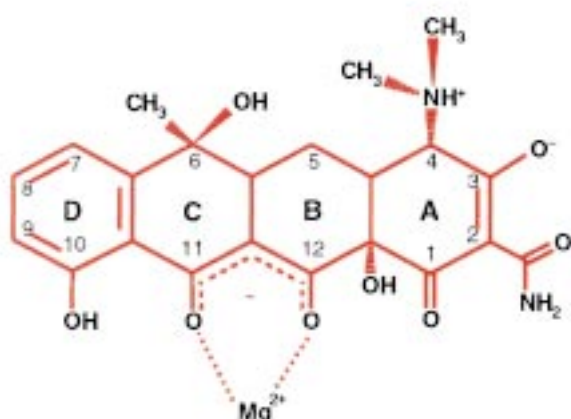
Cover Picture

**Thomas M. Klapötke Heinrich Nöth, Thomas Schütt,
and Marcus Warchhold**

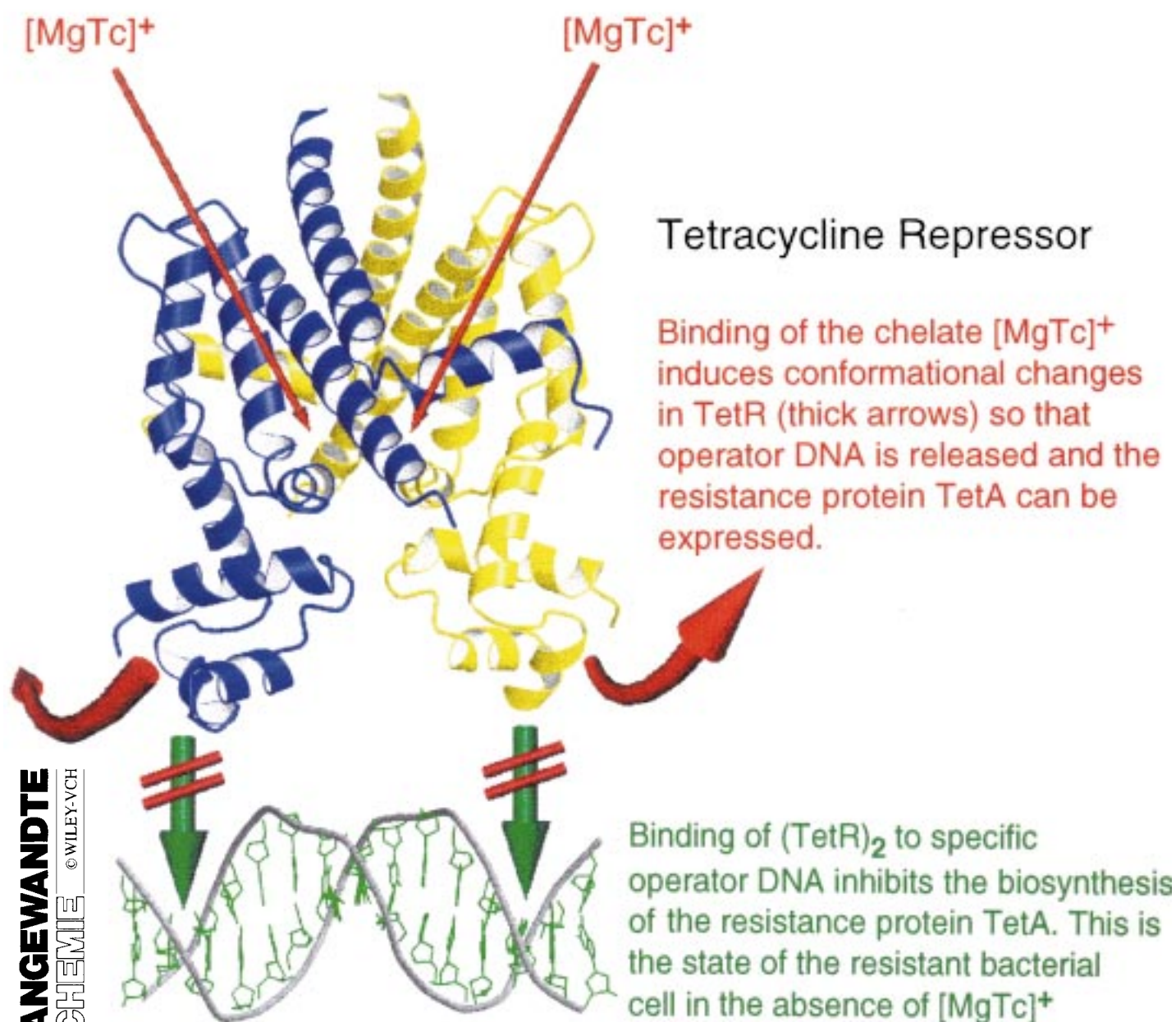
The cover picture shows the X-ray crystallographically determined structure of the hexaazidoarsenate(v) anion, which was produced by the simple reaction of trimethylsilylazide and $[\text{PPh}_4]^+[\text{AsCl}_6]^-$ and can be isolated in the form of the $[\text{PPh}_4]^+$ salt. This is the first X-ray structural analysis of an arsenic azide. The extremely nitrogen-rich anion is almost S_2 symmetric in the crystal. Unexpectedly, the compound obtained shows little inclination to explode. More information about these interesting compounds is reported by T. M. Klapötke et al. on page 2108 ff.



Resistance against the antibiotic tetracycline (Tc) is tightly regulated in gram-negative bacteria by two states of the dimeric tetracycline repressor (TetR)₂: bound to operator DNA and, in the "induced" form, bound to $[\text{MgTc}]^+$.



If Tc enters the (bacterial) cell, it binds Mg^{2+} to form the biologically active chelate $[\text{MgTc}]^+$.



The Tetracycline Repressor—A Paradigm for a Biological Switch

Wolfram Saenger,* Peter Orth, Caroline Kisker, Wolfgang Hillen, and Winfried Hinrichs

The excessive use of antibiotics has enabled bacteria to develop resistance through a variety of mechanisms. The most common bacteriostatic action of the broad-spectrum antibiotic tetracycline (Tc) is by the inactivation of the bacterial ribosome so that the protein biosynthesis is interrupted and the bacteria die. The most common mechanism of resistance in gram-negative bacteria against Tc is associated with the membrane-intrinsic protein TetA, which exports invaded Tc out of the bacterial cell before it can attack its target, the ribosome. The expression of TetA is tightly regulated by the homodimeric Tet repressor (TetR)₂, which

binds specifically with two helix-turn-helix motifs of operator DNA (*tetO*; $K_{\text{ass}} \approx 10^{11} \text{ M}^{-1}$) located upstream from the *tetA* gene on a plasmid or transposon. When Tc diffuses into the cell it chelates Mg^{2+} and the complex $[\text{MgTc}]^+$ binds to (TetR)₂ to form the induced complex (TetR·[MgTc])₂. This process is associated with conformational changes, which sharply reduce the affinity of (TetR)₂ to *tetO*, so that expression of TetA can take place, thus conferring resistance to the bacteria cells against Tc. Crystallographic studies show sequence-specific protein–nucleic acid interactions in the (TetR)₂·*tetO* complex and how the

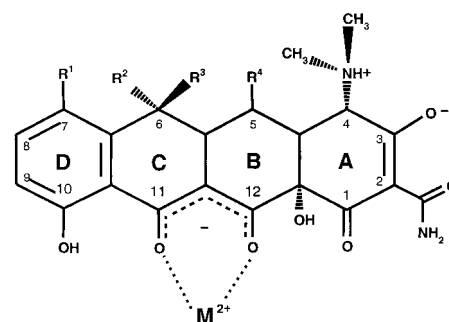
binding of two $[\text{MgTc}]^+$ to (TetR)₂ enforces conformational changes that are stabilized by cooperative binding of two chains of eight water molecules each so that the formed (TetR·[MgTc])₂ is no longer able to recognize and bind to *tetO*. Since the switching mechanisms of the TetR/[MgTc]⁺ system is so tight, it has proven very useful in the regulation of eukaryotic gene expression and may also be applicable in gene therapy.

Keywords: antibiotics • DNA recognition • resistance • tetracycline

1. Introduction: Regulation of Tetracycline Resistance

The ability of bacteria to respond favorably to their environment and to survive under hostile conditions has raised severe problems in contemporary pharmacology and medicine.^[1–3] This situation has arisen because almost all pathogenic bacteria have acquired resistances against the presently known and widely used antibiotics. The resistances are associated with proteins encoded by genes located on moveable genetic elements, such as transposons or resistance

(R) factors,^[4–6] which can be rapidly exchanged and efficiently distributed among bacteria. One of the best known examples of bacterial resistance is directed against the broad-spectrum antibiotic tetracycline (Tc) and its chemically modified analogues (Scheme 1).



Scheme 1. Chemical structure of tetracycline (Tc). In the therapeutically used Tc, substituents may be exchanged as follows: $\text{R}^1 = \text{H}, \text{Cl}, \text{N}(\text{CH}_3)_2$; $\text{R}^2 = \text{CH}_3, \text{H}$; $\text{R}^3 = \text{OH}, \text{H}$; $\text{R}^4 = \text{H}, \text{OH}$. Under physiological conditions a divalent metal ion (M^{2+} ; preferentially Mg^{2+}) is chelated by the deprotonated keto-enol group O11/O12[−] to form $[\text{MgTc}]^+$.

After Tc has diffused through the cytoplasmatic membrane into bacteria of different species it chelates a divalent cation M^{2+} , preferentially Mg^{2+} . The complex $[\text{MgTc}]^+$ (Scheme 1)

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Wolfram Saenger was born 1939 in Frankfurt (Germany). He graduated from the Technische Hochschule Darmstadt in Chemistry and received his Ph.D in 1965 for studies on the kinetics of cyclodextrin inclusion formation with F. Cramer. In his two years postdoctoral work at Harvard University with J. Z. Gougoutas he became acquainted with X-ray crystal structure analysis and joined F. Cramer at the Max-Planck-Institut für experimentelle Medizin in Göttingen. There he set up an independent research group working on structure analyses of oligosaccharides, proteins, and nucleic acids. In 1972, he habilitated at the Universität Göttingen, and since 1981, he has held the chair for crystallography at the Freie Universität Berlin. In 1987, he received the Leibniz Award and in 1989 the Humboldt Award. Besides structural biology, his current interests are in hydrogen bonding and protein aggregation that lead to crystallization.

Peter Orth was born in 1969 in Berlin (Germany). He studied Chemistry at the Lomonossow University in Moscow until 1994. During his Ph.D. he elucidated the mechanism of regulation of

the tetracycline repressor with crystallographic methods in the group of W. Saenger at the Freie Universität Berlin and obtained his Ph.D. in 1998. He proceeded to work in W. Saengers group on the structural characterization of the membrane protein, photosystem II.

Caroline Kisker was born in 1964 in Berlin (Germany). She studied Biochemistry at the Freie Universität Berlin and pursued her Ph.D. thesis in the group of W. Saenger with the structural characterization of the tetracycline repressor in the complex with its inducer tetracycline. For this work she obtained the Karl Ramsauer Award. After completion of her Ph.D. thesis in 1994, she joined D. C. Rees' laboratory at the California Institute of Technology in Pasadena. She elucidated the structure of carbonic anhydrase and studied the sulfite oxidase deficiency at the atomic level. For the last two years she has worked as Assistant Professor at the State University of New York at Stony Brook where she pursues crystallographic and biochemical studies of DNA repair enzymes and enzymes containing the molybdenum cofactor.

Wolfgang Hillen was born in 1948 in Osnabrück (Germany). He studied chemistry at the university of Münster and received his Ph.D in 1977 for a thesis on codon–anticodon interaction with H.-G. Gassen. He was a postdoctoral fellow with R. D. Wells from 1978 until 1980 at the University of Wisconsin, Madison, where he studied sequence-dependent tertiary structures of DNA. He returned to the Technische Hochschule Darmstadt where he earned his Habilitation in 1983 with work on the molecular mechanisms of gene regulation. In 1984 he was appointed as Associate Professor of Physical Biology at the University of Erlangen-Nürnberg. He received the Carl Dülsberg Award of the GDCh in 1986 and the Max Planck Award in 1997. His current research interests are molecular mechanisms of gene regulation, protein–ligand interactions, signaling between physiological parameters and gene regulators, and allosterical conformational changes of proteins.

Winfried Hinrichs was born 1950 in Hamburg (Germany). After training as a laboratory assistant, he studied chemical engineering (1970–1973), then chemistry (1976–1980), and received his Ph.D. in 1983 at the University of Hamburg with a thesis on organic semiconductors (group of G. Klar). He spent a postdoctoral time at the Rijksuniversiteit te Leyden (The Netherlands) and joined the group of W. Saenger in 1986 at the Institut für Kristallographie in the Freie Universität Berlin (Habilitation 1996). After a one year sabbatical in the group of D. Moras in Strasbourg (Université Louis Pasteur, IGBMC) he became professor for biochemistry at the Ernst-Moritz-Arndt-Universität Greifswald in 1999. His scientific work is dominated by X-ray crystal structure analyses with the main interests focused on structure–function relationships of biological macromolecules.

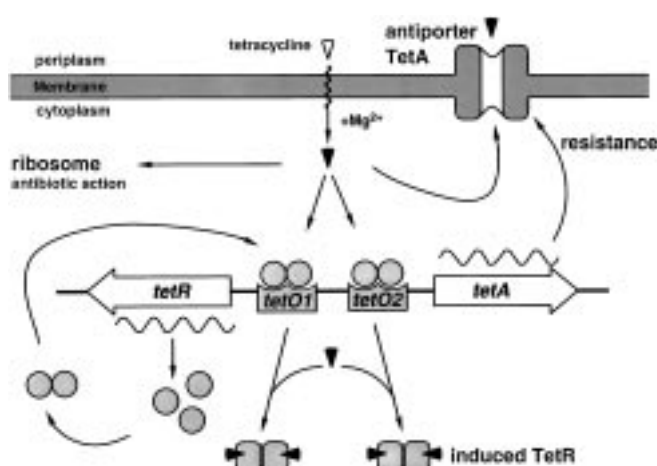
acts by binding to the small (30S) prokaryotic ribosomal subunit ($K_{\text{ass}} \approx 10^6 \text{ M}^{-1}$), thereby inhibiting protein biosynthesis and stalling the growth of the invaded cell.^[7, 8] The presently known resistance mechanisms^[6] against the Tc-type antibiotics involve proteins that either protect the ribosome from attack by $[\text{MgTc}]^+$,^[9, 10] or export invaded Tc out of the bacterial cell.^[11–14] Chemical modification of Tc by resistance proteins has also been demonstrated, but this plays no significant role in resistance.^[15] The export mechanism is most abundant among gram-negative bacteria. It relies on the transport protein TetA, which is embedded in the cytoplasmic membrane and acts as an antiporter by coupling the export of $[\text{MgTc}]^+$ out of the resistant bacterial cell with the uptake of H^+ .^[16, 17] The expression of TetA is tightly regulated by the tetracycline repressor (Tet Repressor, TetR); TetA would be disadvantageous for the cell in the absence of $[\text{MgTc}]^+$, since it interferes with the maintenance of the electrostatic potential across the cell membrane.^[18]

1.1. The Tet Repressor (TetR) and Its Induction by $[\text{MgTc}]^+$

The tetracycline repressor occurs as the homodimer $(\text{TetR})_2$ in which two identical helix-turn-helix (HTH) motifs bind in the absence of $[\text{MgTc}]^+$ to two adjacent major grooves of DNA. $(\text{TetR})_2$ has only a low affinity to nonspecific DNA sequences but binds tightly ($K_{\text{ass}} \approx 10^{11} \text{ M}^{-1}$) to two specific DNA operator sequences, *tetO1* and *tetO2*, which have twofold (palindromic) symmetry of the nucleotide sequence,^[18, 19] to form the complex $[(\text{TetR})_2 \cdot \text{tetO}]$ (here and in the following sections, *tetO* means *tetO1* and/or *tetO2*). This process blocks the transcription of the gene *tetR* (dependent on *tetO1*), which encodes for TetR itself, and of the gene *tetA* (dependent on *tetO1* and *tetO2*), which encodes for the resistance protein TetA (Schemes 2 and 3 A). If Tc diffuses into a resistant bacterial cell carrying the genes *tetR* and *tetA* a chelate $[\text{MgTc}]^+$ is formed with Mg^{2+} . This chelate binds to $[(\text{TetR})_2 \cdot \text{tetO}]$ ($K_{\text{ass}} \sim 10^9 \text{ M}^{-1}$)^[20] and enforces conformational changes in $(\text{TetR})_2$ that weaken its affinity to *tetO* in two steps, depending on conditions: 10^2 – 10^3 -fold for the first and 10^4 – 10^7 -fold for the second bound $[\text{MgTc}]^+$ so that the overall affinity of $(\text{TetR})_2$ to *tetO* is reduced by 6–10 orders of magnitude.^[19] The “induced” complex $(\text{TetR} \cdot [\text{MgTc}]^+)_2$ formed between $(\text{TetR})_2$ and two inducers $[\text{MgTc}]^+$ releases *tetO*, so that expression of the genes *tetR* and *tetA* can now proceed. The antiporter protein TetA is inserted into the cytoplasmic membrane and exports $[\text{MgTc}]^+$, thereby preventing attack of $[\text{MgTc}]^+$ at the ribosomal 30S subunit, which has a 1000-fold lower affinity to $[\text{MgTc}]^+$ than $(\text{TetR})_2$.

1.2. The Tet Repressor is a Unique Biological Switch

The high affinity of $(\text{TetR})_2$ to the DNA operators *tetO1* and *tetO2* is sharply reduced upon the binding of inducer $[\text{MgTc}]^+$ to $(\text{TetR})_2$. This well-defined switching between the two states relevant for regulation of gene expression is outstanding for inducible prokaryotic repressors. This is why



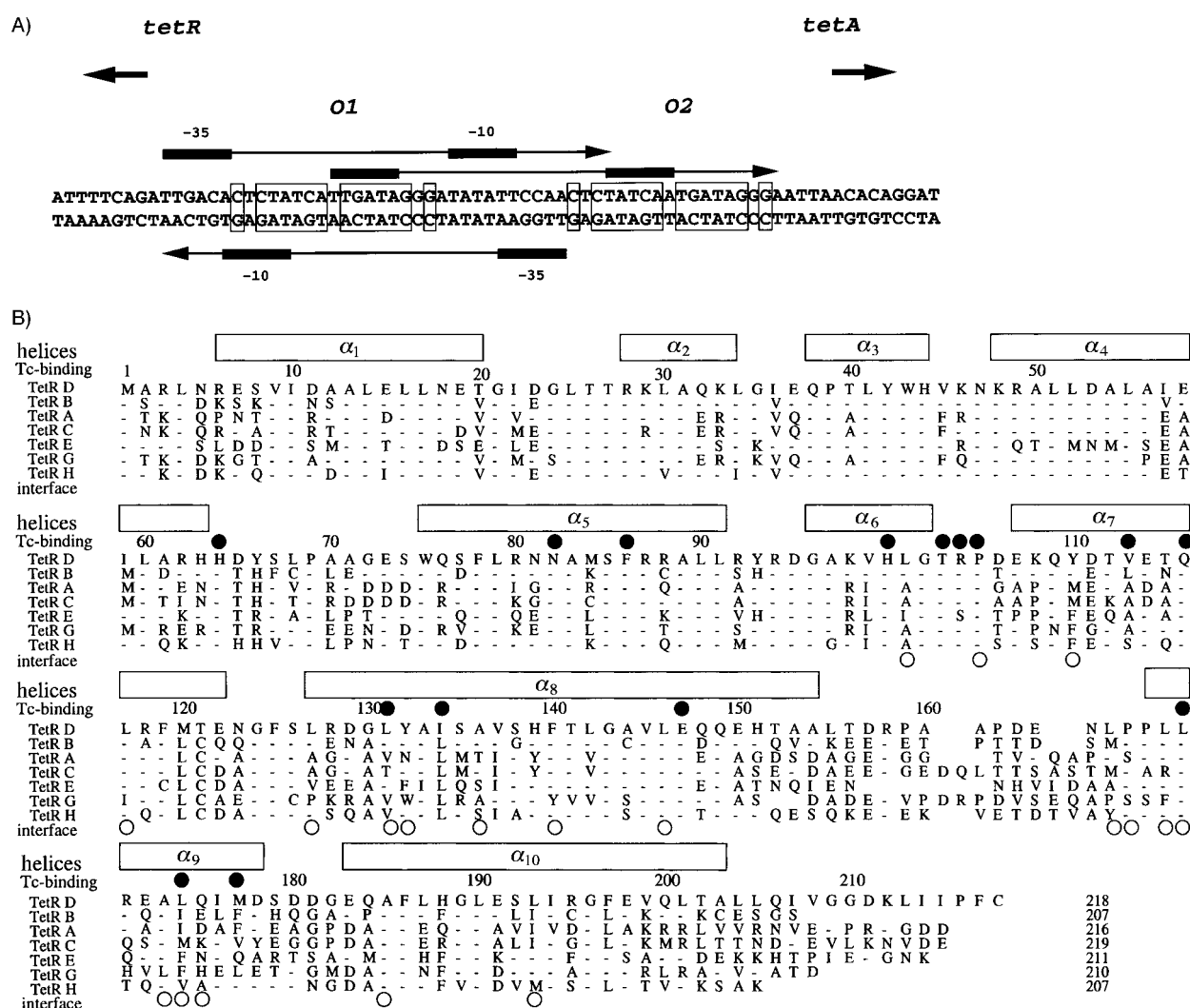
Scheme 2. Schematic description of tetracycline (Tc) resistance. Tet repressor (TetR, depicted as gray spheres) binds as a homodimer to operators *tetO1* and *tetO2*. Tc (open triangle) diffuses into the (bacterial) cell and forms a complex with a divalent cation (Mg^{2+}) to produce $[\text{MgTc}]^+$ (black triangles). If $[\text{MgTc}]^+$ binds to $[(\text{TetR})_2 \cdot \text{tetO}]$, it enforces a conformational change and the “induced” $(\text{TetR} \cdot [\text{MgTc}]^+)_2$ complexes release the operators so that transcription of genes *tetR* (coding for TetR) and *tetA* (coding for the resistance protein TetA) can now proceed (wavy lines represent mRNA). After translation, TetA is inserted into the cytoplasmic membrane and exports invaded $[\text{MgTc}]^+$ out of the cell so that it cannot attack the ribosome. The export of $[\text{MgTc}]^+$ is coupled with the influx of H^+ .

$(\text{TetR})_2/[\text{MgTc}]^+$ is utilized by molecular biologists as an easily controllable switch for the regulation of gene expression in transgenic organisms.^[21–23] X-ray crystal structures of $(\text{TetR})_2$ and of its complexes with several chemically modified $[\text{MgTc}]^+$ and with oligonucleotide duplexes carrying the operator DNA (*tetO1*) sequence have been determined to elucidate the molecular mechanism of TetR regulation at the atomic level.^[24–28] These results form the basis of the following discussion.

2. Biochemical Background: The Tc and TetR Families and the DNA Operators *tetO*

Of the four six-membered rings A–D in the Tc skeleton, only D is aromatic (see Scheme 1). On the “south” side of the Tc molecule there are five hydroxyl/keto groups in a suitable arrangement to coordinate metal ions.^[27] The 1,3-keto-enol groups O11/O12 are deprotonated at physiological pH conditions, and have a high affinity for divalent cations. The binding of Mg^{2+} in aqueous solution ($K \approx 10^3 \text{ M}^{-1}$) suggests that the complex carrying one net positive charge, namely $[\text{MgTc}]^+$, is involved in all relevant biological processes.^[29]

At present, seven highly homologous resistance determinants of the TetR-type are known. They are grouped into the classes A to E, G, and H (Scheme 3 B) and share 45 % to 75 % amino acid sequence identity. As expected, the highest identity/homology is found in positions known to be engaged in binding to the inducer $[\text{MgTc}]^+$ and to the DNA operator. The high sequence identity of amino acids suggests that the seven TetR share the same overall fold with ten α -helices and no β -strands, as deduced from crystallographic studies of TetR



Scheme 3. A) Nucleotide sequences of operators *tetO1* and *tetO2* for the class TetR^D taken from the resistance factor RA1. The boxed operator sequences are palindromic, with one extra base pair A-T or T-A inserted at the position of the twofold symmetry axis. The promoter of *tetA* is indicated above the sequence, that of *tetR* below; the binding regions for RNA polymerase (TATA- and GC-rich boxes) are indicated by thick lines numbered –10 and –35, respectively. The directions for transcription are marked by thick arrowheads. B) Alignment of the seven amino acid sequences of TetR classes A–E, G, and H. Hyphens (–) indicate amino acids identical to those in the top line (TetR^D), every tenth amino acid is numbered. The α -helices α_1 – α_{10} are indicated on top of the sequences. Amino acids marked ● in the line “Tc binding” are in contact with the inducer [MgTc⁺] in the complex (TetR · [MgTc⁺]₂); these amino acids are conserved or type-conserved. Open circles (○) at the bottom line (“interface”) show amino acids involved in monomer–monomer contacts in (TetR^D)₂. The helix-turn-helix (HTH) motif binding to operator DNA (*tetO*) is formed by α_2 and α_3 .

classes B and D, TetR^B and TetR^D, respectively.^[24–28] There is only one segment, between helices α_8 and α_9 , where deletions/insertions are observed and the C-terminus varies; the overall length of the repressor varies from 207 to 218 amino acids. A number of random and site-directed point mutations produced variants of TetR that have contributed to our understanding of the function of these proteins, and these will be discussed in Section 5.

The two DNA operators *tetO1* and *tetO2* are palindromic with a central base-pair inserted on their symmetry axes (see Scheme 3 A). The associated genes *tetR* and *tetA* are arranged in opposite polarity and overlapped by promoters for *tetA* and *tetR* transcription. The distance between the symmetry axes of *tetO1* and *tetO2* depends on the regulon in the different transposons and R factors and varies between 27 and 36 base pairs. As shown by footprint analyses, the recognition helices

α_3 and α_3' of the homodimeric (TetR)₂ insert into two adjacent major grooves on *tetO1* and *tetO2*.^[31, 32]

3. Crystallographic Studies Correlate the Structure and Function of TetR

3.1. Architecture of the (TetR^D)₂ Homodimer

The X-ray crystal structure analyses of the (TetR^D)₂ homodimer in a complex with operator DNA and bound in the induced form as a complex with two molecules of [MgTc]⁺ have shown that the polypeptide chains do not consist of the 218 amino acids corresponding to the nucleotide sequence of the *tetR^D* gene, but that the N-terminal Met1 group and the ten amino acids of the C-terminus are missing (confirmed by

N- and C-terminal sequencing and matrix-assisted laser-desorption/ionization (MALDI) mass spectrometry). These two deletions occur either posttranslationally (Met1) or during preparation (ten C-terminal amino acids) and have no effect on the biological activity of TetR^D.

The two monomers of the (TetR^D)₂ homodimer are identical, each folded into ten α -helices: α_1 – α_{10} for one and α_1' – α_{10}' for the other monomer (Figure 1). The monomers are

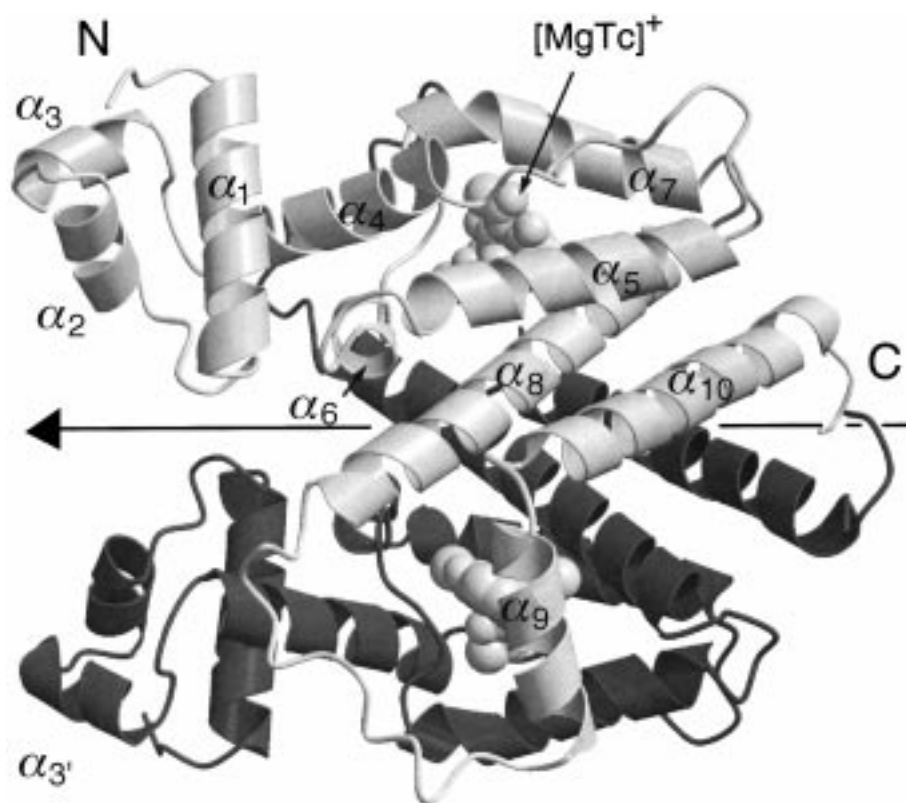


Figure 1. Schematic representation of the (TetR^D)₂ homodimer binding two inducers [MgTc]⁺ to form the induced complex (TetR · [MgTc]⁺)₂. The two monomers of TetR, one depicted in light gray and the other in dark gray, are related by a twofold rotation axis (horizontal arrow). The α -helices are represented by spirals and labeled α_1 to α_{10} . The termini of the polypeptide chain in light gray are marked N and C. The two [MgTc]⁺ are shown in a space-filling representation. The DNA binding domains α_1 – α_3 and α_1' – α_3' are located on the left. They are of the common helix-turn-helix (HTH) type, with α_3 and α_3' the DNA recognition helices; their midpoints are separated by 39.6 Å and only after removal of [MgTc]⁺ are they able to approach by about 3 Å so that they can insert into adjacent major grooves of the operator DNA, *tetO* (see Figure 2A). The large regulatory core domain of (TetR)₂ is located on the right, formed by α_5 – α_{10} and α_5' – α_{10}' , and harbors the two [MgTc]⁺ binding tunnels. The core domain is connected to the DNA-binding domains through helices α_4 and α_4' .

related by a twofold rotation axis coinciding with the long dimension of the (TetR^D)₂ dimer. The (TetR^D)₂ molecule is clearly divided into two N-terminal DNA-binding domains and a regulatory core with globular shape.

The DNA-binding domains consist of the N-terminal three-helix-bundles ($\alpha_1/\alpha_2/\alpha_3$ and $\alpha_1'/\alpha_2'/\alpha_3'$) of which α_2 and α_2' are the supporting helices and α_3 and α_3' are the recognition helices of classical helix-turn-helix (HTH) motifs.^[33] The center-to-center separation of the recognition helices α_3 and α_3' , which determines the ability of (TetR^D)₂ to bind to *tetO*, is controlled by helices α_4 and α_4' (amino acid residues 48–63); they link the DNA-binding domains to the [MgTc]⁺ binding sites in the regulatory core domain (see Sections 3.3.3 and 4).

The central part of the regulatory core domain is a four-helix-bundle consisting of the antiparallel helices α_8 and α_{10} , which cross the dyad related helices α_8' and α_{10}' at an angle of approximately 50°.^[25] If the three-dimensional structures of free (TetR^D)₂,^[26] the complex (TetR^D · [MgTc]⁺)₂, and the complex [(TetR)₂ · *tetO*] are superimposed, then these four α -helices as well as α_5 and α_5' are identified as the most rigid and structurally best-conserved part, which constitutes a scaffold

that determines the architecture of the core domain of (TetR^D)₂. The core domain harbors two tunnelliike [MgTc]⁺ binding cavities formed by α_5 – α_8 , α_8 , and α_9 (and their symmetry-related counterparts α_5' – α_8' , α_8' , and α_9' for the other tunnel).^[26]

In the regulatory core domain, both monomers are clearly separated in a hand-shake motif. Only α_9 wraps around the “other” monomer at the periphery of (TetR^D)₂ and closes the entrance of the [MgTc]⁺ binding tunnel, like a sliding door, in the final step of the recognition and binding process. The two [MgTc]⁺ in the induced repressor (TetR · [MgTc]⁺)₂ are completely buried in the binding tunnels of (TetR)₂ far away (at ≈ 33 Å) from the DNA-recognition helices α_3 and α_3' .

3.2. Structure of the Complex Formed between (TetR)₂ and the Operator DNA

(TetR^D)₂ was co-crystallized with different oligonucleotide duplexes carrying the specific DNA operator sequence *tetOI*.^[34] Crystal structure analyses of complexes containing *tetOI* fragments with blunt ends (13 and 15 base pairs long) or one-nucleotide overhangs (18mer) provide the following picture.^[35]

The *tetO* operator in the complex with (TetR^D)₂ is in the B-DNA conformation and the overall twofold rotation symmetry is retained for both components (Figure 2A). At the center (base pair 0) of the palindromic *tetO* the minor groove facing (TetR^D)₂ is widened and the major groove on the “back side” is correspondingly compressed from 11.7 Å in canonical “straight” B-DNA^[36] to 9.5–10 Å. At base pairs +2 and –2 the DNA is bent “away” from (TetR^D)₂ because at these positions the recognition helices α_3 and α_3' are inserted in the major grooves and widen them to about 14 Å. These distortions of the DNA double helix are compensated for along the trajectory of base pairs 3 to 6 and –3 to –6, respectively, by a bending of the DNA towards the protein so that the overall geometry of DNA appears to be “straight”.

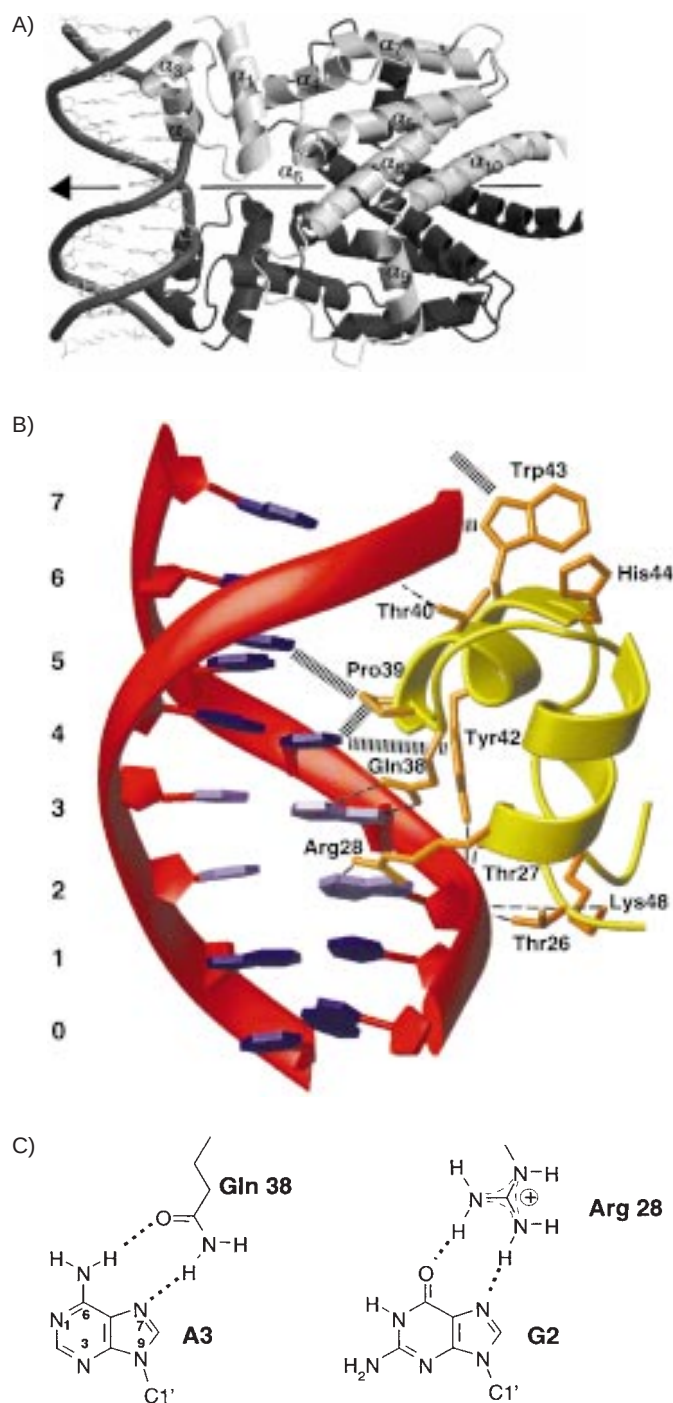


Figure 2. A) Crystal structure of the complex formed between (TetR^D)₂ and operator DNA *tetOI*. The α-helices of (TetR^D)₂ are indicated schematically by spirals (as in Figure 1) and *tetOI* by two tubes for the sugar–phosphate backbones with attached nucleosides. The complex has twofold rotation symmetry, indicated by the horizontal arrow. Note the distortion of the DNA double helix as shown by kinks in the sugar–phosphate backbone; see text. B) Detailed view of the interactions formed between one half of the operator *tetOI* and the HTH motif (α₂ and α₃). Note the specific hydrogen bonds (---) formed between conserved Arg28 (N-terminus of α₂), Gln38 (N-terminus of α₃), and bases G2 and A3, respectively, and the van der Waals contacts (|||||) between conserved Pro39, Tyr42, Trp43, and C5-methyl groups of bases T4 and T5. T4 is on one strand of the DNA double helix, T5 on the other. C) Illustration of the hydrogen bonds (••••) in the specific interactions Arg28–G2 and Gln38–A3. Some atoms of the purine ring A3 are numbered.

Sequence-specific contacts between amino acid side chains of the (TetR)₂ helix–turn–helix motifs and bases in adjacent major grooves of operator DNA involve G2, A3, and T4 in one strand of the double helix and T5 in the other (Figure 2B). The contacts are contributed by bidentate hydrogen bonding between side chains of Arg28 (on α₂) and Gln38 (on α₃) interacting with bases G2 and A3, respectively (Figures 2B, C), and by van der Waals contacts between side chains of Pro39 and Tyr42 (both on α₃) and the C5-methyl groups of bases T4 and T5. The affinity of *tetO* to (TetR)₂ is greatly reduced if these partners are exchanged for others, as shown by a series of genetic experiments.^[29] These sequence-specific interactions are augmented by a number of non-specific interactions between (TetR)₂ and the sugar–phosphate backbone of *tetO* involving Thr26 and Thr27 on α₂, Thr40 and Trp43 on α₃, and Lys48 at the N-terminus of α₄. Since all these amino acids are conserved among the seven TetR classes (Scheme 3B), the interactions illustrated in Figure 2B are likely to be identical for the entire *tetO*/(TetR)₂ system.

3.3. The Induced Complex (TetR^D·[MgTc]⁺)₂

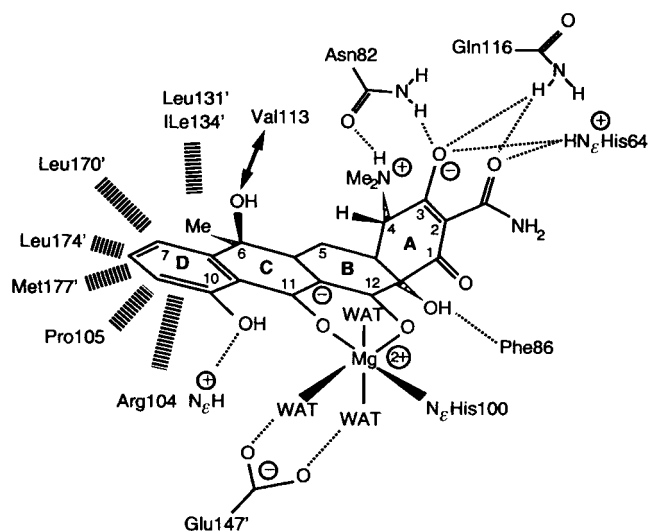
3.3.1. Binding the Inducer [MgTc]⁺ to (TetR^D)₂

If Tc invades a bacterial cell that is resistant to tetracycline, it binds as [MgTc]⁺ with high affinity ($K_{\text{ass}} \approx 10^9 \text{ M}^{-1}$) to (TetR^D)₂ in the complex formed between (TetR^D)₂ and operator DNA.^[30] This binding is accompanied by conformational changes in (TetR^D)₂ (see Section 3.3.2), which abolish the specific interactions between (TetR^D)₂ and *tetO* and reduce the binding affinity to operator DNA by 6–10 orders of magnitude.^[19] (TetR·[MgTc]⁺)₂ dissociates from *tetO* so that gene expression of *tetR* and *tetA* can proceed (Scheme 2).

The inducer [MgTc]⁺ binds to the two tunnelling cavities in the regulatory core domain of (TetR^D)₂.^[24–26] The tunnels have a narrower opening formed by α₅ and α₇ (α₅ and α₇) and a wider opening defined by the loop α₆–α₇ and by α₉ (α₆–α₇ and α₉).^[26] Crystallographic studies have shown that [MgTc]⁺ has to enter the wider opening head-on with ring A of Tc so that hydrogen bonds can form between the functional groups on ring A and the amino acids near the narrower opening of the tunnel (Scheme 4). After [MgTc]⁺ is fully inserted, α₉ (α₉) shifts laterally, like a sliding door, and closes the “entrances” of the tunnels.^[26] The complex (TetR·[MgTc]⁺)₂ is the “induced” form of (TetR^D)₂, which is unable to bind to operator DNA.

3.3.2. Binding of [MgTc]⁺ Triggers a Sequence of Conformational Changes in (TetR^D)₂

The amino acids of (TetR^D)₂, which are hydrogen bonded to ring A of Tc, are in comparable positions if the crystal structures of free (TetR^D)₂^[26] the complex [(TetR^D)₂·*tetO*],^[35] and the induced form (TetR^D·[MgTc]⁺)₂^[24–26] are superimposed. Larger structural changes are found with amino acids engaged in hydrophobic interactions, especially those located on α₉ (Leu170, Leu174, and Met177, see Scheme 4). The



Scheme 4. Schematic representation of the interactions between $(\text{TetR})_2$ and the inducer $[\text{MgTc}]^+$. Amino acids of the two TetR molecules are distinguished by ('), hydrogen bonds are indicated by dotted lines (---), and van der Waals contacts by thick dashed lines (|||||). Mg^{2+} is octahedrally coordinated by O11 and O12 of Tc, by His100- N_ϵ , and by three water molecules (WAT). Note the unusual $\text{O}-\text{H}\cdots\pi$ interaction between the OH group of Tc and the aromatic side chain of Phe86; the interaction between Val 113 and OH group at position 6 of Tc is less favorable (indicated by a double arrow).

major conformational changes leading to induction of $(\text{TetR}^{\text{D}})_2$, however, concern the coordination of Mg^{2+} .

In the absence of $[\text{MgTc}]^+$ the binding tunnel of $(\text{TetR}^{\text{D}})_2$ is filled with (disordered) water molecules that cannot be located in electron density maps. Helix α_6 is only two turns long, being formed by $^{96}\text{Gly-Ala-Lys-Val-His-Leu-Gly}^{102}$. In the seven classes of TetR these amino acids are conserved or type-conserved (Lys98, Val99, and Leu101; see Scheme 3B). Upon insertion of $[\text{MgTc}]^+$, α_6 is displaced by about 1.5 Å in the direction of its C-terminus, and the C-terminal turn of α_6 is peeled off to form the type II β -turn $^{100}\text{His-Leu-Gly-Thr}^{103}$ (Figure 3). This conformational change is triggered by direct coordination of His100- N_ϵ to Mg^{2+} and simultaneous formation of a hydrogen-bonding pattern between Thr103- O_γ , an aqua ligand of Mg^{2+} , and the peptide oxygen atom of His100. As a structural consequence, the loop connecting α_6 and α_7 is pushed by about 2.5 Å so that the methylene side chains of Arg104 and Pro105 form part of the hydrophobic cavity contacting rings C and D of Tc.^[26, 35] In addition, the N-terminus of α_7 is overwound so that the common 3.6_{13} α -helix is changed to a 3_{10} helix. The associated rearrangements of main-chain and side-chain atoms of the loop N-terminal to α_7 are required for the cooperative binding of the chain of water molecules described below.

3.3.3. A Cooperative Zipper of Eight Water Molecules Tightens α_4 in the Induced Position

The conformational changes that occur after induction of TetR^{D} by $[\text{MgTc}]^+$ and dissociate the complex $[(\text{TetR}^{\text{D}})_2 \cdot \text{tetO}]$ have also been verified by immunological and genetic studies.^[37, 38] Crystallographic analyses show that they are

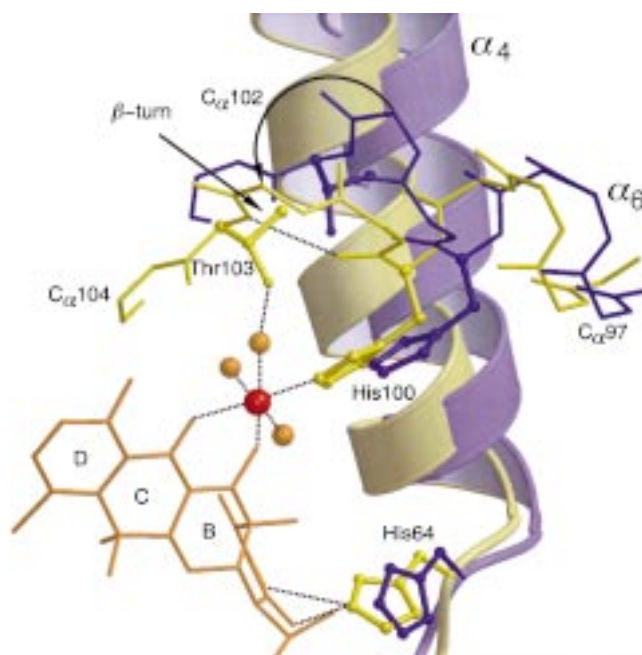


Figure 3. Comparison of the $[\text{MgTc}]^+$ binding site without the inducer (blue), showing the arrangement of some of the relevant amino acids, the two-turn-long α_6 (wire representation), and α_4 (continuous spiral) connecting the core domain of $(\text{TetR}^{\text{D}})_2$ with the DNA binding domain. If $[\text{MgTc}]^+$ enters the binding site to form the induced complex, the amino acids of TetR move into positions drawn yellow. Note the modifications at the C-terminus of α_6 where an α -helical turn is peeled off to form the type II β -turn (indicated by the straight arrow) and Gly102 flips over (shown by the curved arrow); in addition, α_6 is shifted by about 1.5 Å to the left (in the direction of the C-terminus).

stabilized by two zigzag chains of eight water molecules, one in each of the two monomers in the $(\text{TetR} \cdot [\text{MgTc}]^+)_2$ complex (Figure 4).^[28] The water molecules are linked by cooperative hydrogen bonds and tether α_4 , α_4 , and the DNA binding domains in a position that abolishes or prevents the specific interactions with operator DNA described in Figure 2A. The zigzag chain of water molecules is hydrogen bonded on one side to the peptide groups of Arg104 in the loop connecting α_6 and α_7 , as well as to the side chain of Gln109 on α_7 and the amide substituent at position 2 of Tc and, on the other side to the peptide oxygen atoms of groups 53, 56, 57, 60, and 64 of α_4 . These functional groups are thus stitched like a zipper. Compared with the $[(\text{TetR}^{\text{D}})_2 \cdot \text{tetO}]$ complex, where in the absence of $[\text{MgTc}]^+$ the functional groups of α_4 and the loop connecting α_6 and α_7 are in different positions, α_4 is now rotated approximately 5° about His64^[35] in a pendulumlike movement (see Figure 3). In this rotation, His64 acts as a C-terminal pivot, which is anchored tightly by $[\text{MgTc}]^+$ to the side chains of conserved amino acids Asn82 and Ser85, both located on the scaffold α_5 .

4. Regulation of $(\text{TetR}^{\text{D}})_2$ is Associated with an Induction-Triggered Rotation of DNA Binding Domains

Superposition of the three-dimensional structures of $(\text{TetR}^{\text{D}})_2$ in the complexes with operator DNA *tetO* and with the inducer $[\text{MgTc}]^+$ reveals the essential features of $(\text{TetR}^{\text{D}})_2$

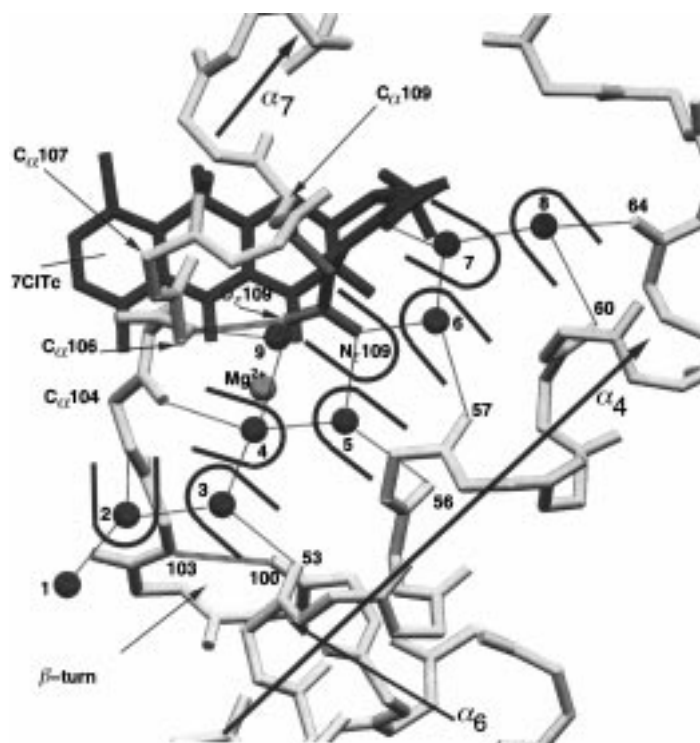


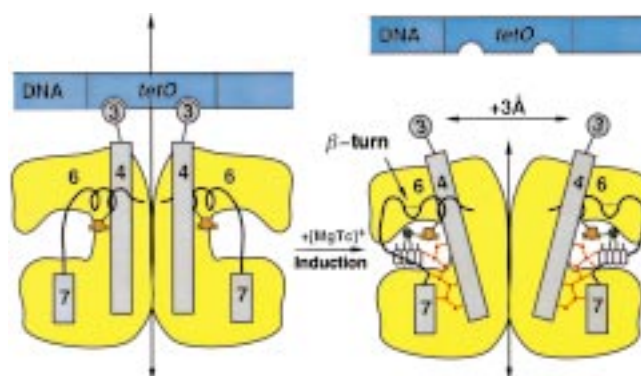
Figure 4. The orientation of α_4 in the induced complex $(\text{TetR}^{\text{D}} \cdot [\text{MgTc}]^+)_2$ is stabilized by a chain of eight cooperatively hydrogen-bonded water molecules (zipper) labeled 1–8 and marked by U shapes. The chain of water molecules is hydrogen bonded on one side to main-chain atoms of the loop segment connecting α_6 and α_7 (Arg104NH $\cdots$ Wat2 and Arg104O $\cdots$ Wat4, Gln109N $\cdots$ Wat5/Wat6, and at position 2 of $[\text{MgTc}]^+$ -amide-NH $_2 \cdots$ Wat7) and on the other side by hydrogen bonds to the peptide oxygen atoms of groups of 53, 56, 57, 60, and 64 of α_4 .

regulation as shown in Scheme 5. The regulatory core domains of these structures are nearly identical except for helices α_6 , α_6' , α_9 , and α_9' . The latter move and close the binding tunnels like sliding doors after insertion of $[\text{MgTc}]^+$, whereas the C-terminal helical turns of α_6 and α_6' are peeled off during induction to permit coordination to the Mg^{2+} ion of the two bound inducers $[\text{MgTc}]^+$. This process triggers rotation of helices α_4 and α_4' in the pendulumlike movement that shifts the attached DNA binding domains in opposite directions, following the dictate of the inherent twofold rotation symmetry of the $(\text{TetR}^{\text{D}})_2$ homodimer.

Helices α_4 and α_4' are tightly fixed in their positions in the induced complex $(\text{TetR}^{\text{D}} \cdot [\text{MgTc}]^+)_2$ by two cooperative chains of eight water molecules each. We assume that the latter provides the force to rotate α_4 and α_4' as well as the attached DNA-binding domains so that the distance between the midpoints of the DNA recognition helices α_3 and α_3' increases from 36.6 Å in the complex $[(\text{TetR}^{\text{D}})_2 \cdot \text{tetO}]$ to 39.6 Å in the induced complex $(\text{TetR}^{\text{D}} \cdot [\text{MgTc}]^+)_2$. Since this is too far to permit binding of α_3 and α_3' into adjacent major grooves of B-DNA, *tetO* is released and expression of genes *tetR* and *tetA* can proceed.

5. Point Mutations Support and Extend Structural Studies on TetR^B

A set of about 100 different point mutations of *tetR^B* was used to investigate the in vivo binding to operator *tetO1* and



Scheme 5. The structure–function relationship of $(\text{TetR}^{\text{D}})_2$ (yellow) in the complex with operator DNA *tetO* (left) and with inducer $[\text{MgTc}]^+$ (right). $(\text{TetR}^{\text{D}})_2$ is drawn schematically with helices α_3 , α_4 , and α_7 as cylinders and α_6 as a spiral. His100 is outlined in orange, Tc is indicated by four fused hexagons (right), Mg^{2+} by a green sphere, and the zipper formed by hydrogen-bonded water molecules in red. In $[(\text{TetR}^{\text{D}})_2 \cdot \text{tetO}]$ (left), α_3 and α_3' of $(\text{TetR}^{\text{D}})_2$ are bound to adjacent major grooves of *tetO*; α_6 is two turns long, α_4 and α_4' lie vertical. If two $[\text{MgTc}]^+$ enter the binding tunnels in $(\text{TetR}^{\text{D}})_2$ to form $(\text{TetR}^{\text{D}} \cdot [\text{MgTc}]^+)_2$ (right), His100-N $_e$ coordinates to Mg^{2+} , the C-terminal turns of α_6 and α_6' peel off to form β -turns. These changes trigger pendulumlike movements of α_4 and α_4' , which are stabilized in their final positions by the water zippers. These movements drive α -helices α_3 and α_3' approximately 3 Å apart so that *tetO* is released and gene expression may occur. Both complexes $[(\text{TetR}^{\text{D}})_2 \cdot \text{tetO}]$ and $(\text{TetR}^{\text{D}} \cdot [\text{MgTc}]^+)_2$ show twofold rotational symmetry indicated by the vertical arrows located between TetR^D monomers.

induction by $[\text{MgTc}]^+$.^[39, 40] The screening system consisted of *tetO1* inserted in the promoter region of the *lacZ* gene, which codes for β -galactosidase, so that binding/release of the $(\text{TetR}^{\text{B}})_2$ variants could be easily monitored by the activity of the expressed β -galactosidase. The amino acid substitutions detected were almost evenly distributed over the entire polypeptide chain of TetR^B except for the DNA-binding domain formed by α_1 , α_2 , and α_3 . This observation suggests that association of $(\text{TetR}^{\text{B}})_2$ with *tetO1* critically responds to any substitution of the amino acids in the DNA binding domain. Several other modifications of the polypeptide chain were accompanied by severe interference in the recognition and binding of $[\text{MgTc}]^+$. The variants of TetR^B showed that noninducible phenotypes may be associated with a) reduced affinity of the inducer $[\text{MgTc}]^+$ as a result of the amino acid substitutions in TetR^B and b) interference with the conformational changes required for induction.

The binding of $[\text{MgTc}]^+$ to $(\text{TetR}^{\text{B}})_2$ is reduced by several orders of magnitude if amino acids His64, Asn82, Phe86, His100, and Gln116, which are engaged in hydrogen bonding to $[\text{MgTc}]^+$ (see Scheme 4), are substituted by other amino acids, and consequently these variants are no longer inducible but they still bind to *tetO1*. By contrast, substitution of amino acids engaged in van der Waals contacts to $[\text{MgTc}]^+$, such as Arg104, Pro105, Leu113, and Leu117, only marginally reduces the affinity to $[\text{MgTc}]^+$, but may largely or even completely abolish induction, which suggests that the conformational changes associated with successful induction are inhibited or even blocked.

Another set of mutations was directed against a few amino acids, such as Asp23, Asp95, Lys98, and Glu150, which connect the regulatory core domain and the DNA-binding

domains through formation of hydrogen bonds. Substitution of these amino acids reduces, but does not abolish, inducibility and has no effect on the affinity of $(\text{TetR}^B)_2$ for $[\text{MgTc}]^+$. In a comparable approach to probe the monomer–monomer interface in $(\text{TetR}^B)_2$, substitution of the amino acids located on the four-helix bundle $\alpha_8/\alpha_{10}/\alpha_8/\alpha_{10}$ and directed towards the “other” monomer were investigated further. These variants show similar affinities to $[\text{MgTc}]^+$ as wild-type $(\text{TetR}^B)_2$ but still bind to *tetOI*, that is, the inducibility is largely abolished; the extent is dependent on the steric bulk of the side chains of the substituted amino acids.

These and other^[38] mutational studies on TetR^B not only give credit to the biological significance of the crystallographic work, but they extend considerably our understanding of the processes associated with induction by $[\text{MgTc}]^+$.

6. The Use of TetR as a Regulatable Switch in Eukaryotic Gene Expression

Transcription of DNA into complementary mRNA is controlled by promoters to which RNA polymerase binds in the presence of a defined number of transcription factors. Since “strong” promoters slow down bacterial growth, a switch was introduced, as in the common *tac* promoter, to initiate transcription at a point suitable to produce proteins in large quantities. This promoter consists of an optimized DNA sequence for enhanced binding of RNA polymerase and an operator DNA that is specific for the Lac repressor.^[41] Coexpression of the Lac repressor in a bacterium carrying the *tac* promoter switches off the expression of the gene located downstream of this promoter as it binds the Lac repressor, thereby preventing transcription. If the inducer isopropyl- β -D-thiogalactopyranoside (IPTG) is added, the Lac promoter is released from its operator and gene expression is switched on.

This system works well in prokaryotes, but eukaryotes require concentrations of IPTG several hundred times higher,^[42] which interferes with the growth of animal cells. These adverse effects can be avoided with TetR since the affinity for its inducer $[\text{MgTc}]^+$ is 1000-fold higher than that of IPTG for the Lac repressor,^[21, 22] and the concentration of $[\text{MgTc}]^+$ required for induction is so low that it is not toxic to the cell. Another advantage of Tc is that it can pass through the eukaryotic cell membrane without requiring transport molecules.

It proved possible to employ the TetR system for switching on and for switching off gene expression when $[\text{MgTc}]^+$ was added.^[22] In *switching on*, *tetO* was inserted in a promoter close to the TATA box (see Scheme 3 A) of a target gene so that binding of $(\text{TetR})_2$ blocks transcription until it is switched on by the addition of $[\text{MgTc}]^+$. In *switching off*, a fusion protein formed between TetR and an eukaryotic activator domain is bound in the absence of $[\text{MgTc}]^+$ to *tetO* located upstream of the TATA box of a target gene so that transcription is activated. If $[\text{MgTc}]^+$ is added, this fusion protein dissociates from *tetO* and transcription is switched off because the activator is now removed.

Another interesting use of TetR in gene regulation has been opened by the “reverse TetR”, which *requires* Mg^{2+} complexes of the Tc analogues anhydrotetracycline or doxycycline (in which the OH group at C6 is missing) for optimal, tight binding to *tetO*.^[43] In the “reverse TetR”, four amino acids are substituted, of which two, in positions 95 and 102, are conserved in the TetR family (see Scheme 3 B): Glu71 Lys, Asp95 Asn, Leu101 Ser, and Gly102 Asp. Since no crystal structures of “reverse TetR” have been determined so far, the molecular mechanisms underlying its phenotype are unknown. From the available structural information on TetR and its complexes with *tetO* and $[\text{MgTc}]^+$, however, it is clear that the Gly102 Asp substitution is of main importance. As shown in Section 3.3.2, the induction of TetR is associated with the formation of the type II β -turn ¹⁰⁰His-Leu-Gly-Thr¹⁰³. For steric reasons this β -turn can only form with Gly102, and not with Asp102, so that the “reverse TetR” is not inducible. In addition, the Asp95 Asn substitution supports this effect since a hydrogen bond between Asn95 and Glu23 may stabilize the relative orientations between α_1 (Glu23) and the regulatory domain (Asn95) so that induction is less favorable. The reason why the “reverse TetR” binds even tighter to *tetO* in the presence of anhydrotetracycline and doxycycline than in the absence of these Tc analogues remains elusive.

A fusion protein constructed of the “reverse TetR” and a transcriptional activator was constitutively expressed in HeLa cells. In the presence of anhydrotetracycline and doxycycline it bound tightly to *tetO* inserted upstream of a target gene, which was expressed over 1000-fold more than in the absence of this construct.^[43] This experiment has shown that “reverse TetR” and TetR will be suited for the regulation of gene expression even in transgenic animals and possibly in gene therapy.

A combination of genes for TetR and “reverse TetR” in the same cell line leads to the possibility of regulating two genes simultaneously;^[44] additional specialized constructs are being designed to keep the basal repression level of target genes at a minimum.^[45] A very valuable feature of the TetR system is that it shows practically no leaking, that is, the switching function is very precise. For this reason, TetR is frequently employed in the regulation of gene expression in transgenic organisms. At present, it is one of the best understood repressor–operator systems and serves as a solid basis for further studies probing into structure–function relationships.

7. Summary and Outlook

A series of crystallographic studies on the system $(\text{TetR}^D)_2/\text{tetO}/[\text{MgTc}]^+$ have disclosed how the binding of two inducer molecules $[\text{MgTc}]^+$ to the $(\text{TetR})_2/\text{tetO}$ complex leads to the release of *tetO* so that transcription of the genes *tetR* and *tetA* can proceed. The key to the events, which reduce the high affinity of the $(\text{TetR}^D)_2/\text{tetO}$ complex ($K_{\text{ass}} \approx 10^{11} \text{ M}^{-1}$) by about ten orders of magnitude, is the insertion of $[\text{MgTc}]^+$ into the two specific binding tunnels in $(\text{TetR}^D)_2$. This process is followed by direct coordination of Mg^{2+} to the His100-N ϵ atom, which triggers a number of conformational changes in $(\text{TetR}^D)_2$ and culminates in a pendulumlike rotation of helices

α_4 and α_4' to which the DNA binding domains are attached; they move about 3 Å apart, thereby releasing *tetO*. The force that stabilizes this particular conformation is provided in part by the formation of two cooperative chains of eight water molecules each (zipper), which form 2×17 hydrogen bonds. This would certainly provide sufficient energy to drive the conformational changes of $(\text{TetR} \cdot [\text{MgTc}]^+)_2$ that causes the release of *tetO*, but the number of hydrogen bonds has to be taken with caveat as these are not the only hydrogen bonds that are formed or modified during the induction of $(\text{TetR}^D)_2$.

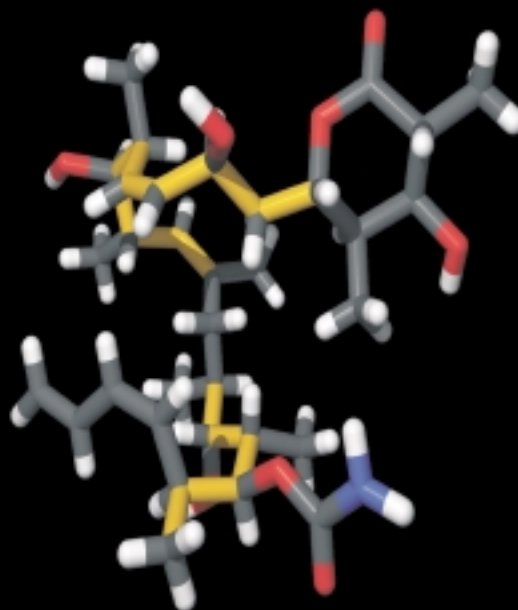
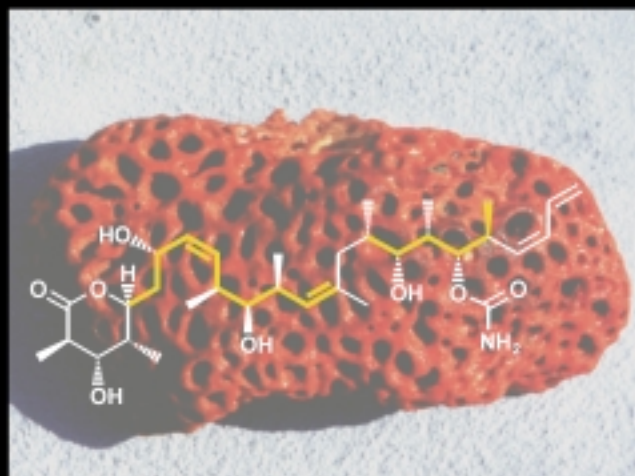
The $(\text{TetR})_2/\text{tetO}/[\text{MgTc}]^+$ system is unique because the tight, leakage-free repression of the *tetA* and *tetR* genes is abandoned even at low, subtoxic concentrations of $[\text{MgTc}]^+$. This favorable property has been utilized in transgenic organisms to regulate the expression of certain target genes. If fusion proteins between TetR and transcriptional activators are employed, $(\text{TetR})_2$ can also be used to switch gene expression on and to switch it off if $[\text{MgTc}]^+$ is added. Another variation on this theme is the "reverse TetR", which binds to *tetO* only in the presence of $[\text{MgTc}]^+$. With TetR, a versatile tool has been found that has already been applied successfully for gene regulation in a number of transgenic plants and laboratory animals.^[46] It has even opened future avenues in gene therapy since Tc is nontoxic to the mammalian cell and extensive documentation exists concerning its safety in its pharmacological application to humans.

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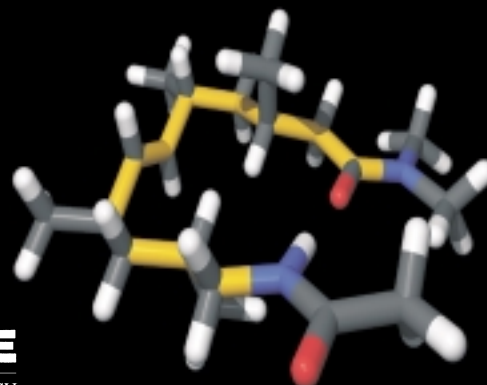
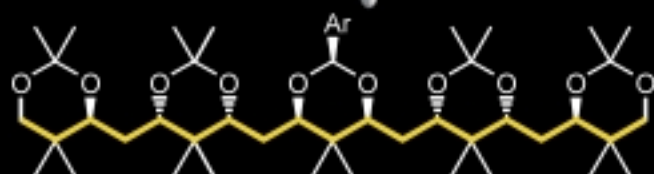
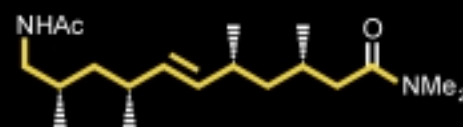
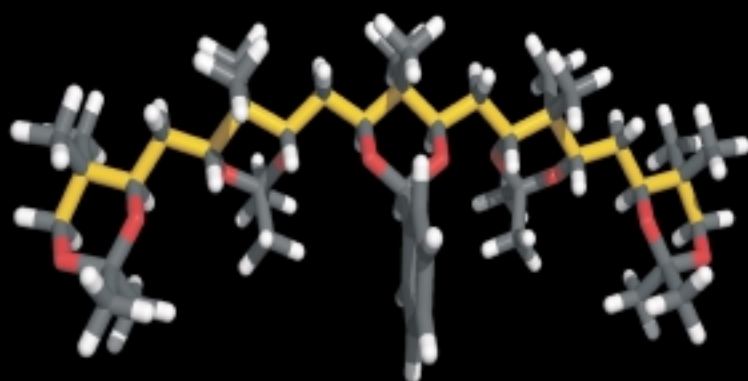
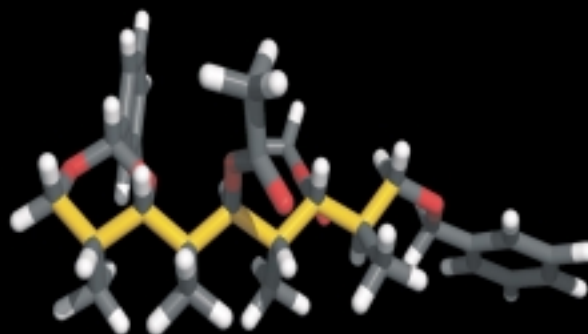
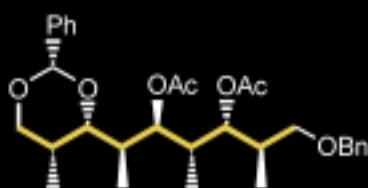
Received: October 4, 1999 [A 364]

- [1] J. S. Parkinson, E. C. Kofoed, *Annu. Rev. Genet.* **1992**, 26, 71–112.
- [2] J. S. Parkinson, *Cell* **1993**, 73, 857–871.
- [3] R. Johnson, J. Adams, *Tree* **1992**, 7, 295–299.
- [4] H. C. Neu, *Science* **1992**, 257, 1064–1072.
- [5] S. B. Levy in *Antimicrobial Drug Resistance* (Ed.: L. E. Bryan), Academic Press, New York, **1984**, pp. 191–240.
- [6] S. B. Levy, *ASM News* **1988**, 54, 418–421.
- [7] A. Wissmann, W. Hillen, *Forum Mikrobiol.* **1989**, 292–299.
- [8] B. Epe, P. Wolley, *EMBO J.* **1984**, 3, 121–126.
- [9] V. Burdett, *J. Biol. Chem.* **1991**, 266, 2872–2877.
- [10] R. Sanchez-Pescador, J. T. Brown, J. T. Roberts, M. S. Urden, *Nucl. Acids Res.* **1988**, 16, 1218.
- [11] B. Mendez, C. Tachibana, S. B. Levy, *Plasmid* **1980**, 3, 99–108.
- [12] K. Tovar, A. Ernst, W. Hillen, *Mol. Gen. Genet.* **1988**, 215, 76–80.
- [13] J. Zhao, T. Aoki, *Microbiol. Immunol.* **1992**, 36, 1051–1060.
- [14] D. Schnappinger, W. Hillen, *Arch. Microbiol.* **1996**, 165, 359–369.
- [15] B. S. Speer, A. A. Salyers, *J. Bacteriol.* **1989**, 171, 148–151.
- [16] B. Eckert, C. F. Beck, *J. Bacteriol.* **1989**, 171, 3557–3559.
- [17] M. D. Marger, M. H. Saier, *Trends Biochem. Sci.* **1993**, 18, 13–20.
- [18] B. Eckert, C. F. Beck, *J. Biol. Chem.* **1989**, 264, 11663–11670.
- [19] T. Lederer, M. Takahashi, W. Hillen, *Anal. Biochem.* **1995**, 232, 190–196.
- [20] W. Hillen, C. Gatz, L. Altschmied, K. Schollmeier, I. Meier, *J. Mol. Biol.* **1983**, 169, 707–721.
- [21] C. Gatz, A. Kaiser, R. Wendenburg, *Mol. Gen. Genet.* **1991**, 227, 229–237.
- [22] S. Freundlieb, U. Baron, A. L. Bonin, M. Gossen, H. Bujard, *Methods Enzymol.* **1997**, 283, 159–173.
- [23] E. Wirtz, C. Clayton, *Science* **1995**, 268, 1179–1183.
- [24] W. Hinrichs, C. Kisker, M. Düvel, A. Müller, K. Tovar, W. Hillen, W. Saenger, *Science* **1994**, 264, 418–420.
- [25] C. Kisker, W. Hinrichs, K. Tovar, W. Hillen, W. Saenger, *J. Mol. Biol.* **1995**, 247, 260–280.
- [26] P. Orth, F. Cordes, D. Schnappinger, W. Hillen, W. Saenger, W. Hinrichs, *J. Mol. Biol.* **1998**, 279, 439–447.
- [27] P. Orth, W. Saenger, W. Hinrichs, *Biochemistry* **1999**, 38, 191–198.
- [28] P. Orth, D. Schnappinger, P. E. Sum, G. A. Ellestad, W. Hillen, W. Saenger, W. Hinrichs, *J. Mol. Biol.* **1999**, 285, 455–462.
- [29] K. H. Jogun, J. J. Stezowski, *J. Am. Chem. Soc.* **1976**, 98, 6018–6026.
- [30] J. Degenkolb, M. Takahashi, G. A. Ellestad, W. Hillen, *Antimicrob. Agents Chemother.* **1991**, 35, 1591–1595.
- [31] W. Hillen, C. Berens, *Annu. Rev. Microbiol.* **1994**, 48, 345–369.
- [32] R. Baumeister, V. Helbl, W. Hillen, *J. Mol. Biol.* **1992**, 226, 1257–1270.
- [33] S. C. Harrison, *Nature* **1992**, 353, 715–719.
- [34] P. Orth, C. Alings, D. Schnappinger, W. Saenger, W. Hinrichs, *Acta Crystallogr. Sect. D* **1998**, 54, 99–101.
- [35] P. Orth, Ph.D. Thesis, Freie Universität Berlin, **1998**.
- [36] W. Saenger, *Principles of Nucleic Acid Structure*, Springer, New York, **1983**.
- [37] E. Pook, S. Grimm, A. Bonin, T. Winkler, W. Hillen, *Eur. J. Biochem.* **1998**, 258, 915–922.
- [38] B. Tiebel, L. M. Aung-Hilbrich, D. Schnappinger, W. Hillen, *EMBO J.* **1998**, 17, 5112–5119.
- [39] L. D. Smith, K. P. Bertrand, *J. Mol. Biol.* **1988**, 203, 949–959.
- [40] G. Müller, B. Hecht, V. Helbl, W. Hinrichs, W. Saenger, W. Hillen, *Nat. Struct. Biol.* **1995**, 2, 693–703.
- [41] H. A. De Boer, L. J. Comstock, M. Vasser, *Proc. Natl. Acad. Sci. USA* **1983**, 80, 21–25.
- [42] J. Figge, C. Wright, C. J. Collins, T. M. Roberts, D. M. Livingstone, *Cell* **1988**, 52, 713–722.
- [43] M. Gossen, S. Freundlieb, G. Bender, G. Müller, W. Hillen, H. Bujard, *Science* **1995**, 268, 1766–1769.
- [44] U. Baron, D. Schnappinger, M. Gossen, W. Hillen, H. Bujard, *Proc. Natl. Acad. Sci. USA* **1991**, 88, 1013–1018.
- [45] K. Forster, V. Helbl, T. Lederer, S. Urlinger, N. Wittenberg, W. Hillen, *Nucl. Acids Res.* **1999**, 27, 708–710.
- [46] H. B. Blau, F. M. V. Rossi, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 797–799.

Conformation Design



Nature does it - what about us ?



Conformation Design of Open-Chain Compounds

Reinhard W. Hoffmann*

At the brink of the 21st century, chemistry is increasingly concerned with the function that molecules fulfil as drugs, receptors, or—as ensemble of molecules—as materials. The capability of compounds to fulfil such functions cannot sufficiently be described by using only the terms composition and configuration. A decisive role is played in addition by the conformation of the molecules, which serves as the link between molecular composition and molecular function. Expressions

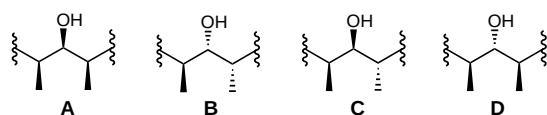
such as “active conformation” or “competent conformation” allude to this aspect. Chemists have to develop an understanding how a flexible molecule adopts the conformation (a distinct shape) which is optimal for the function in question and how this process can be controlled. On the outset of such considerations, we may ask how nature succeeded in the process of evolution to endow flexible molecules with a preference to adopt the conformation which is optimal for

the function it has to serve. In this review, I report on how we have reached a crude level of understanding of conformation design in nature with reference to the class of polyketide natural products, how we developed these insights into a conformation design of open-chain compounds, and which applications are already in sight.

Keywords: alkane • conformation analysis • polyketide • structure–activity relationships

1. Introduction

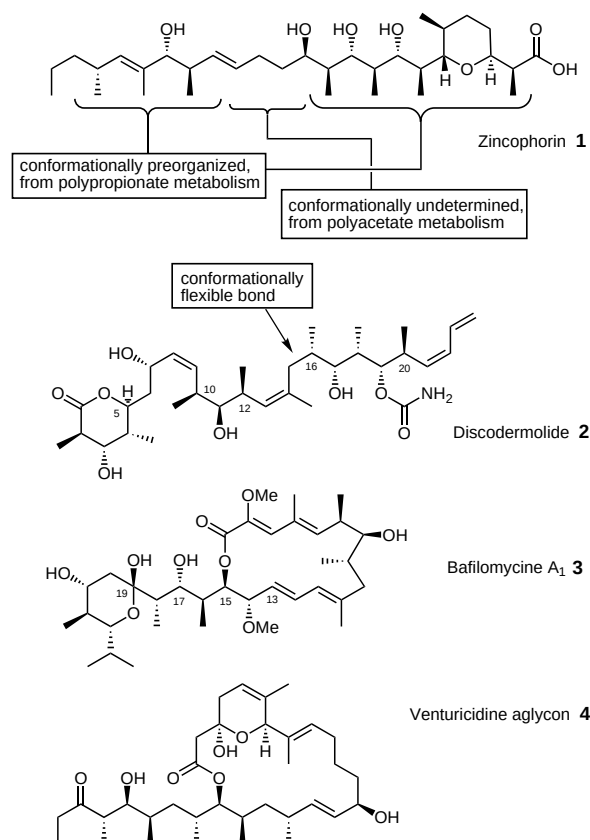
When working for more than two decades on the development of methods for the stereoselective synthesis of “stereotriads”,^[1] one obviously takes note of the large number of biologically active natural products, which contain such stereotriads as a characteristic structural element (Scheme 1).



Scheme 1. The four possible stereotriads **A–D** found in polypropionate natural products.

These are principally the polyketide natural products,^[2] which are biogenetically derived from propionyl–coenzyme A through the fatty acid metabolism. Some examples may illustrate their structural diversity (Scheme 2).

An inspection of such structures certainly induced many to wonder what is the purpose of those numerous methyl side

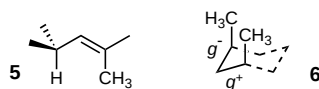


Scheme 2. Some “polyketide” natural products.

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groups. In any case, nature could have made more extensive use of the polyacetate metabolism! What is the advantage gained in natural evolution to have individual stereotriads of a particular, rather than a different, configuration? There must have been a bonus for nature to develop the complex machinery^[3] of the polypropionate metabolism. Such considerations strengthen the notion that the presence of the numerous methyl side groups is somehow connected with the backbone conformation of these molecules. The methyl groups do not affect the flexibility of the backbone, yet they reduce the number of low energy local conformers with the result that such molecules preferentially populate certain conformations. In short, we deal with “flexible molecules with a defined shape”.^[4]

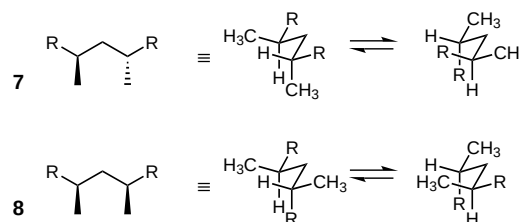
Two principles became evident^[5, 6] that nature uses to destabilize undesired conformations: one is to avoid 1,3-allylic strain;^[7] the other is to avoid *syn*-pentane interactions. The significance^[8] and the mode of operation^[9] of 1,3-allylic strain became clear to us during the 1980s. In a substance such as **5**, a single conformation of the vinylic bond is almost completely (>90%) populated in which the H–C–C=C dihedral angle lies within $0 \pm 30^\circ$ (Scheme 3). The substructure **5** occurs, for instance, in zincophorin **1**^[10] and in discodermolide **2**^[11] (Scheme 2).



Scheme 3. Preferred conformation of a 1,1-disubstituted allylic system **5** and of a pentane **6** destabilized by a *syn*-pentane interaction.

A destabilizing *syn*-pentane interaction is created when a hydrocarbon chain is folded such that a g^+ dihedral angle is followed by one of g^- along the backbone^[12] (see structure **6**). This places, in the case of **6**, two methyl (end)groups into a similar spatial proximity, as found in a 1,3-diaxial arrangement on a cyclohexane ring. The conformation shown in **6** is no minimum on the energy hypersurface; rather, the molecule relaxes by increasing the backbone dihedral angles to about 90° . The resulting conformers are still higher in energy (by about 14 kJ mol^{-1}) than other unstrained conformers.^[13] For this reason, linear hydrocarbon chains in alkanes adopt conformations that are free of such *syn*-pentane interactions.

The methyl side groups in the polyketide natural products cause the substructures of the style of **7** and **8** to have only two local conformations that are free of such destabilizing *syn*-pentane interactions^[14] (Scheme 4).



Scheme 4. Biconformational segments of 2,4,6,... *n*-polymethylated alkane chains.

Perusal of numerous crystal structures of polyketide natural products showed that indeed the substructures **7** and **8** are present almost completely in one of the two low energy backbone conformations.^[4] The fact that these expected conformations are present in the solid state does not, by itself, allow any conclusions regarding the nature of preferred conformations present in solution. Information on this aspect could be gained, for example, from vicinal ^3J coupling constants along the molecular backbone. Such data are hardly available, since the structural elucidation of polyketide natural products took place in a period in which high field NMR spectroscopy had not reached a level which would permit routine measurement of such coupling constants. If one wanted to learn more about the preferred conformations of polyketide natural products, the NMR spectra of representative examples would have to be reinvestigated with the more refined methods available today.

The limited data available do not give a uniform picture. For instance, a marked alteration of vicinal coupling constants^[18] has been reported for the C-13 to C-19 chain of bafilomycin A₁ **3** as well as of the related compounds elaiomycin,^[15] concanamycin,^[15, 16] and hygrolidine.^[17] This indicates the preferential population of a single local backbone conformation^[19] at the four bonds of the skeleton between C-14 and C-18. It is unknown as to what extent this is supported by the formation of intramolecular hydrogen bonds.

The few vicinal coupling constants reported for discodermolide **2** do not allow any statement regarding a preferred



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backbone conformation. The data for discodermolide tetraacetate^[20] reveal that the two local conformations are each populated about equally in both of the stereotriads C-10/C-12 and C-16/C-20. In contrast, the backbone segment C-4/C-10 appears to populate a single distinct conformation.

Such observations nurtured our speculation that nature used the methyl side groups in polyketide natural products to give these compounds a predisposition to adopt the conformation necessary for the biological function that they may have. Buoyed by such speculation, we attempt to “translate” the relative configuration of the numerous stereogenic centers in the polyketide natural products into the corresponding preferred backbone conformation.^[4] Eventually, we had to overcome several conceptual barriers before we made the transgression from this curiosity-driven pastime to arrive at the statement:

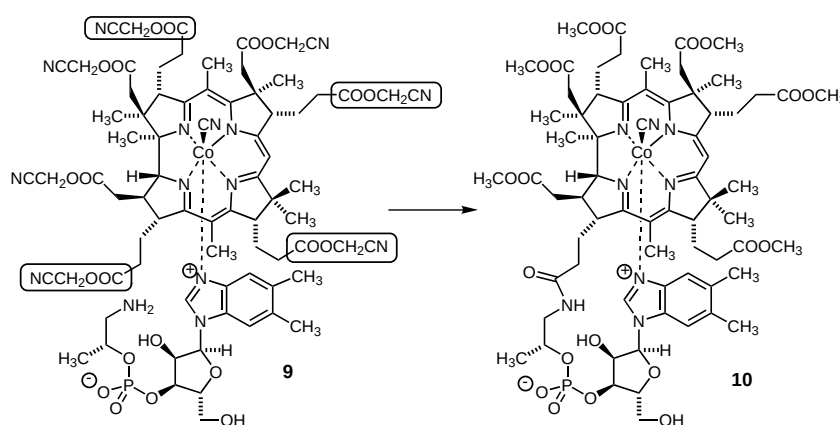
Conformation design: nature does it—what about us?

From today’s standpoint, not only does it matter if we understand nature’s conformation design but whether we can reliably mimic it with model compounds and control the conformation and biological activity of designed molecules. The long term goal is to finely tune the conformational preorganization of a drug molecule in order to learn where the optimum between the extremes “rigid” and “unordered” lies. Only on the basis of such studies may we recognize why nature chose flexible backbones with a higher or lower preference to populate a preferred conformation. We should focus first on the local conformational preferences in small backbone segments. This might allow to address questions of whether it suffices for nature to reach optimal biological activity for drugs by maintaining a high conformational preference in individual local backbone segments or whether a lower global conformational preference of the backbone of a drug molecule is more important in order to optimize biological activity.

We are, with hindsight, ashamed of how long it took to realize that nature, in demonstrating a conformation design in polyketide natural products, provides us with a toolkit to effect a conformation design of other flexible molecules of interest. Already in the early 1980s, when investigating the allylboration of chiral α -substituted aldehydes, we recognized how the stereoselectivity of the reaction may be controlled by avoiding *syn*-pentane interactions across a newly forming bond.^[21] On establishing the structure of the resulting complex, we realized that these molecules adopt conformations that avoid *syn*-pentane interactions, a result that manifested itself in conspicuous ¹³C NMR chemical shifts.^[22] We discussed these results at length with Professor J. K. Whitesell, who was, at that time, a visiting professor in Marburg. This led to insights into the interrelation of the relative configuration of adjacent stereogenic centers, the resulting conformational preferences, and ¹³C NMR chemical shifts.^[23] These insights were used later to predict one of these parameters based on

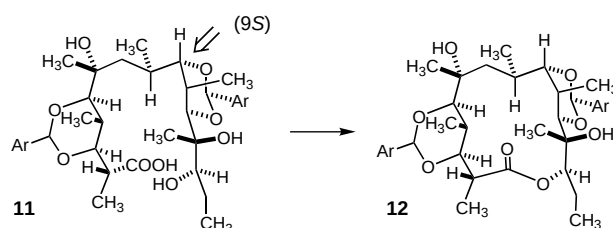
the knowledge of the other two.^[24] When pursuing the synthesis of polyketide natural products in the 1980s, the NMR spectra of several synthetic intermediates made us conscious of the interrelation between the kind of preferred local backbone conformation and the relative configuration at stereogenic centers along the backbone. However, it did not occur to us at this stage to initiate a program on the conformation design of flexible molecules.

At the end of the 1980s, the significance of the proper conformational preorganization was generally recognized when it came to effect ring closure reactions to form macrocyclic compounds. A key example is given by the ring closure of the vitamin B₁₂ precursor **9** to give, in a position-selective manner, the macrocycle **10**^[25] (Scheme 5).



Scheme 5. The final ring-closing reaction in the synthesis of vitamin B₁₂.

Another characteristic example was evident from the efforts to form the 14-membered lactone ring of erythronolide: Studies from the groups of Woodward,^[26] Stork,^[27] Yonemitsu,^[28] and Paterson^[29] underlined the importance of the (9*S*) configuration of the precursor **11** to populate that conformation,^[30] which allows the ends to meet and thereby permits the closure of the lactone ring to form **12** (Scheme 6).

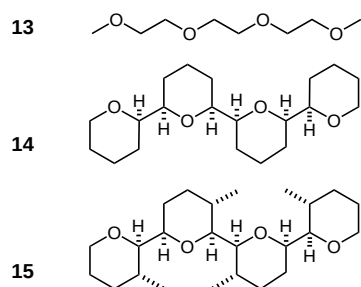


Scheme 6. Ring closure during the synthesis of the macrocycle of erythronolide.

Despite all these insights, we did not envision the possibility to synthesize flexible molecules with a predetermined conformation. We happened, at the end of the 1980s, “to stand repeatedly at a door”, to use the imagery of Hückel^[31], “and had the door handle in our hands, but failed to open this door.” Eventually in 1991, on inspecting crystal structures of numerous polyketide natural products did the pieces fall into place and we realized that conformation design should be feasible and rewarding. Through the “open door” we envi-

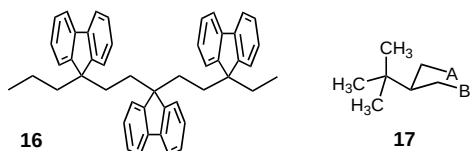
sioned the possibilities similar to viewing a new fertile valley. Thus, we looked at who was tilling the land there.

Most conspicuous were the contributions guided by Still,^[6, 32] who is to be recognized as the pioneer of intentional conformation design. He showed that the number of low energy conformations of the polyether chain **13** can be significantly reduced by ring annellation, that is, by the incorporation of two neighbouring chain bonds into a ring (Scheme 7). By judicious placement of methyl substituents, he reached molecule **15**, which has only a single low energy conformation. This preferred conformation made this compound a specific ligand for the enantioselective recognition of amino acids.^[33, 34] By further optimization of the ligand **15**,^[35–37] Still reached a leading position in conformation design of complexing ligands.^[38]



Scheme 7. Conformational organization of a polyether chain by ring annellation and buttressing substituents.

Also clearly directed at conformation design were the studies led by Alder,^[39] who defined the conformation-controlling role of quaternary centers. He had entered this research area more or less by chance,^[40] but then developed fascinating concepts for stabilizing the extended conformation of (linear) polymer chains like **16** and of macrocyclic two-dimensional networks^[40] (Scheme 8).

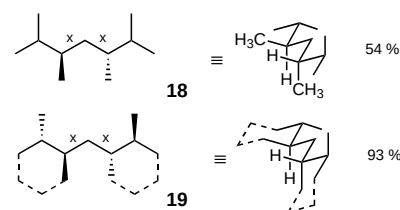


Scheme 8. Effects of buttressing substituents on the folding of alkane chains.

There were signs of conformation design in the studies of DeClercq et al., who, by placing a *tert*-butyl group on a chain molecule **17**, made it energetically cheaper for the end groups A and B to find themselves in close proximity^[41] (Scheme 8).

Over discussions with colleagues and with their kind advice we gradually became aware of earlier contributions to the field, which must now be considered as forerunners of a conformation design: This holds for the review of Anderson^[42] and for the pioneering studies of Luisi,^[43] in which the consequences of *syn*-pentane interactions are clearly delineated and have even been intentionally applied.^[44] This holds also for the numerous single observations on preferred conformations of certain hydrocarbon backbones, which have

been or could be ascribed to the avoidance of *syn*-pentane interactions, for instance, the studies by Firl,^[45] Kishi,^[46] Sih,^[47] Allinger,^[48] and Yamamoto.^[49] The classical studies^[50] on the conformation of polymer chains of polypropylene and other vinylic polymers should be mentioned in this context. In all these papers the conformational preferences and their causes have been clearly reported and discussed. The next step, however, to use this information to achieve an intentional conformation design, was still required. As a prelude, we started in 1991 with Thomas Sander and Martin Brumm to evaluate the conformational preferences of polysubstituted heptane chains by force field calculations.^[51] On compound **18**, for example, the calculations showed that no single conformation exceeded a population of 10%, yet the calculations on the two central backbone bonds (marked on **18**) predicted a modest conformational preference of 54 % for the conformation shown (Scheme 9). We realized that this conformational preference can be augmented significantly by ring

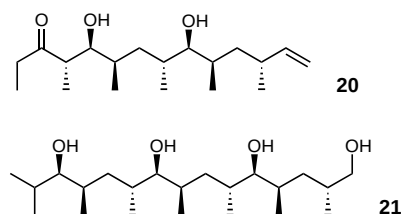


Scheme 9. Preferred conformation at the bonds marked with × and the percentage of these conformations in the total conformer population (calculated with the MM3* force field).

annellation (see structure **19**), which reduces the number of freely rotating bonds. This led to our study of the bis(tetrahydropyranyl)methanes **28**.^[52, 53] Our investigation at this stage were poorly focussed since we lacked information on problems for the solution of which conformation design would provide an essential element. In this situation, we resorted to writing a review before we had made any major contributions to the field. We hoped that this review^[4] would be read by colleagues in bioorganic chemistry and drug research and that we would this way receive suggestions to interesting problems. The response did not meet our expectations: A search in the Science Citation Index shows that this review was largely ignored by people in biomedical research. Thus, in the end, we had to go ahead by ourselves.

2. Tracing Nature: 2,4-Dimethylpentane Derivatives

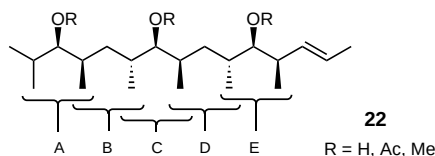
The period started in which in our group Ulrike Rolle and then Richard Göttlich scouted out the possibilities of conformation design with great enthusiasm. Sobering results followed quickly. For instance, the crystal structure of venturicidine benzoate^[54] and the conspicuous pattern of methyl substituents in the venturicidine aglycon **4** led us to believe that alkane chains with a substituent pattern similar to that in venturicidine, such as **20**, should preferentially populate a conformation with a fully extended backbone (Scheme 10).



Scheme 10. Examples for polymethylated alkane chains with a substituent pattern corresponding to venturicidine.

For this reason, we developed an efficient method to synthesize compounds with structures similar to those found in venturicidine^[55] and reached, for instance, the tetraol **21**.^[55–57] With **21** in hand, we could not measure the vicinal $^3J_{\text{H,H}}$ coupling constants^[58] which would allow a conformation analysis. The relevant ^1H NMR signals could not be resolved even using a 500 MHz instrument and—as we see it today—compound **21** has no tendency to populate a fully extended conformation!

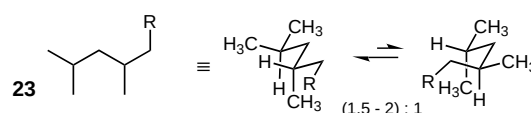
We reacted to both failures aggressively: In a collaboration with Professor Berger—resulting in the dissertation of Thomas Fäcke— ^{13}C NMR editing methods were developed through which $^3J_{\text{H,H}}$ coupling constants could be determined even when the ^1H signals overlaid.^[59] Moreover, methods were developed which, for compounds with ^{13}C in its natural abundance, allow the determination of ^{13}C – ^{13}C coupling constants,^[60] which in turn give information on the conformation of a molecular backbone. Armed in this manner, we tackled the conformation analysis of the intermediate **22**,^[57] which lies on the synthetic pathway of **21**, the largest such compound amenable to such an analysis (Scheme 11). The



Scheme 11. Synthetic analogues to the polypropionate part of venturicidine.

result^[61] was a complete surprise for us: There was none or only a low, local conformational preference in the segments A and C. The high conformational preference hoped for in the *tt* conformation was, however, found in the segments B and D. In segment E, a preference for the *gg* conformation was found, a conformation in which the main chain bends to align the alkenyl residue sideways. This taught us how remote we were from a real understanding of the factors which determine the conformation in such compounds.

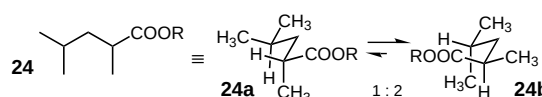
In due course—initially more or less random, eventually quite focused—we synthesized and analyzed numerous model compounds. In this manner, we slowly recognized the factors which determine the local conformations in compound **22**, which allowed us to rationalize the initially surprising results. The results may be summarized as follows: Placing a residue *R* at the end of a 2,4-dimethylpentane segment (see structure **23**) favors the population of the extended conformation, yet the effects remain relatively small ($< 2:1$)^[61] (Scheme 12).



Scheme 12. Conformational preference of a 2,4-dimethylpentane skeleton (*R* = alkyl residue).

The connection of several 2,4-dimethylpentane units to give polymethylated alkane chains like **22** is synonymous with the extension of a 2,4-dimethylpentane unit. It is therefore no surprise that a connection of dimethylpentane segments by itself does not generate a high conformational preference. It should be mentioned that syndiotactic polypropylene has been found to crystallize as two polymorphs, one with an extended local conformation for all dimethylpentane segments,^[62] and one with a doubly bent conformation for every second dimethylpentane segment.^[63] In short, a 2,4,6,...*n*-polymethylated alkane chain has, for each dimethylpentane segment, only two low energy conformations. Compounds that populate a single backbone conformation cannot therefore be reached simply by placing methyl substituents at every other carbon atom at a chain, at least when they are arranged in a syndiotactic manner (compare also the situation in discodermolide tetraacetate discussed in the previous Section).

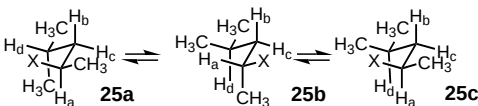
If one replaces one of the methyl groups in 2,4-dimethylpentane by a vinyl ester (**24**) or aldehyde residue, that is, residues bound via an sp^2 -hybridized carbon atom, the conformer **24b** is preferentially populated (but only by a small margin; Table 1).^[64] In **24b**, the less bulky sp^2 -hybridized residue takes a position lateral to the main chain. These statements are based on a comparison of the $^3J_{\text{H,H}}$ coupling constants with those calculated for the individual low energy conformers (Scheme 13).



Scheme 13. Conformational preference of a 2,4-dimethylpentanoate.

The position of the conformer equilibrium is determined by the fact that in **24a** the methyl group is oriented sideways to the main chain, where it experiences a *gauche* interaction with the main chain. In conformer **24b**, the ester group lies in this position. If a substituent is positioned at the end of the chain, it is free of such *gauche* interactions with the main chain. Consequently, the sterically less demanding substituent (compare the *A* values^[65] describing the equatorial/axial equilibrium for monosubstituted cyclohexanes) will adopt the position lateral to the main chain. The effects, however, remain small. Even if they may be augmented or overcompensated by further substituents, local conformer preferences in excess of 80% are attained in this manner only with difficulty.^[64]

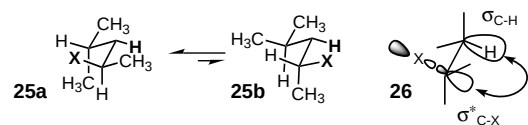
A further variation in the 2,4-dimethylpentane system lies in the substitution of one or two of the methyl groups by halogen atoms or other heteroatom-bound groups (*X* in **25**;

Table 1. Estimated conformer populations for 2-substituted 4-methylpentanes from $^3J_{\text{H,H}}$ coupling constants and from force field calculations.


X =	Contribution (%) of the a, b, c conformers to the total conformer population ^[a]	NMR coupling constants [Hz]				Solvent	Estimated a:b from NMR data
		H _a H _b	H _a H _c	H _b H _d	H _c H _d		
COOMe	53 : 32 : 11	8.0	5.6	n.d.	n.d. ^[b]	CDCl ₃	1.7 : 1
CH = CH ₂	71 : 19 : 9	8.2	6.2	n.d.	n.d.	CDCl ₃	1.5 : 1
C ₆ H ₅	68 : 20 : 10	8.5	5.6	n.d.	n.d.	CDCl ₃	2 : 1
CH = O	57 : 32 : 5	7.4	6.5	n.d.	n.d.	CDCl ₃	1.2 : 1
OH	53 : 24 : 15	8.2	4.9	6.0	8.2 ^[c,d]	CDCl ₃	1.6 : 1
		8.2	4.9	6.0	8.2	DMSO	1.6 : 1
OMe	47 : 28 : 12	7.4	5.6	6.4	7.4 ^[d]	CDCl ₃	1.4 : 1
NH ₂	50 : 34 : 7	7.8	5.9	6.4	7.8 ^[c]	DMSO	1.4 : 1
NH ₃ ⁺ AcO ⁻	39 : 54 : -	5.9	8.6	8.2	6.2 ^[c]	DMSO	1 : 1.7
NHMe	54 : 32 : 10	5.7	7.7	8.0	6.0	DMSO	1 : 1.6
		6.3	7.4	7.9	6.5	CDCl ₃	1 : 1.3
NMe ₂	58 : 31 : 8	7.1	7.1	7.1	7.1 ^[c]	DMSO	1 : 1
		5.7	8.0	7.9	6.3	CDCl ₃	1 : 1.5
NPhth ^[e]	78 : 7 : 10	9.9	4.8	4.8	10.3 ^[c]	C ₆ D ₆	3.7 : 1
Cl	63 : 29 : 4	9.3	4.8	5.4	8.6	CDCl ₃	2.2 : 1

[a] The values, estimated with the MM3* force field, do not total 100 % on account of other conformation types. [b] n.d. = not determined. [c] Coupling constants were assigned by deuterium labeling. [d] The calculated (Macromodel) *gauche* coupling constants for the hydrogen atom positioned *a* to the oxygen atom depends on the spatial alignment of the OR group. These coupling constants were not used in the estimation of the **a:b** ratio. [e] NPhth = phthalimidyl.

Scheme 14). For 2-chloro- and 2-bromo-4-methylpentane **25** (X = Cl, Br), it had been shown earlier by IR studies^[66] that conformer **25a** with the halogen atom lateral to the main chain predominates. An analysis of NMR coupling constants for a series of 2-heteroatom-substituted 4-methylpentanes **25**^[67] showed that the conformational preferences are rather small (see Table 1). The largest effects were recorded for electronegative residues such as phthalimidyl-, chloro-, or bromo-substituents. This indicates that it is not only steric effects that dominate the conformer equilibrium. Perhaps there is an additional stabilization of **25a** by an $\sigma_{\text{C-H}}-\sigma^*_{\text{C-X}}$ hyperconjugation^[68] (see structure **26**), which could not occur in **25b** on account of the absence of a C–H bond antiperiplanar to C–X.



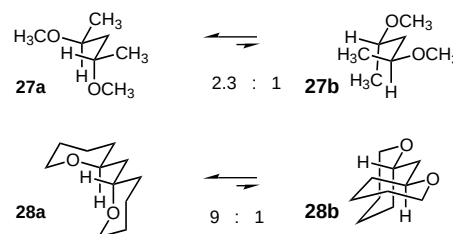
Scheme 14. Conformational preference of 2-X,4-methylpentanes.

However small the conformational preference were in the monosubstituted compounds **25**, higher preferences may be expected for 2,4-di-heterosubstituted pentanes. For a the *d,l*-stereoisomer, the conformational preferences found in the monosubstituted derivatives **25** should reinforce one another (they should counterbalance in the *meso*-isomers!). A starting point for further scrutiny was the dimethoxy compound **27**, on which previous investigations^[69] indicated a 2.3:1 conformation preference towards positioning the methoxy groups lateral to the main chain. We surmised that the free rotation

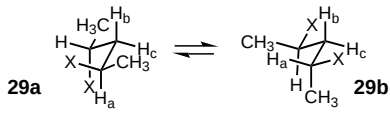
of the methoxy groups compromises the conformational preference to favor **27a**. This is a consequence of the methyl groups of the two methoxy units in **27b** may orient in three positions in space. In conformer **27a** only two arrangements are free of *syn*-pentane interactions. This increases statistically the population of the (undesired) conformer **27b** in the conformer equilibrium. By tying the methoxy groups into a ring, like in **28**, this effect can be avoided. Indeed, the bis(tetrahydropyranyl)methane **28** synthesized by B. Colin Kahrs showed clearly a more marked conformer preference of about 9:1^[52] (Scheme 15).

The tendency of electronegative heteroatoms to adopt a position lateral to the main hydrocarbon chain^[70] has been exploited by Dirk Stenkamp in a systematic study^[67, 71] of 2,4-disubstituted pentanes (**29**, Table 2).

The conformational preferences for the chloro- and bromo compounds, estimated from the vicinal $^3J_{\text{H,H}}$ coupling constants, agree with previous reports.^[73] The dichloro compound **30** had been investigated before as a model compound for the local conformer preferences in polyvinylchloride.^[74] This

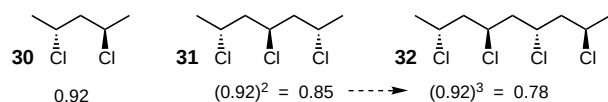


Scheme 15. Tying freely rotatable groups into rings increases the conformational preferences of a molecular skeleton.

Table 2. The $^3J_{\text{H,H}}$ coupling constants and the derived conformer population for 2,4-disubstituted pentanes.


X =	NMR coupling constants [Hz]		a : b
	H _a H _b	H _a H _c	
OMe	7.8	4.6	2.3 : 1 ^[69]
OAc	9.0	3.8	3.5 : 1 ^[72]
C≡C-SiMe ₃	10.6	4.3	4.5 : 1
N ₃	10.5	3.0	8 : 1
Cl	10.6	2.2	11.5 : 1
Br	10.8	4.4	11.5 : 1
NPhth	11.8	4.5	19 : 1

shifted our attention to syndiotactic 2,4,6,...*n*-polychlorinated alkanes, which might possess a marked preference for the fully extended conformation (Scheme 16). Compound **31** had already been evaluated by experiment and calculations.^[75] We prepared **31** anew^[76] and determined its $^3J_{\text{H,H}}$ coupling constants: These revealed in each of the segments of **31** the



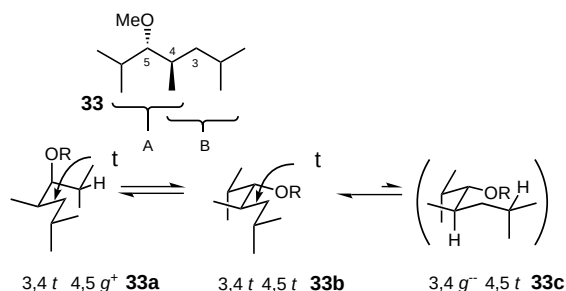
Scheme 16. Conformational preferences of fully extended alkane chains in compounds which model syndiotactic polyvinylchloride.

same conformational preference (0.92) as in the simple dichloropentane **30**. For the total molecule of **31**, the conformational preference amounts to only 0.85 ($= (0.92)^2$). On this basis, one would predict a total conformer preference of 0.78 for **32**, which thereby indicates the limitations of any conformation design along those lines. An acceptable total conformer preference for a larger molecule (arbitrarily set to > 0.8) can only be reached if the local conformer preference in each of the segments is very high. In this respect, the diphthalimidyl compound **29** (Table 2) is promising.

3. Conformation Control by Inductor Groups

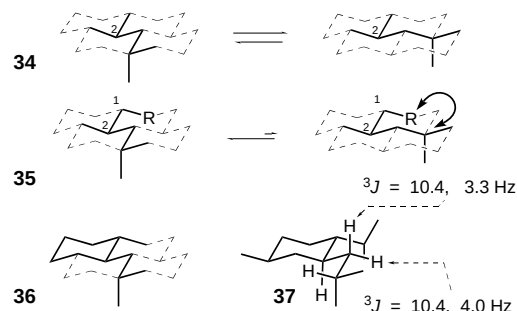
In reconsidering the results obtained so far, we reexamined those cases in which we had (accidentally) encountered a high conformational preference. This held for the segments B and D in compound **22**. To account^[61] for this high conformational preference, reference to the model compound **33** will be discussed: It is the “*anti*”-configuration of the methoxy and the methyl groups, in which the methoxy group at C-5 is placed in a position which allows only a single, low energy conformation in the neighbouring dimethylpentane segment B. The other local conformation in segment B (**33c**) is destabilized by a *syn*-pentane interaction with the methoxy group at C-5! The methoxy substituent therefore induces a preferred *trans*-conformation of the C-3/C-4 bond (see **33a** and **33b** in Scheme 17).

We may conclude that the methoxy substituent serves as an inductor group as it selectively destabilizes one of the two backbone conformations in the neighbouring segment. Inductor groups—the groups that influence the population of a single conformation at a close by (but not necessarily



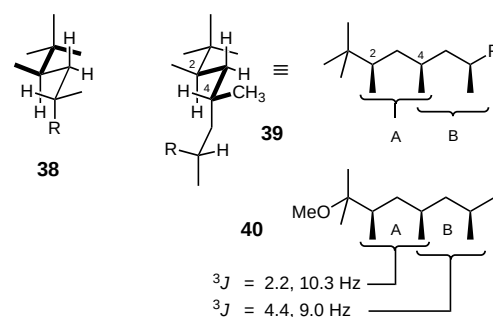
Scheme 17. Effect of an *anti*-positioned methoxy substituent in segment A on the conformational preference in segment B of compound **33**.

adjacent) bond—were known. We refer to the effects of a *tert*-butyl group or, more generally, of quaternary centers.^[39–41] With Martin Stahl and Ulrich Schopfer, we evaluated the requirements for inductor groups that would allow a conformation design in 2,4,6,...*n*-polysubstituted alkane chains.^[77] The following principle became clear: Introduction of a substituent at C-1 of a 2,4-dimethylpentane **34** destabilizes one of the two backbone conformations, provided the substituent is held antiperiplanar to the C-2 methyl group (see structure **35** in Scheme 18).



Scheme 18. Effect of a C-1-positioned inductor substituent on the conformer equilibrium in 2,4-dimethylpentane.

A simple means to hold a substituent at the position indicated in **35** is to annelate a six-membered ring in a chair conformation, as **36**. A compound of this type, **37**, was synthesized by Philipp Reiss.^[78] The large difference in the $^3J_{\text{H,H}}$ coupling constants demonstrates the predominance of a single conformation, as predicted by calculations with the MM3* force field from the Macromodel program.^[79] There is a foolproof possibility to hold a substituent antiperiplanar to the C-2 methyl group, as indicated in **35**, by choosing compound **38**, that is, by making use of the *tert*-butyl effect:^[39–41] Due to the quaternary center at C-1, there is always one residue antiperiplanar to the C-2 methyl group (Scheme 19).

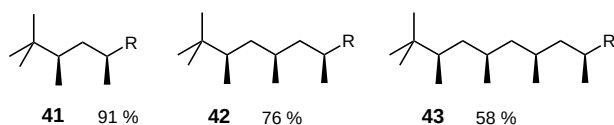


Scheme 19. Transmission of the conformation induction that originates from a *tert*-butyl residue from a dimethylpentane segment to a neighbouring one.

More fascinating was the insight that compound **38** may serve as such an inductor group to an attached second dimethylpentane segment **39**. The *tert*-butyl group induces a preferred conformation in segment A of compound **39**. This conformation places C-2 antiperiplanar to the C-4 methyl group, which should induce a preferred conformation in segment B of **39**.

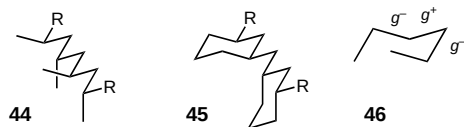
This prediction was tested with compound **40**,^[80] which contains a methoxy group, the presence of which would prevent overlay of the relevant ^1H NMR signals. The coupling constants recorded for **40** show that a local conformational preference prevails not only in segment A but also in segment B.

Such a transmission of the folding information from one to the next dimethylpentane segment requires that the methyl side groups are arranged in an isotactic manner, as in the structures **41** and **42**. Isotactic polypropylene crystallizes in a single modification, in which the chain adopts the expected helical conformation.^[81] This is exactly the conformation which results from transmission of the folding information from adjacent segments. As soon as there is a single syndiotactic unit in a 2,4,6,...*n*-polysubstituted alkane chain, the transmission of the folding information is interrupted at this point. "Downstream" from such a syndiotactic unit, the chain can adopt a multitude of low energy conformations.^[77] But, even in a homogeneously isotactic substituted alkane, this transmission soon reaches its limitations. This was foreshadowed in the difference between the coupling constants for the segments A and B in compound **40**. Force field calculations indicate a rapid decrease in the overall conformational preference with an increase in the number *n* of adjacent dimethylpentane segments: By three segments, the total conformer preference falls below 60 % because of the number of individual, undesired conformers enduring a single *syn*-pentane interaction increases by $n(n+1)$ (Scheme 20).



Scheme 20. Calculated preference (percentage of the total conformer population) for attaining the fully extended conformation of derivatives of isotactic oligopropylene.

It is evident that the loss of entropy, which accompanies the population of a single conformation, will increase with the number of the freely rotating bonds and hence with the size of the molecule. Ring annellation reduces the entropy loss associated with the population of a single conformation (compare compounds **44** and **45**, which have the same number of bonds between the R groups; Scheme 21). In essence, ring



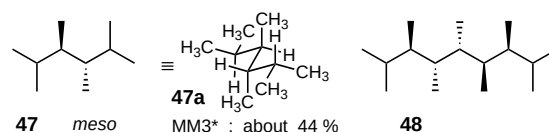
Scheme 21. The number of freely rotating backbone bonds may be reduced by annellation.

annellation is no more than cheating. Yet nature is doing just the same by using the tetrahydropyrane ring frequently found in polyketide natural products and, in the end, we too did just the same in optimizing the conformational preference in going from 2,4-dimethoxypentane **27** to the bis(tetrahydropyranyl)methane **28**.

4. Round Three: 2,3,4,5-Tetramethylhexane Derivatives

When trying to endow larger molecules with the preference for a single conformation, the main problem is the concomitant loss in entropy. The Gibbs–Helmholtz equation ($\Delta G = \Delta H - T\Delta S$), which has to be applied to conformer equilibria as well, suggests an escape route: The well behaved molecule, in the sense of enhancing the population of a single conformation, involves a loss in entropy. Nevertheless, enhanced population of the desired conformation may be attained if we penalize the undesired conformations with destabilizing enthalpy (a higher value of ΔH). We will simply increase the penalty for misbehaviour. In the molecules discussed in Section 3, each undesired backbone conformation was penalized by a simple *syn*-pentane interaction. We should address molecules in which any undesired conformation is penalized by two *syn*-pentane interactions or, similarly, by a *syn*-hexane interaction. A *syn*-hexane arrangement corresponds to a folding of a hexane backbone with a $g^-g^+g^-$ sequence of dihedral angles; see structure **46**. According to calculations on this compound,^[13] this arrangement is not a minimum on the energy hypersurface^[82] because of the presence of two *syn*-pentane interactions.

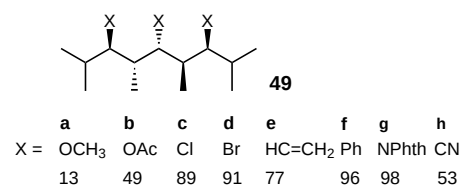
We considered *meso*-2,3,4,5-tetramethylhexane **47**, which has a backbone in which rotation from the conformation **47a** about the central C-3/C-4 bond creates two *syn*-pentane interactions simultaneously^[77] (Scheme 22). Unfortunately,



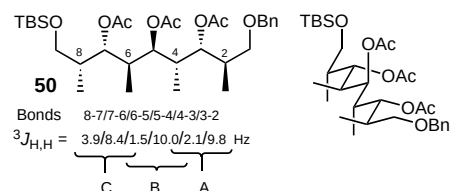
Scheme 22. Preferred conformation of *meso*-2,3,4,5-tetramethylhexane.

calculations on **47** predict merely a conformational preference of 44 %. This arises from the eight destabilizing *gauche* interactions present in the minimum energy conformation **47a**. These *gauche* interactions decrease the energy difference to the next higher undesired conformer. Any extension of such a system in the direction of larger compounds, such as **48**, appears to be unattractive at first sight. On the other hand, it is conceivable to make some compromises by selecting buttressing substituent groups, which, when positioned laterally to the main chain, cause only small (at least, smaller than methyl) *gauche* interactions. Such substituents, such as oxygen, chloro, vinyl, phenyl, or phthalimidyl residues, have been identified in Section 2. MM3* calculations indicate that high conformational preferences can indeed be reached with such substituents for extended molecules such as **49**^[83] (Scheme 23).

This encouraged Dirk Stenkamp to synthesize compound **50**,^[83] which, according to the $^3J_{\text{HH}}$ coupling constants, possesses a high conformational preference in the segments A and B ($>90\%$; Scheme 24). This can be traced back to the antiperiplanar placement of C-7-OAc/C-6-CH₃ (controls segment B) and C-5-OAc/C-4-CH₃ (controls segment A). The



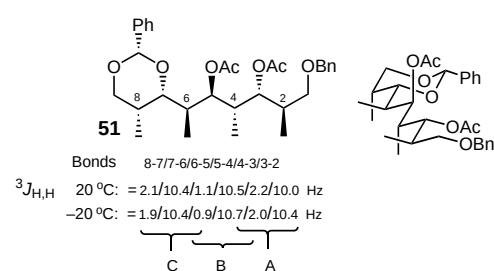
Scheme 23. Conformational preference (percentage of the total conformer population) for attaining a fully extended conformation of analogues of 2,3,4,5,6,7,8-heptomethylnonane.



Scheme 24. The ³J_{H,H} coupling constants and preferred conformations of polyoxygenated, polymethylated alkanes **50**. TBS = *tert*-butyldimethylsilyl, Ac = acetyl, Bn = benzyl.

conformational preference in segment C is high but not as high as in the segments A and B. This can be attributed to the situation that the C-7/C-8 backbone bond in segment C no longer corresponds to a central bond in a tetramethylhexane **47**. Alternatively, since the C-9-OR and C-8-CH₃ groups are not necessarily antiperiplanar, the effect of C-9-OR as an inductor group on the conformation in segment C is only moderate. Such an antiperiplanar arrangement of C-9-OR and C-8-CH₃ may be enforced by combining the C-7 and C-9 oxygen-containing functional groups into an acetal ring.

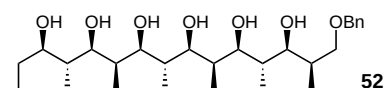
These considerations led to the synthesis of compound **51**, in which the conformational preference in each of the segments exceeds 90%. That the conformational preference does not yet exceed 95% can be recognized from the temperature dependence^[83] of the vicinal coupling constants: Their difference is increased slightly upon lowering the temperature from +20 to −20 °C (Scheme 25).



Scheme 25. The ³J_{H,H} coupling constants and preferred conformation of compound **51**.

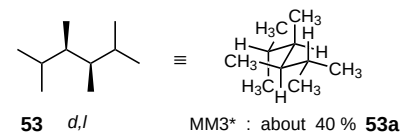
These results indicate how far a conformation design can be pushed based on highly substituted alkane chains. Another example, **52**, is provided from Paterson's group.^[84] In compound **52**, the slightly curved conformation of the main chain is stabilized by a sequence of hydrogen bonded bridges (Scheme 26).

The *meso*-tetramethylhexane **47** is not the only starting point that can attain molecular backbones with a high



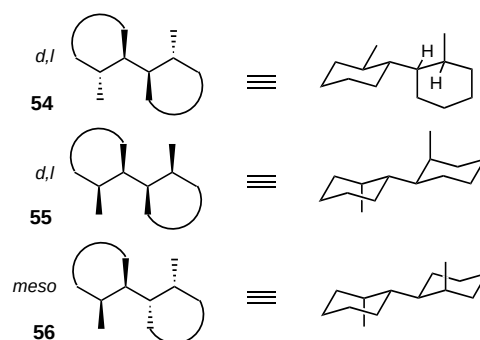
Scheme 26. Polyhydroxylated and polymethylated alkane chains having a high conformational preference for attaining a fully extended conformation.

conformational preference. The (*R*,R**)-2,3,4,5-tetramethylhexane **53** likewise constitutes a system in which rotation about the central bond out of the conformation **53a** generates two simultaneous *syn*-pentane interactions (Scheme 27).



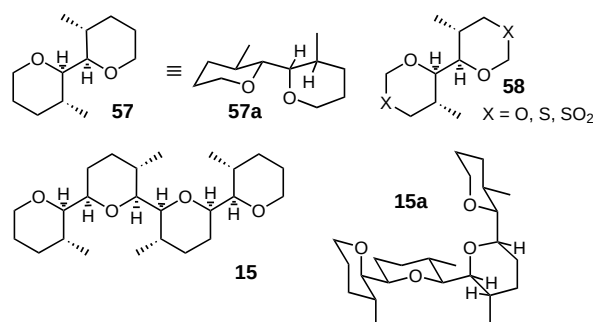
Scheme 27. Preferred conformation of *d,l*-2,3,4,5-tetramethylhexane.

Beginning with **53**, we would like to illustrate yet another way to avoid the dilemma of destabilizing the desired conformation **53a** by eight *gauche* interactions, which rendering the energy difference to the next higher conformation too small. Annellation of six-membered rings may help here too, since, for each inter-ring bond in a cyclohexane ring, one *gauche* arrangement is incorporated into the ring and is no longer destabilizing. Double annellation of six-membered rings of **53** generates either **54** or **55**. Likewise, ring annellation to the *meso*-compound **47** generates **56** (Scheme 28).



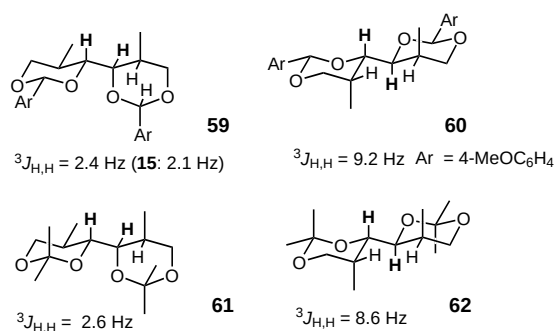
Scheme 28. Possible annellation of six-membered rings to 2,3,4,5-tetramethylhexane and preferred conformations of the resulting compounds.

Force field calculations indicate that all of these derivatives of dicyclohexyl should have a high conformational preference at the inter-ring bond. The largest energy difference between the most stable and the second-most stable conformation is calculated for compound **54**, in which the methyl groups are in an equatorial position. Still et al. had realized the high conformational preference of this system prior to our studies: They used the oxygenated derivatives **57**^[33, 37] as well as the acetals **58**^[34–36] to arrive at conformationally preorganized host molecules, such as **15**, to allow the enantioselective complexation of amino acids (Scheme 29).



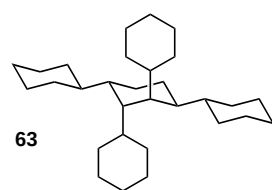
Scheme 29. Conformationally preorganized bis- and oligotetrahydropyrane derivatives investigated by Still et al.

Trixi Brandl, from our research group, synthesized and analyzed the benzylidene acetals **59** and **60** as well as the acetonides **61** and **62**. The $^3J_{\text{H,H}}$ coupling constants at the interring bond were taken from the ^{13}C side bands in the ^1H NMR spectrum. The values given in Scheme 30 attest to the high conformational preference.



Scheme 30. Conformationally preorganized 4,4'-di-1,3-dioxane derivatives.

A further dicyclohexyl derivative with a high conformational preference at the inter-ring bonds is the *ctc*-tetracyclohexylcyclohexane **63** described by Biali et al (Scheme 31).^[85]

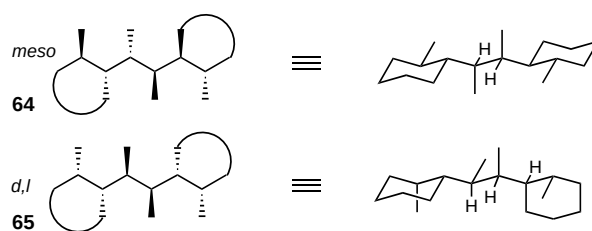


Scheme 31. 1,2,3,4-Tetracyclohexylcyclohexane with a high conformational preference.

The effective six-ring annelation can also be applied to the homologues of the tetramethylhexanes **47** and **53**. The larger molecules **64** and **65** display, according to force field calculations, a high conformational preference at all the skeletal bonds linking the two cyclohexane rings. The high conformational preference can be ascribed to the fact that any 120° rotation about these skeletal bonds of the conformation shown generates simultaneously two *syn*-pentane interactions (Scheme 32).

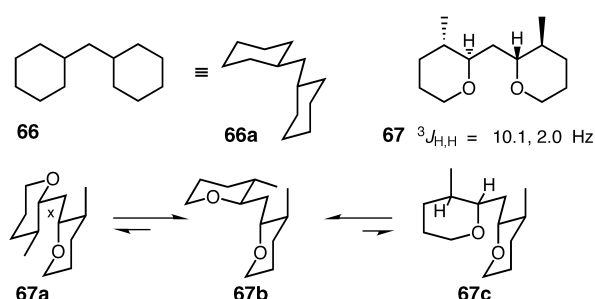
5. Dicyclohexylmethane Derivatives

The same situation can also be attained by suitable placement of methyl substituents at a dicyclohexylmethane skeleton **66**. By extension of the studies on the related



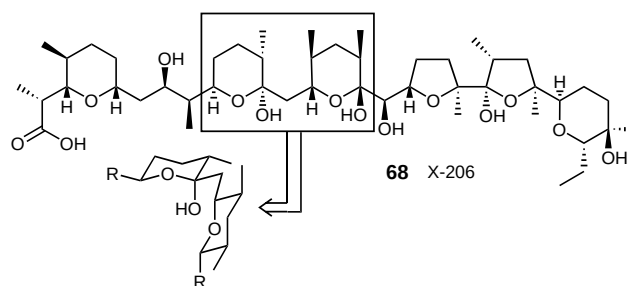
Scheme 32. Possible annellation of six-membered rings to hexamethyloctane and preferred conformations of the resulting compounds.

bis(tetrahydropyranyl)methane **28**, two methyl groups were introduced to give **67** (Scheme 33). The preferred conformation is **67b**. A $+120^\circ$ rotation around the bond $\times$ leads to a *syn*-hexane destabilization ($\rightarrow$ **67a**). A -120° rotation generates conformer **67c**, which is destabilized by a lesser *syn*-pentan interaction between two oxygen atoms. Kahrs determined the relevant $^3J_{\text{H,H}}$ coupling constants which showed that **67** possesses a higher conformational preference than the parent compound **28**.^[86]



Scheme 33. Conformational preferences of dicyclohexylmethane and bis(tetrahydropyranyl)methane derivatives.

Structures related to **67** are found in natural products: Such a bis(tetrahydropyranyl)methane unit is central to the structure of the antibiotic X-206 (**68**; Scheme 34).^[88] Here, the

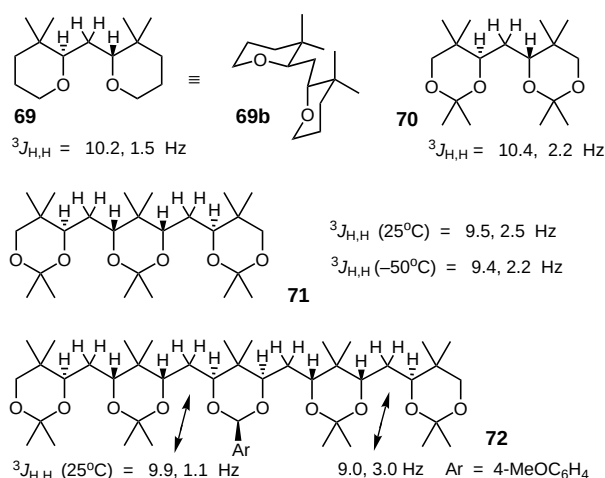


Scheme 34. A bis(tetrahydropyranyl)methane unit in the antibiotic X-206.

bis(tetrahydropyranyl)methane unit carries optimally positioned, buttressing methyl substituents. The resulting high local conformational preference is critical to a distinct folding of the total molecule into a form optimal for binding and transporting cations. In this case, conformational control is ostensibly linked to the molecular function.

The conformational preference of the skeleton **67** may be further improved by placing two additional methyl groups to generate **69** (Scheme 35). In **69**, the conformer corresponding

to **67c** is now stabilized as well by a *syn*-hexane situation. The coupling constants observed for **69**^[52] document the outstanding preference of this system to adopt conformation **69b**. This holds likewise for the corresponding acetone **70**.^[53] When advancing to the corresponding trimer **71** and the pentamer **72**, which have been synthesized by Thomas



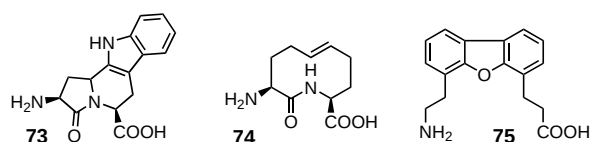
Scheme 35. Bis(tetrahydropyranyl)methane derivatives carrying buttressing substituents to reach a high conformational preference.

Trieselmann, one recognizes a small decrease in the differences of the coupling constants. This attests to the lowering of conformational preferences in larger molecules (the given coupling constants in **71** or **72** represent the average over two backbone segments!). Quite clearly, conformation control in larger molecules is a battle against the unavoidable loss of entropy. Nonetheless, compounds **70**–**72** from our third round of conformation design demonstrate how far effective conformation control can be extended towards larger compounds.

6. Applications of Conformation Design

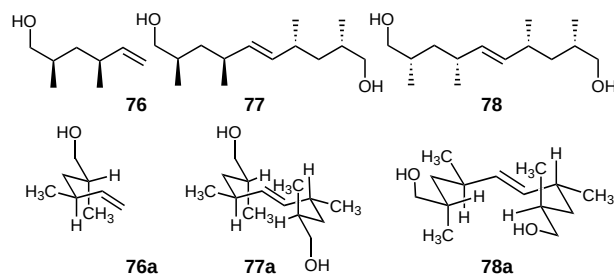
All our investigations described in the previous Sections were motivated by the desire to delineate the scope of a conformation design and to demonstrate that we can understand, as well as reproduce, nature's conformation designs. In the area of peptides, nature demonstrates how closely tied any biological activity is to a distinct backbone conformation, the "active conformation".^[89] For some of the biologically active peptides, one has well founded concepts as to the spatial disposition of the pharmacophoric groups. One tries to mimic these arrangements in rigid analogues as used in the development of peptidomimics.^[90] This may be briefly illustrated for β -turn mimics.^[91] A β -turn is a folding motif of a peptide chain, in which the chain direction is reversed in space to allow the formation of β -sheets. When designing β -turn mimics, one concentrates on rigid analogues, like **73**,^[92] or ones of limited conformational mobility, such as **74**^[93] or **75**^[94] (Scheme 36).

Conformationally mobile β -turn mimics would approximate the situation found in a natural β -turn more closely. It is



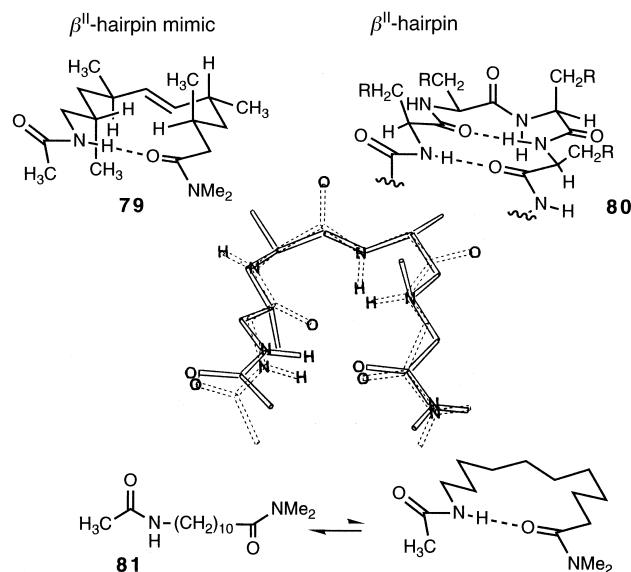
Scheme 36. Examples of β -turn mimics.

therefore a challenge for conformation design to develop fully flexible but conformationally preorganized β -turn mimics. We considered the alcohol **76** as the starting point for such an endeavour. Ulrich Schopfer showed—in congruence with the principles delineated in Section 2—that **76** populates conformation **76a** by about 80 % (Scheme 37). We then synthesized the diols **77** and **78**, in which two modules of the type **76** are joined in a heterochiral (**77**) and homochiral (**78**) manner. Compound **77** is expected to preferentially populate the Z-shaped conformation **77a**, while the stereoisomeric diol **78** populates, according to NOE experiments, the U-shaped conformation **78a**.^[95]



Scheme 37. Conformationally preorganized derivatives of *trans*-5-decene-1,10-diol.

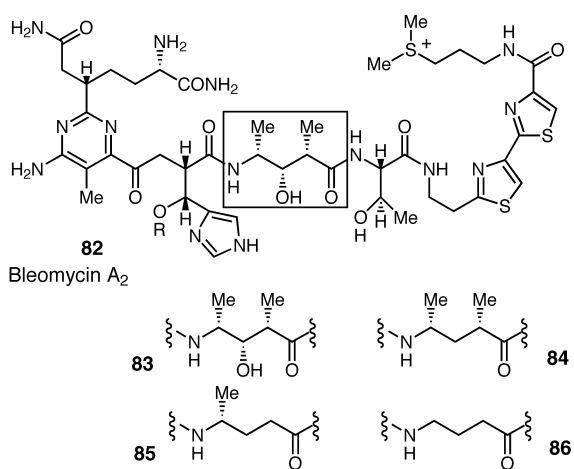
Conceptually, it is only a small step from **78** to **79**. An overlay of the most stable computed conformation of **79** with the structure of a prototypical β^{II} -hairpin^[96] demonstrates the marked similarity of the three-dimensional structures (Scheme 38).



Scheme 38. Comparison of the structures of a β -hairpin mimic and of a β -hairpin, as well as a 10-acetamidodecanoic amide, which is not preorganized conformationally.

Compound **79**, synthesized by Ulrich Schopfer and Trixi Brandl showed a high preference for a conformation with an intramolecular amide hydrogen bridge in its ^1H NMR and IR spectra, which corresponds to that of a β -hairpin.^[97] Compound **79** allows an easy identification of the elements of conformation design: the four methyl groups and the double bond. The analogous compound **81**, devoid of these elements, has the same distance between the amide moieties. Further, **81** is multiconformational as it possesses both intra- and intermolecularly NH-bridged conformations.

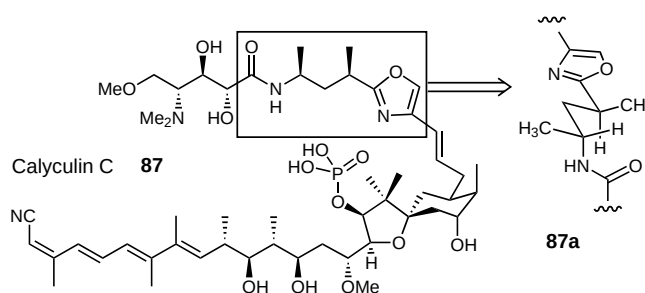
Other nonnatural amino acids, for example, β -^[98, 99] and γ -^[100] amino acids or certain THF-amino acids^[101] may be substituted in such a manner, that peptides derived thereof may have distinct spatial structures.^[99] Conformation design of such nonnatural amino acids is of particular interest because we have hitherto no information what advantage nature takes in using flexible backbones with preferred conformations instead of rigid modules in most biologically active compounds. Such information could be obtained by studying the consequences of changes in the disposition towards the active conformation of a given active compound while maintaining a constant distance between the pharmacophoric groups. Such a study has recently been reported for bleomycin A₂ **82**, in which a specific amino acid **83** serves as a conformationally preorganized linker unit between the two halves of the molecule containing the pharmacophoric groups^[102] (Scheme 39). Replacement of the linker **83** by other γ -amino acids **84** to **86** leads to a diminishing tendency



Scheme 39. Bleomycin A₂ and structural analogues.

to populate a preferred conformation and a commensurate drop in biological activity by a factor of ten.^[103] From these results, the group of Boger was able to clearly demonstrate a relationship between the tendency inherent in the backbone to populate a distinct active conformation with the biological activity.

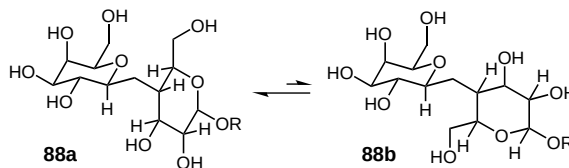
A derivative of an amino acid enantiomeric to **84** is found in the molecular backbone of calyculin C **87**.^[104] This unit has a high conformational preference and causes a distinct folding of the molecule (compare **87a**; Scheme 40). Surprisingly, the conformational preference of this linker unit is much higher in



Scheme 40. Calyculin C with a conformationally preorganized segment.

the natural product than in related model compounds like **84**.^[105] The reason for this remains to be clarified.

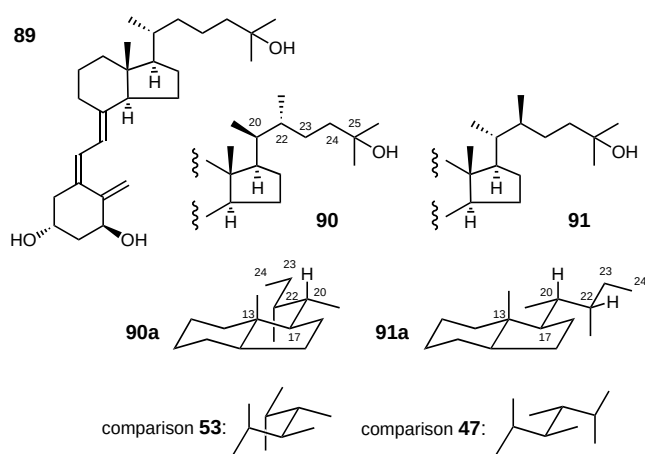
The interaction of a low molecular mass active compounds with a protein receptor leads to a bound molecule, the conformation of which is not necessarily identical with the most stable conformation in the unbound state. In these cases, the receptor selects one conformation from a pool of rapidly interconverting conformers of the guest. This one conformation corresponds to the active conformation. This has been reported for the binding of C-lactose **88** to bovine heart galectine.^[106] The Curtin–Hammett principle therefore applies to the binding process; the statistical chance of each conformer to bind is the same averaged over the conformer population (Scheme 41).



Scheme 41. The lowest energy conformers of C-lactose.

Of more interest are those cases in which a biologically active compound is bound to two receptors in different conformations. C-Lactose was found to bind to ricine in the *anti*-conformation **88a**, whereas binding to bovine heart galectine occurred in the *syn*-conformation **88b**.^[106, 107] This opened the possibility to synthesize rigid analogues of either **88a** or **88b** to achieve selective binding to the different receptors. Unfortunately, increasing the rigidity of biological active compounds, such as disaccharides, by adding bridging rings is not without further problems, as it does not guarantee a higher Gibbs free energy of binding to the corresponding receptor protein.^[108] It is therefore of interest to establish whether selectivity in the binding to different receptors can be attained by using conformationally flexible, but conformationally preorganized, analogues of the natural guest molecule.

For this reason, the following example from vitamin D research should be highlighted: 1 α ,25-Dihydroxy vitamin D₃ (**89**; Scheme 42) has multifaceted biological activities affecting the calcium and phosphorus metabolisms, cell differentiation, and regulation of the immune system. For ther-



Scheme 42. Conformationally preorganized analogues of 1 α ,25-dihydroxy vitamin D₃.

apeutic applications, it is necessary to address these biological responses separately. Thus, vitamin D₃ analogues are desired which lead to only one of these responses. It has been found that the steroid side chain of **89** is in a different conformation when bound to vitamin D receptor protein (VDR), which regulates calcium metabolism, or when bound to the vitamin D binding protein (DBP). This highlights that there are different and distinct active conformations of the C-17/C-25 side chain of vitamin D₃ and opens the opportunity to modify binding selectivity by conformation design.

By a change of the configuration of **89** at C-20, introduction of a further conformation-determining methyl group at C-22 produces **90** (its preferred conformation is indicated as **90a**), which binds more strongly to VDR than the parent **89**. Compound **90** is devoid of activity against DBP. The stereoisomeric compound **91** has only a low activity against VDR but binds strongly to DBP.^[109] Force field calculations suggest^[110] that in **90** the preferred conformation of C-13/C-17/C-20/C-22 is >90% *gauche* but in **91** >90% *trans*.^[111] The next chain segment C-20/C-22/C-23/C-24 remains biconformational. In order to rationalize the conformational preferences of **90** and **91**, it is instructive to recognize the tetramethylhexane substructures **53** in compound **90** and **47** in compound **91**.

7. Retrospect and Outlook

There is an optimal time for every research project. The investigations towards a conformation design could not have been carried out twenty years earlier because neither computer hardware nor software was sufficient to calculate conformer populations routinely. Further, NMR spectroscopy had not reached a state that allowed conformation analyses of molecules of the size (up to about 90 skeleton atoms) considered here. To successfully tackle this project, the methods of stereoselective synthesis should have reached a state of reliability which permitted not only the synthesis and study of single molecules of interest but also of whole sets of stereoisomeric compounds. It was only at the beginning of the

1990s that developments permitted us to address all of the questions associated with conformation design.

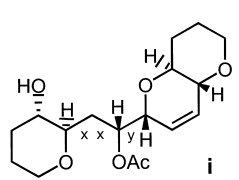
Now at the beginning of this new century, biologists are increasingly thinking in chemical terms, that is, on a molecular level by considering single molecules and their individual function. Organic chemists are moving their frontiers, in the context of biochemical problems, towards ever larger molecules and defined molecular assemblies; these systems are synthesized and studied because of their biological properties. The structure and properties of such molecules are related through their folding, to which conformation is the key. Conformation analyses of large molecules is now within reach using advanced NMR techniques. As soon as one can investigate the interrelation between conformation and properties of larger molecules, conformation design of such entities will move into focus. This will require the cooperation of synthetic chemists, computational chemists, spectroscopists, and physical chemists. From the perspective of biology and chemistry moving closer together, the research topic covered here marks a development from the chemistry of the 1990s to that of the 21st century. Conformation is the mediator between structure and function (molecular properties, biological activity). Control of conformation by conformation design is what nature has been doing ever from the beginning. It is our task to learn to do it likewise.

I would like to thank my co-workers, the names of whom were mentioned in the text, for their courage to undertake pioneering studies in an uncharted area, for their creative contributions, and for their perseverance. These studies were initially supported by the VW Stiftung and later by the Deutsche Forschungsgemeinschaft. Constant support came from the Fonds der Chemischen Industrie. I would like to thank all of these organizations for putting us in a position to carry out this work.

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- [1] a) R. W. Hoffmann, *Angew. Chem.* **1982**, *94*, 569–580; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 555–566; b) R. W. Hoffmann, *Angew. Chem.* **1987**, *99*, 503–517; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 489–503; c) R. W. Hoffmann, G. Dahmann, M. W. Andersen, *Synthesis* **1994**, 629–638.
- [2] D. O'Hagan, *The Polyketide Metabolites*, Ellis Horwood, Chichester, **1991**.
- [3] a) J. Cortes, S. F. Haydock, G. A. Roberts, D. J. Bevirt, P. F. Leadley, *Nature* **1990**, *348*, 176–178; b) S. Donadio, M. J. Staver, J. B. McAlpine, S. J. Swanson, L. Katz, *Science* **1991**, *252*, 675–679; c) R. Pieper, C. Kao, C. Khosla, G. Luo, D. E. Cane, *Chem. Soc. Rev.* **1996**, 297–302.
- [4] R. W. Hoffmann, *Angew. Chem.* **1992**, *104*, 1147–1157; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1124–1134.
- [5] a) W. C. Still, P. Hauck, D. Kempf, *Tetrahedron Lett.* **1987**, *28*, 2817–2820; b) P. K. Somers, T. J. Wandless, S. L. Schreiber, *J. Am. Chem. Soc.* **1991**, *113*, 8045–8056.
- [6] D. A. Evans, R. P. Polniaszek, K. M. DeVries, D. E. Guinn, D. J. Mathre, *J. Am. Chem. Soc.* **1991**, *113*, 7613–7630.
- [7] F. Johnson, *Chem. Rev.* **1968**, *68*, 375–413.
- [8] R. W. Hoffmann, *Chem. Rev.* **1989**, *89*, 1841–1860.
- [9] J. L. Broecker, R. W. Hoffmann, K. N. Houk, *J. Am. Chem. Soc.* **1991**, *113*, 5006–5017.
- [10] H. A. Brooks, D. Gardner, J. P. Poyser, T. J. King, *J. Antibiot.* **1984**, *37*, 1501–1504.

- [11] A. B. Charette, H. Juteau, H. Lebel, C. Molinaro, *J. Am. Chem. Soc.* **1998**, *120*, 11943–11952.
- [12] The dihedral angles which describe the folding of a hydrocarbon chain may be defined in an idealized way with reference to a Newman projection: *trans* (t) = 180°, *gauche*⁺ (g^+) = 60°, *gauche*[−] (g^-) = 300°.
- [13] S. Tsuzuki, L. Schäfer, H. Goto, E. D. Jemmis, H. Hosoya, K. Siam, K. Tanabe, E. Osawa, *J. Am. Chem. Soc.* **1991**, *113*, 4665–4671.
- [14] a) W. C. Still, D. Cai, D. Lee, P. Hauck, A. Bernardi, A. Romero, *Lect. Heterocycl. Chem.* **1987**, *9*, S33–S42; b) G. Quinkert, E. Egert, C. Griesinger, *Aspekte der Organischen Chemie, Vol. 1*, VCH, Weinheim, **1995**, p. 131.
- [15] K. U. Bindseil, A. Zeeck, *J. Org. Chem.* **1993**, *58*, 5487–5492.
- [16] H. Kinashi, K. Someno, K. Sakaguchi, T. Higashijima, T. Miyazawa, *Tetrahedron Lett.* **1981**, *22*, 3857–3860.
- [17] H. Seto, H. Akao, K. Furihata, N. Otake, *Tetrahedron Lett.* **1982**, *23*, 2667–2670.
- [18] G. Werner, H. Hagenmaier, K. Albert, H. Kohlshorn, *Tetrahedron Lett.* **1983**, *24*, 5193–5196.
- [19] G. H. Baker, P. J. Brown, R. J. J. Dorgan, J. R. Everett, S. V. Ley, A. M. Z. Slawin, D. J. Williams, *Tetrahedron Lett.* **1987**, *28*, 5565–5568.
- [20] a) S. P. Gunasekera, M. Gunasekera, R. E. Longley, G. K. Schulte, *J. Org. Chem.* **1990**, *55*, 4912–4915; b) S. P. Gunasekera, M. Gunasekera, R. E. Longley, G. K. Schulte, *J. Org. Chem.* **1991**, *56*, 1346.
- [21] R. W. Hoffmann, U. Weidmann, *Chem. Ber.* **1985**, *118*, 3966–3979.
- [22] R. W. Hoffmann, U. Weidmann, *Chem. Ber.* **1985**, *118*, 3980–3992.
- [23] J. K. Whitesell, B. Hildebrandt, *J. Org. Chem.* **1985**, *50*, 4975–4978.
- [24] a) B. Hildebrandt, H. Brinkmann, R. W. Hoffmann, *Chem. Ber.* **1990**, *123*, 869–873; b) M. Stahl, U. Schopfer, G. Frenking, R. W. Hoffmann, *J. Org. Chem.* **1996**, *61*, 8083–8088; c) M. Stahl, U. Schopfer, *J. Chem. Soc. Perkin Trans. 2* **1997**, 905–908; d) M. Stahl, U. Schopfer, G. Frenking, R. W. Hoffmann, *Mol. Phys.* **1997**, *92*, 569–579.
- [25] A. Eschenmoser, *Angew. Chem.* **1988**, *100*, 5–40; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 5–39.
- [26] R. B. Woodward, E. Logusch, K. P. Nambiar, K. Sakan, D. E. Ward, B.-W. Au-Yeung, P. Balaram, L. J. Browne, P. J. Card, C. H. Chen, R. B. Chênevert, A. Fliri, K. Frobel, H.-J. Gais, D. G. Garratt, K. Hayakawa, W. Heggie, D. P. Hesson, D. Hoppe, I. Hoppe, J. A. Hyatt, D. Ikeda, P. A. Jacobi, K. S. Kim, Y. Kobuke, K. Kojima, K. Krowicki, V. J. Lee, T. Leutert, S. Malchenko, J. Martens, R. S. Matthews, B. S. Ong, J. B. Press, T. V. Rajan Babu, G. Rousseau, H. M. Sauter, M. Suzuki, K. Tatsuta, L. M. Tolbert, E. A. Truesdale, I. Uchida, Y. Ueda, T. Uyehara, A. T. Vasella, W. C. Vladuchick, P. A. Wade, R. M. Williams, H. N. C. Wong, *J. Am. Chem. Soc.* **1981**, *103*, 3213–3215.
- [27] G. Stork, S. D. Rychnovsky, *J. Am. Chem. Soc.* **1987**, *109*, 1565–1566, 6904.
- [28] O. Yonemitsu, *Organic Synthesis in Japan; Past, Present, and Future* (Ed.: R. Noyori) Tokyo Kagaku Dozin, Tokyo, **1992**, pp. 557–565.
- [29] I. Paterson, A. Ward, P. Romea, R. D. Norcross, *J. Am. Chem. Soc.* **1994**, *116*, 3623–3624.
- [30] “Stereoselective synthesis of polyketide natural products—achievements and future development”: R. W. Hoffmann in *Stereocontrolled Organic Synthesis* (Ed.: B. M. Trost) Blackwell Scientific Publications, London, **1994**, pp. 259–274.
- [31] W. Hüchel, *Ber. Dtsch. Chem. Ges.* **1949**, *82*, I–X.
- [32] P. W. Smith, W. C. Still, *J. Am. Chem. Soc.* **1988**, *110*, 7917–7919.
- [33] T. Iimori, S. D. Erickson, A. L. Rheingold, W. C. Still, *Tetrahedron Lett.* **1989**, *30*, 6947–6950.
- [34] G. Li, W. C. Still, *J. Org. Chem.* **1991**, *56*, 6964–6966.
- [35] a) G. Li, W. C. Still, *Tetrahedron Lett.* **1993**, *34*, 919–922; b) G. Li, W. C. Still, *Tetrahedron Lett.* **1992**, *33*, 5929–5932.
- [36] S. Tang, W. C. Still, *Tetrahedron Lett.* **1993**, *34*, 6701–6704.
- [37] a) X. Wang, S. D. Erickson, T. Iimori, W. C. Still, *J. Am. Chem. Soc.* **1992**, *114*, 4128–4137; b) S. D. Erickson, M. H. J. Ohlmeyer, W. C. Still, *Tetrahedron Lett.* **1992**, *33*, 5925–5928; c) G. Li, W. C. Still, *J. Am. Chem. Soc.* **1993**, *115*, 3804–3805; d) M. T. Burger, A. Armstrong, F. Guarnieri, D. Q. McDonald, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 3593–3594.
- [38] See: S. Kudis, G. Helmchen, *Angew. Chem.* **1998**, *110*, 3210–3212; *Angew. Chem. Int. Ed.* **1998**, *37*, 3047–3050.
- [39] a) R. W. Alder, C. M. Maunders, A. G. Orpen, *Tetrahedron Lett.* **1990**, *31*, 6717–6720; b) R. W. Alder, P. R. Allen, D. Hnyk, D. W. H. Rankin, H. E. Robertson, B. A. Smart, R. J. Gillespie, I. Bytheway, *J. Org. Chem.* **1999**, *64*, 4226–4232.
- [40] R. W. Alder, P. R. Allen, K. R. Anderson, C. P. Butts, E. Khosravi, A. Martin, C. M. Maunders, A. G. Orpen, C. B. St. Purcain, *J. Chem. Soc. Perkin Trans. 2* **1998**, 2083–2107.
- [41] S. Cauwberghs, P. J. De Clercq, *Tetrahedron Lett.* **1988**, *29*, 2493–2496.
- [42] “Conformation analysis of acyclic and alicyclic saturated hydrocarbons”: J. E. Anderson in *The Chemistry of Alkanes and Cycloalkanes* (Eds.: S. Patai, Z. Rapoport), Wiley, New York, **1992**, pp. 95–133.
- [43] P. L. Luisi, *Naturwissenschaften* **1977**, *64*, 569–574.
- [44] S. Pucci, M. Aglietto, P. L. Luisi, *J. Am. Chem. Soc.* **1967**, *89*, 2787–2788.
- [45] J. Firl, G. Kresze, T. Bosch, V. Arndt, *Liebigs Ann. Chem.* **1978**, 87–97.
- [46] M. D. Lewis, Y. Kishi, *Tetrahedron Lett.* **1982**, *23*, 2343–2346.
- [47] D. V. Patel, F. VanMiddlesworth, J. Donaubaue, P. Gannett, C. J. Sih, *J. Am. Chem. Soc.* **1986**, *108*, 4603–4614.
- [48] a) N. L. Allinger, J. Allinger, *Struktur Organischer Moleküle*, Thieme, Stuttgart, **1974**, p. 128; see also: b) O. Golan, Z. Goren, S. E. Biali, *J. Am. Chem. Soc.* **1990**, *112*, 9300–9307.
- [49] Y. Yamamoto, H. Nemoto, R. Kikuchi, H. Komatsu, I. Suzuki, *J. Am. Chem. Soc.* **1990**, *112*, 8598–8599.
- [50] a) H. Tadokoro, *Structure of Crystalline Polymers*, Wiley, New York, **1979**, chap. 2; b) A. E. Tonelli, *Annu. Rep. NMR Spectrosc.* **1997**, *34*, 185–229, and references therein.
- [51] R. W. Hoffmann, T. Sander, M. Brumm, *Chem. Ber.* **1992**, *125*, 2319–2324.
- [52] R. W. Hoffmann, B. C. Kahrs, J. Schiffer, J. Fleischhauer, *J. Chem. Soc. Perkin Trans. 2* **1996**, 2407–2414.
- [53] R. W. Hoffmann, B. C. Kahrs, *Tetrahedron Lett.* **1996**, *37*, 4479–4482.
- [54] M. Brufani, S. Cerrini, W. Fedeli, C. Musu, L. Cellai, W. Keller-Schierlein, *Experientia* **1971**, *27*, 604–606.
- [55] R. W. Hoffmann, U. Rolle, R. Göttlich, *Liebigs Ann.* **1996**, 1717–1724.
- [56] R. Göttlich, Diploma thesis, University of Marburg (Germany), **1993**.
- [57] R. Göttlich, T. Fäcke, U. Rolle, R. W. Hoffmann, *J. Chem. Soc. Perkin Trans. 2* **1996**, 2059–2063.
- [58] The $^3J_{\text{H,H}}$, $^3J_{\text{H,C}}$, and $^3J_{\text{C,C}}$ coupling constants are a function of the dihedral angle as defined by the Karplus relationship. They are, therefore, conformation dependent. Since rotation around the skeletal bonds is rapid on the NMR timescale, the experimental coupling constants are the population-weighted average of the coupling constants of the individual conformers. In biconformational situations, that is, conformer equilibria in which predominantly two conformers contribute (> 80%), an estimate of the position of the conformer equilibrium can be made by comparison of the experimental coupling constant with those calculated for the two contributing conformers.
- [59] T. Fäcke, S. Berger, *Magn. Reson. Chem.* **1995**, *33*, 144–148.
- [60] T. Fäcke, S. Berger, *J. Am. Chem. Soc.* **1995**, *117*, 9547–9550.
- [61] R. W. Hoffmann, R. Göttlich, *Liebigs Ann.* **1997**, 2103–2111.
- [62] G. Natta, *Angew. Chem.* **1956**, *68*, 393–403.
- [63] G. Natta, M. Peraldo, G. Allegra, *Makromol. Chem.* **1964**, *75*, 215–216.
- [64] R. Göttlich, U. Schopfer, M. Stahl, R. W. Hoffmann, *Liebigs Ann.* **1997**, 1757–1764.
- [65] J. A. Hirsch, *Top. Stereochem.* **1967**, *1*, 199–222.
- [66] a) G. A. Crowder, *J. Mol. Struct. (THEOCHEM)* **1979**, *53*, 297–300; b) G. A. Crowder, R. M. P. Jaiswal, *J. Mol. Struct. (THEOCHEM)* **1983**, *99*, 93–100; c) F. Bentley, N. T. McDevitt, A. L. Rozek, *Spectrochim. Acta* **1964**, *20*, 105–126.
- [67] R. W. Hoffmann, D. Stenkamp, T. Trieselmann, R. Göttlich, *Eur. J. Org. Chem.* **1999**, 2915–2927.
- [68] a) N. D. Epiotis, R. L. Yates, J. R. Larson, C. R. Kirmaier, F. Bernardi, *J. Am. Chem. Soc.* **1977**, *99*, 8379–8388; b) T. K. Brunck,

- F. Weinhold, *J. Am. Chem. Soc.* **1979**, *101*, 1700–1709; c) P. Dionne, M. St. Jacques, *J. Am. Chem. Soc.* **1987**, *109*, 2616–2623; d) D. Parker, K. Senanayake, J. Vepsäläinen, S. Williams, A. S. Batsanov, J. A. K. Howard, *J. Chem. Soc. Perkin Trans. 2* **1997**, 1445–1452; e) H. Senderowitz, L. Golender, B. Fuchs, *Tetrahedron* **1994**, *50*, 9707–9728; f) W. T. Borden, *Chem. Commun.* **1998**, 1919–1925; g) P. R. Rablen, R. W. Hoffmann, D. A. Hrovat, W. T. Borden, *J. Chem. Soc. Perkin Trans. 2* **1999**, 1719–1726.
- [69] K. Matsuzaki, K. Sakota, M. Okada, *J. Polym. Sci. Part A* **1969**, *7*, 1444–1449.
- [70] For 1,3-difluoropropane in the gaseous state, see: R. Glaser, I. Adin, D. Shiftan, Q. Shi, H. M. Deutsch, C. George, K.-M. Wu, M. Froimowitz, *J. Org. Chem.* **1998**, *63*, 1785–1794.
- [71] See also: R. Göttlich, B. C. Kahrs, J. Krüger, R. W. Hoffmann, *Chem. Commun.* **1997**, 247–251.
- [72] D. Doskocilova, B. Schneider, *Coll. Czech. Chem. Commun.* **1964**, *29*, 2290–2308.
- [73] a) P. E. McMahon, R. L. McCullough, *Trans. Faraday Soc.* **1964**, *60*, 2089–2096; b) P. E. McMahon, *Trans. Faraday Soc.* **1965**, *61*, 197–200; c) P. E. McMahon, W. C. Tincher, *J. Mol. Spectrosc.* **1965**, *15*, 180–198; d) B. Schneider, J. Stokr, D. Doskocilova, S. Sykora, J. Jakes, M. Kolinsky, *J. Polym. Sci. Ser. C* **1969**, 1073–1084; e) T. Moritani, Y. Fujiwara, *J. Chem. Phys.* **1973**, *59*, 1175–1189.
- [74] T. Shimanouchi, S. Tsuchiya, S. Mizushima, *J. Chem. Phys.* **1959**, *30*, 1365–1366.
- [75] a) D. Doskocilova, J. Stokr, B. Schneider, H. Pivcova, M. Kolinsky, J. Petranek, D. Lim, *J. Polym. Sci. Part C* **1967**, *16*, 215–228; b) P. J. Flory, C. J. Pickles, *J. Chem. Soc. Faraday Trans. 2* **1973**, *69*, 632–642; c) A. E. Tonelli, F. C. Schilling, W. H. Starnes, Jr., L. Shepherd, I. M. Plitz, *Macromolecules* **1979**, *12*, 78–83.
- [76] R. W. Hoffmann, D. Stenkamp, *Tetrahedron* **1999**, *55*, 7169–7176.
- [77] R. W. Hoffmann, M. Stahl, U. Schopfer, G. Frenking, *Chem. Eur. J.* **1998**, *4*, 559–566.
- [78] P. Reiss, Diploma thesis, University of Marburg (Germany), **1998**.
- [79] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Cauffield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
- [80] R. Göttlich, PhD thesis, University of Marburg (Germany), **1996**.
- [81] G. Natta, P. Corradini, *J. Polym. Sci.* **1959**, *39*, 29–46.
- [82] Such a conformation relaxes to another conformation. This second conformation is still quite strained and lies about 25 kJ mol⁻¹ above the most stable all-*trans* conformation.^[13]
- [83] D. Stenkamp, R. W. Hoffmann, R. Göttlich, *Eur. J. Org. Chem.* **1999**, 2929–2936.
- [84] a) I. Paterson, J. P. Scott, *Tetrahedron Lett.* **1997**, *38*, 7441–7444; b) I. Paterson, J. P. Scott, *Tetrahedron Lett.* **1997**, *38*, 7445–7448; c) I. Paterson, J. P. Scott, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1003–1014.
- [85] I. Columbus, R. E. Hoffman, S. E. Biali, *J. Am. Chem. Soc.* **1996**, *118*, 6890–6896.
- [86] Compound **i**, related to **67**, has a high conformational preference (about 85%) at the bonds marked *x* and adopts a conformation corresponding to **67b**. At the bond *y*, two conformers are populated in a *gauche* ordering of the oxygen functions.^[87]
- 
- [87] R. M. Rodriguez, E. Q. Morales, M. Delgado, C. G. Espinola, E. Alvarez, R. Pérez, J. D. Martin, *Org. Lett.* **1999**, *1*, 725–728.
- [88] a) J. F. Blount, J. W. Westley, *J. Chem. Soc. Chem. Commun.* **1975**, 533–533; b) A. C. O'Sullivan, F. Struber, *Helv. Chim. Acta* **1998**, *81*, 812–827.
- [89] a) H. Kessler, *Angew. Chem.* **1982**, *94*, 509–520; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512–523; b) H. Kessler, *Trends in Drug Research, Proceedings of the 7th Noordwijkerhout–Camerino Symposium* (Ed.: V. Claassen), Elsevier, Amsterdam, **1990**, pp. 73–84.
- [90] J. Gante, *Angew. Chem.* **1994**, *106*, 1780–1802; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699–1720.
- [91] a) J. P. Schneider, J. W. Kelly, *Chem. Rev.* **1995**, *95*, 2169–2187; b) G. Müller, G. Hessler, H. Y. Decornez, *Angew. Chem.* **2000**, *112*, 926–928; *Angew. Chem. Int. Ed.* **2000**, *39*, 894–896.
- [92] D. Andreu, S. Ruiz, C. Carreno, J. Alsina, F. Albericio, M. A. Jiménez, N. de la Figuera, R. Herranz, M. T. Carcia-López, R. González-Muniz, *J. Am. Chem. Soc.* **1997**, *119*, 10579–10586.
- [93] B. E. Fink, P. R. Kym, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **1998**, *120*, 4334–4344.
- [94] F. Jean, E. Buisine, O. Melnyk, H. Drobecq, B. Odaert, M. Hugues, G. Lippens, A. Tartar, *J. Am. Chem. Soc.* **1998**, *120*, 6076–6083.
- [95] R. W. Hoffmann, U. Schopfer, M. Stahl, *Tetrahedron Lett.* **1997**, *38*, 4055–4058.
- [96] a) J. B. Ball, R. A. Hughes, P. F. Alewood, P. R. Andrews, *Tetrahedron* **1993**, *49*, 3467–347; b) A β -turn comprises a peptide chain of four amino acids with a hydrogen bond between the NH group of the fourth amino acid to the carbonyl group of the first amino acid. A β -hairpin has, at both the N-terminal and the C-terminal end of a β -turn, at least one further amino acid which forms additional hydrogen bonds between one another.
- [97] U. Schopfer, M. Stahl, T. Brandl, R. W. Hoffmann, *Angew. Chem.* **1997**, *109*, 1805–1807; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1745–1747.
- [98] a) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015–2022; b) D. Seebach, K. Gademann, J. V. Schreiber, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel, H. Widner, *Helv. Chim. Acta* **1997**, *80*, 2033–2038; c) K. Gademann, B. Jaun, D. Seebach, R. Perozzo, L. Scapozzo, G. Folkers, *Helv. Chim. Acta* **1999**, *82*, 1–11; d) D. H. Appella, J. J. Barchi, Jr., S. R. Durell, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 2309–2310; e) B. W. Gung, D. Zou, A. M. Stalcup, C. E. Cottrell, *J. Org. Chem.* **1999**, *64*, 2176–2177; f) D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* **1999**, *111*, 1700–1703; *Angew. Chem. Int. Ed.* **1999**, *38*, 1595–1597; g) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 6206–6212; h) S. Abele, K. Vögtli, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1539–1558; i) S. Abele, P. Seiler, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1559–1571; j) M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1774–1783.
- [99] S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180.
- [100] a) S. Hanessian, X. Luo, R. Schaum, S. Michnick, *J. Am. Chem. Soc.* **1998**, *120*, 8569–8570; b) T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 983–1002; c) S. Hanessian, X. Luo, R. Schaum, *Tetrahedron Lett.* **1999**, *40*, 4925–4929.
- [101] a) H. Wagner, K. Harms, U. Koert, S. Meder, G. Boheim, *Angew. Chem.* **1996**, *108*, 2836–2839; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2643–2646; b) M. D. Smith, T. D. W. Claridge, G. E. Tranter, M. S. P. Sanson, G. W. J. Fleet, *Chem. Commun.* **1998**, 2041–2042; c) T. D. W. Claridge, D. D. Long, N. L. Hungerford, R. T. Aplin, M. D. Smith, D. G. Marquess, G. W. Fleet, *Tetrahedron Lett.* **1999**, *40*, 2199–2202.
- [102] T. Owa, A. Haupt, M. Otsuka, S. Kobayashi, N. Tomioka, A. Itai, M. Ohno, T. Shiraki, M. Uesugi, Y. Sugiura, K. Maeda, *Tetrahedron* **1992**, *48*, 1193–1208.
- [103] a) D. L. Boger, T. M. Ramsey, H. Cai, S. T. Hoehn, J. Stubbe, *J. Am. Chem. Soc.* **1998**, *120*, 9149–9158; b) D. L. Boger, H. Cai, *Angew. Chem.* **1999**, *111*, 470–500; *Angew. Chem. Int. Ed.* **1999**, *38*, 448–476.
- [104] Y. Kato, N. Fusetani, S. Matsunaga, K. Hashimoto, K. Koseki, *J. Org. Chem.* **1988**, *53*, 3930–3932.
- [105] a) R. W. Hoffmann, M. A. Lazaro, F. Caturra, E. Framery, I. Valancogne, C. A. G. N. Montalbetti, *Tetrahedron Lett.* **1999**, *40*, 5983–5986; b) R. W. Hoffmann, F. Catura, M. A. Lazaro, E. Framery, M. C. Bernabeu, I. Valancogne, C. A. G. N. Montalbetti, *New J. Chem.* **2000**, *24*, 187–194.
- [106] J. L. Asensio, J. F. Espinosa, H. Dietrich, F. J. Canada, R. R. Schmidt, M. Martin-Lomas, S. Andre, H.-J. Gabius, J. Jimenez-Barbero, *J. Am. Chem. Soc.* **1999**, *121*, 8995–9000.
- [107] J.-F. Espinosa, F. J. Canada, J. L. Asensio, M. Martin-Pastor, H. Dietrich, M. Martin-Lomas, R. R. Schmidt, J. Jimenez-Barbero, *J. Am. Chem. Soc.* **1996**, *118*, 10862–10871.

- [108] a) M. Wilstermann, J. Balogh, G. Magnusson, *J. Org. Chem.* **1997**, 62, 3659–3665; b) D. R. Bundle, R. Alibés, S. Nilar, A. Otter, M. Warwas, P. Zhang, *J. Am. Chem. Soc.* **1998**, 120, 5317–5318; c) S. A. Wakowich-Sgarbi, D. R. Bundle, *J. Org. Chem.* **1999**, 64, 9080–9089.
- [109] a) K. Yamamoto, W. Y. Sun, M. Ohta, K. Hamada, H. F. DeLuca, S. Yamada, *J. Med. Chem.* **1996**, 39, 2727–2737; b) K. Yamamoto, H. Oozumi, K. Umesono, A. Verstuyf, R. Bouillon, H. F. DeLuca, T. Shinki, T. Suda, S. Yamada, *Bioorg. Med. Chem. Lett.* **1999**, 9, 1041–1046.
- [110] See: M. Gonzales-Sierra, D. A. Bustos, E. A. Ruveda, A. C. Olivieri, M. Grasselli, *Can. J. Chem.* **1988**, 66, 71–75.
- [111] D. Van Haver, P. J. De Clercq, *Bioorg. Med. Chem. Lett.* **1998**, 8, 1029–1034.
- [112] See: W. L. Duax, J. F. Griffin, D. C. Rohrer, C. M. Weeks, *Lipids* **1980**, 15, 783–792.

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A Diborane Story**

Pierre Laszlo*

Just as with ferrocene,^[1] the formula first written for diborane B₂H₆ was orthodox, conforming with the usual paradigmatic rules regarding molecular structure. However, it was wrong. Correcting the mistake showed an extremely unusual bonding picture for the molecule. Just as in the ferrocene case, it opened up a whole new chapter of chemistry, later recognized with the award of a Nobel prize to William N. Lipscomb in 1976 for “his studies on the structure of boranes illuminating problems of chemical bonding.”

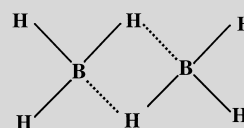
I shall highlight here only a few episodes from the diborane story. Act 1: In 1937, a former student of Linus Pauling, Simon H. Bauer—an outstanding physical chemist still very much active in research at Cornell at the age of 88—applied the technique of electron diffraction, a tool that he had learned to use at Caltech with Pauling, to diborane. He found and reported a structure analogous to that of ethane, which he therefore wrote as H₃B–BH₃.^[2] In 1942 Bauer reiterated his contention of the ethane-like structure for diborane.^[3]

At the same time of the early 1940s, H. I. Schlesinger, a chemistry professor at the University of Chicago, was also working on boron compounds. One of the reasons was his involvement in the Manhattan Project: it was thought that separation of the isotopes of uranium could be devised using such derivatives. In any case, Schlesinger was very much interested in the structure of diborane.

Thus Schlesinger wrote a letter to Linus Pauling at Caltech on January 3 1941.^[4] Let me quote:

As a result of our work on the metallo borohydrides I definitely feel that a structure for diborane quite different from those generally proposed, would aid in correlating many of the observations we have made. ... Curiously enough I have just now received a reprint of a Russian article on hydrides of boron... I gather from some of the formulae in the article that the author has come to a conclusion very similar to mine.

The structure I have in mind is a bridge structure, in which the two boron atoms are joined to each other through an unusual type of hydrogen bond, perhaps best represented by the following formula



From hindsight we know this Schlesinger formula to be the correct one. Also, he ought to have shared in the recognition for proposing such a bridged structure. End of Act II.

Act III was brief. Pauling wrote back by return mail (January 7 1941)^[4]

I do not feel very friendly toward the structure which you mention in your letter for the diborane molecule. So long as the suggested structure remains vague and indefinite, it is not easy to say that it is eliminated by electron diffraction data or other data. However, the force constant for the B–B vibration is I think much stronger than would be expected for a structure of this type, in which there is no direct B–B bond.

In other words, at this point Pauling, with his considerable insider knowledge of structural chemistry, not only shot down the correct structure that Schlesinger had come up with, but he also indirectly convinced him not to bring it into print. What a shame!^[5] When Schlesinger did publish his review, he had summarized the chemical and physical properties of boron hydrides in terms of an ethane-like structure containing electron-deficient boron–hydrogen bonds.^[6]

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[**] I thank the Ava Helen and Linus Pauling Archive at Oregon State University in Corvallis, Oregon, and its director Clifford Mead for permission to quote from Pauling's correspondence, and Professor Roald Hoffmann for a useful discussion.

Act IV occurred soon afterwards. An undergraduate student by the name of H. Christopher Longuet-Higgins was at Balliol College, in Oxford. He was only in his second year of study when he became interested in this question of the diborane structure. He too came up with the same bridged structure after examining all the physical evidence and after thinking out an adequate theoretical description. His paper, signed jointly with one of the professors of chemistry from Balliol, R. P. Bell (a reputed physical chemist), appeared in 1943 in the *Journal of the Chemical Society*.^[7] Longuet-Higgins would go on to become one of the worlds leading chemical theoreticians.

There was even a precursor found (as is almost invariably the rule with science discoveries^[8]): Diltthey had anticipated the bridged structure as early as 1921^[9] and this was revived by Russian scientists in the early 1940s^[10, 11] (Schlesinger was referring to them in his letter to Pauling).

Act V saw the consolidation of the bridged structure of Schlesinger, Longuet-Higgins, and others (such as Diltthey and the Russian scientists). In 1947–48 Price confirmed the earlier (1940, 1941) infrared spectroscopic evidence by Stitt^[12, 13] and interpreted it in favor of the bridged structure.^[14, 15] Shoolery brought to bear clinching nmr evidence.^[16] Three different theorists (Mulliken, Pitzer, and Walsh) gave molecular-orbital and valence-bond descriptions of the bridged structure in 1946 and 1947.^[17–19] In 1951 Hedberg and Schomaker did another electron-diffraction study, which showed that the data ruled out the open structure and was consistent with the bridged structure only.^[20]

Yet, in the second edition of *The Nature of the Chemical Bond*, which came out in 1945, Linus Pauling devoted a full 3.5 pages to presenting diborane as an ethane-like structure on the strength of both G. N. Lewis's conceptualization^[21] of this molecule with "six electron pair bonds resonating amongst seven positions" and of S. H. Bauer's electron diffraction work.^[22] True, his influence on structural chemistry was on the wane: in the felicitous wording of Mary Jo Nye, his valence-bond approach to molecular structure "earned most chemists' allegiance, at least up until 1940."^[23]

Both stories, that of ferrocene^[1] and that of diborane, have a strong resemblance. They both bear out an account of scientific revolutions in the style of Kuhn.^[24] Both compounds were anomalies within the existing paradigms. Their representations disobeyed "conventional wisdom" to make use of the expression pioneered by Galbraith.^[25] Accordingly, they suffered from a birth defect. Both were incorporated into existing knowledge, at a high cost. Their formulas were inconsistent with some of the experimental evidence, some of the data had been inadvertently misread because of the paradigmatic bias.

Both stories are inconsistent with the notion, central to a sociologist of science such as Bloor, of a Symmetry Principle.^[26] According to this methodological edict the historian or the sociologist of science should give equal footing to the two types of theories of nature, whether they are winners or losers. There is, to the contrary, blatant dissymmetry in both the ferrocene^[1] and the diborane case. Their first investigators could not bring themselves to see, let alone accept, the correct

structure because the existing knowledge did not allow it. In order to make room for the correct formulation, two moves had to occur: a theoretical description for the novel entity had to be provided, and those parts of the experimental evidence earlier glossed over because they did not jibe with the postulated structure had to be retrieved from the waste basket. As an empirical natural philosopher, I must credit Thomas Kuhn with an operationally valid description, and I must beg to disagree with Bloor's symmetry principle: in both cases, the difference between the winning side and the losing side was not historically contingent and socially determined.

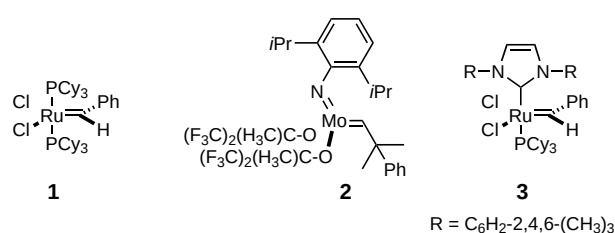
- [1] P. Laszlo, R. Hoffmann, *Angew. Chem.* **2000**, *112*, 127; *Angew. Chem. Int. Ed.* **2000**, *39*, 123.
- [2] S. H. Bauer, *J. Am. Chem. Soc.* **1937**, *59*, 1096.
- [3] S. H. Bauer, *Chem. Rev.* **1942**, *31*, 46.
- [4] This letter is held in the Pauling Archive at Oregon State University, in Corvallis.
- [5] The more so that valence-bond theory provides a fine description of diborane, accounting nicely for the strong boron–boron bond. Pauling was also mistaken in one comment: the force field is overdetermined and the molecular vibrations can be fit without a B–B bond. I am grateful to Roald Hoffmann for this clarification.
- [6] H. I. Schlesinger, A. B. Burg, *Chem. Rev.* **1942**, *31*, 1. Alfred Stock's dogma of an ethane-like structure for diborane also cast its long shadow.
- [7] H. C. Longuet-Higgins, R. P. Bell, *J. Chem. Soc.* **1943**, 250.
- [8] Here is an example: Bernal and Kauffman have shown (including experimentally!) that Edith Humphrey had prepared enantiomerically pure crystals during her Ph.D. in Alfred Werner's Zürich laboratory, which Werner never bothered to make polarimetric measurements on. Had he done so, he would have been provided with an unequivocal proof of the soundness of his coordination theory, for which he won the Nobel Prize: I. Bernal, G. B. Kauffman, *J. Chem. Educ.* **1987**, *67*, 604; I. Bernal, *The Chemical Intelligencer* **1999**, 28–31. For priority and multiple discoveries, see P. Laszlo, *La découverte scientifique*, PUF, Paris, **1999**.
- [9] W. Diltthey, *Z. Angew. Chem.* **1921**, *34*, 596.
- [10] B. V. Nekrassov, *J. Gen. Chem. URSS* **1940**, *10*, 1021; B. V. Nekrassov, *J. Gen. Chem. URSS* **1940**, *10*, 1156.
- [11] Y. K. Syrkin, M. E. Dyatkina, *Acta Physicochim. URSS* **1941**, *14*, 547. These insightful and brave scientists suffered much as a consequence of the infamous 1951 resonance theory conference and scandal (Roald Hoffmann, private communication).
- [12] F. Stitt, *J. Chem. Phys.* **1940**, *8*, 981.
- [13] F. Stitt, *J. Chem. Phys.* **1941**, *9*, 780.
- [14] W. C. Price, *J. Chem. Phys.* **1947**, *15*, 614.
- [15] W. C. Price, *J. Chem. Phys.* **1948**, *16*, 894.
- [16] J. N. Shoolery, *Discuss. Faraday Soc.* **1955**, *19*, 215.
- [17] R. S. Mulliken, *Chem. Rev.* **1947**, *41*, 207.
- [18] K. S. Pitzer, *J. Am. Chem. Soc.* **1946**, *67*, 1126.
- [19] A. D. Walsh, *J. Chem. Soc.* **1947**, 89.
- [20] K. Hedberg, V. Schomaker, *J. Am. Chem. Soc.* **1951**, *73*, 1482.
- [21] G. N. Lewis, *J. Chem. Phys.* **1933**, *1*, 17.
- [22] L. Pauling, *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, NY, USA, **1945**, p. 259.
- [23] M. J. Nye, *Before Big Science. The Pursuit of Modern Chemistry and Physics 1800–1940*, Twayne & Prentice Hall International, New York, **1996**, p. 184.
- [24] T. S. Kuhn, *The Structure of Scientific Revolutions*, University of Chicago Press, **1996**.
- [25] J. K. Galbraith, *The Affluent Society*, Houghton Mifflin, Boston, MA, USA, **1958**.
- [26] D. Bloor, *Knowledge and Social Imagery*, University of Chicago Press, Chicago, **1976** (2nd edition with a new foreword, **1991**).

Synthesis of Medium-Sized Rings by the Ring-Closing Metathesis Reaction

Martin E. Maier*

The construction of rings represents a central theme in natural product synthesis.^[1] Among the various available options, such as cycloaddition reactions, ring transformations, and cyclization reactions, the latter are probably the most commonly used because of the number of possible initiators and terminators. However, not every ring size is accessible with the same ease. Because of enthalpic (increasing strain in the transition state) and entropic influences (probability of the chain ends meeting), medium-sized rings are the most difficult to prepare. Among the many cyclization reactions, the olefin metathesis reaction (ring-closing olefin metathesis, RCM) has gained enormous popularity in recent years.^[2–7] In this reaction, nonconjugated dienes are converted to cyclic alkene structures in the presence of a metal carbene complex. Catalysts developed by Grubbs and Schrock are commonly used for that purpose due to their high reactivity.

The Grubbs catalyst **1** (Cy = cyclohexyl) is very easy to prepare, commercially available, and air stable.^[8] On the other hand, the preparation of the Schrock catalyst **2** is a more elaborate undertaking.^[9] Nonetheless, **2** is also commercially available. Recently, novel air- and water-tolerant ruthenium complexes like **3** were introduced and shown to exhibit increased metathesis activity as compared to **1**.^[10, 11] These complexes are even able to induce cyclization to tetrasubstituted cycloalkenes.

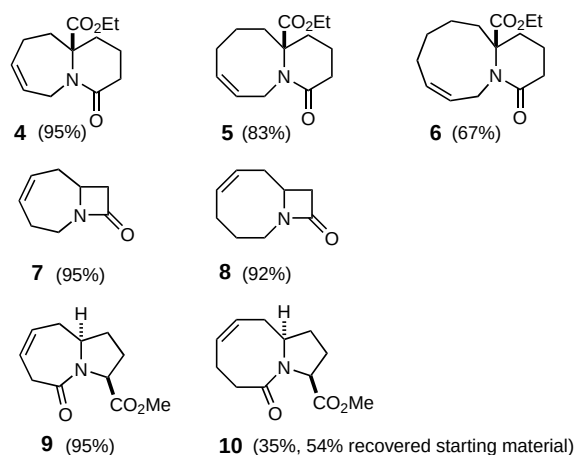


A great advantage of the RCM is the involvement of relatively stable double bonds. This facilitates synthesis and handling of the cyclization precursors. In light of this, it is no wonder that the McMurry cyclization^[12, 13] of dialdehydes has some serious competition.

An elegant application of the RCM reaction has been the formation of the 16-membered macrocycle of the epothilones.^[14–20] Other natural product syntheses in which the RCM reaction has been instrumental include those of roseophilin,^[21–23] and manzamine-type alkaloids,^[24] among others.^[7] In addition, an RCM reaction was used in a total synthesis of the streptogramin antibiotic (–)-griseoviridin to combine a terminal 1,3-dienyl group and an alkenyl group to provide the macrocyclic ring with a 1,3-diene.^[25]

In contrast, only recently were successful RCM reactions for medium-sized rings reported. In order to facilitate the desired cyclization, several features were installed in the substrate that provide some sort of conformational constraint. These constraints were achieved either by using preexisting rings (cyclic conformational constraints) or acyclic conformational constraints. In the following text, I will illustrate some of this work.

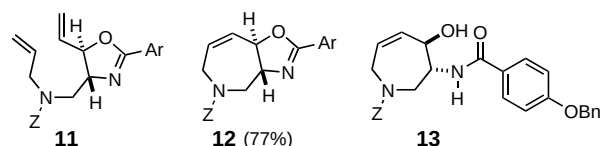
Attachment of olefinic side chains to lactams facilitates the RCM cyclization, as shown by several groups.^[26] For example, seven- to nine-membered bicyclic lactams **4–6** were formed easily using the Grubbs catalyst **1** in dichloromethane at room temperature.^[27] Similarly, macrocyclic rings could be annulated onto a β -lactam (**7, 8**)^[28] and onto a pyrrolidine ring system (**9, 10**).^[29] Compounds of this type are of interest as turn-inducing structures in small peptides.



In order to establish a seven-membered nitrogen heterocycle carrying a 1,2-amino alcohol by an RCM cyclization, the group of Cook et al. used an oxazoline ring as a constraint.^[30] The substrate **11** was prepared from D-serine utilizing a

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palladium-catalyzed equilibration of vinyloxazolines to control the stereochemistry of the vicinal amino alcohol. As deduced from force-field calculations, the torsional angle of the side chains in **11** is about 95°, which is easily accommodated by the bicyclic structure **12**. Accordingly, the alkene metathesis reaction with 10 mol % of the Grubbs catalyst in refluxing dichloromethane furnished the heterocycle **12** in 77% yield. Compound **12** was transformed into the hexahydroazepine **13**, which is an intermediate in the synthesis of balanol, a potent protein kinase C inhibitor.



The RCM approach turned out to be very useful for the synthesis of medium-sized oxacycles. Such cyclic ethers occur in a number of biologically active natural products such as the brevetoxins. If the olefinic side chains are positioned on the ring vicinal to each other, the cyclizations are very facile. Examples include the *trans*-fused polyether systems **14** (Figure 1).^[31, 32] Only for the nine-membered ring does a decreased yield point to some steric hindrance in the transition state. Further, the oxygen atoms seem not to interfere with the catalytic cycle. Macrocyclic spiro rings of type **15** could be additionally attached to a sugar derivative.^[33]

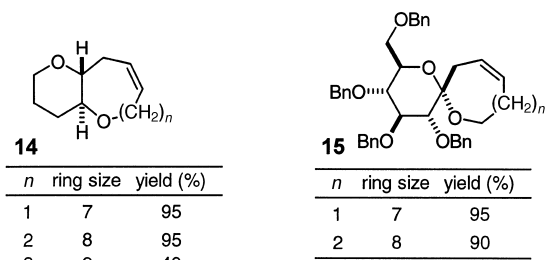
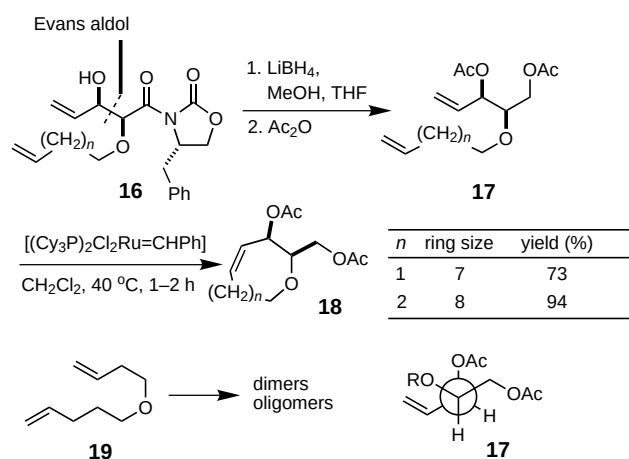


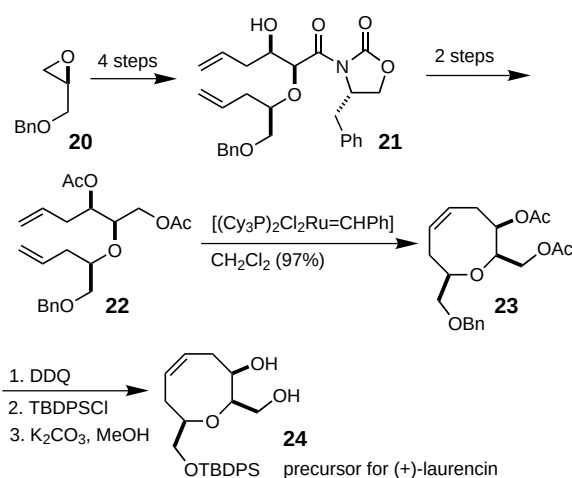
Figure 1. Grubbs catalyst **1** in the preparation of oxacyclene. Bn = benzyl.

Besides using a ring in order to limit the conformational freedom of a chain, appropriately positioning substituents on the chain carrying the two olefinic groups can boost the macrocyclization. The group of Crimmins reported on the use of the *gauche* effect of 1,2-dioxygen substituents to facilitate the ring closure (Scheme 1).^[34] They also demonstrated the power of the RCM approach by establishing some very simple routes to chiral ether cyclization substrates. For example, an Evans aldol reaction of glycolate imides with acrolein led to the α,β -dihydroxy acid derivative **16**. Subsequent reduction and acetylation provided the dienes **17**, which could in turn be easily cyclized with excellent yields by the ruthenium carbene complex **1**. Since the ether **19** gave only dimers and oligomers, it was suggested that the vicinal stereo centers in **17** induce conformations where the olefinic chains are positioned *gauche* (see the Newman projection of **17**).^[34, 35]

This concept was applied to the synthesis of a key precursor of the natural product (+)-laurencin (Scheme 2). One stereo-



Scheme 1. Use of the *gauche* effect of 1,2-dioxygen substituents to enhance the ring-closing reaction. Ac = acetyl.

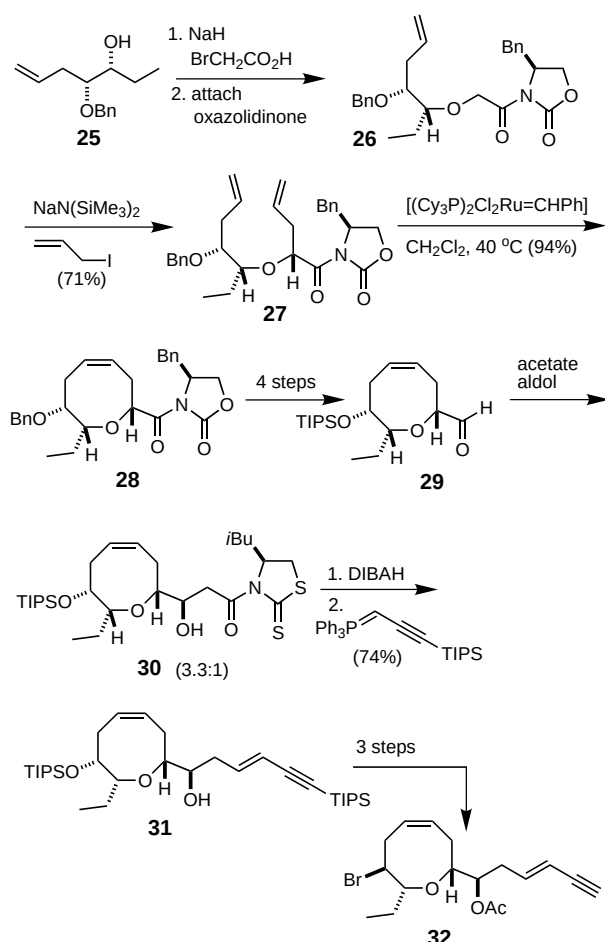


Scheme 2. Synthesis of the laurencin precursor **24**. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; TBDPS = *tert*-butyldiphenylsilyl.

center was fashioned from (*R*)-benzyl glycidyl ether **20**, whereas the other two came from an aldol reaction. As in the model studies, the eight-membered ring **23** formed with an excellent yield.

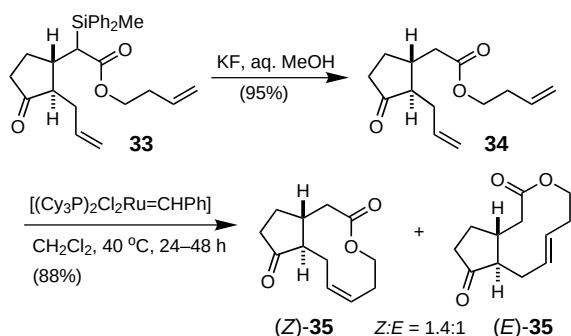
Subsequently, the same group published a more direct synthesis of (+)-laurencin, in which the ethyl side chain was included early in the synthesis (Scheme 3).^[36] Instead of aldol reactions, asymmetric alkylation reactions were used to set two of the stereocenters. Thus, the diol **25** was prepared by allylation of a benzyl glycolate imide followed by a chelation-controlled ethylation of the derived aldehyde. Another allylation of **26** provided the diene **27** that cyclized to the oxocene **28**. Further steps were needed to change the protecting group, generate the aldehyde **29**, and to elongate the side chain. The addition of the chlorotitanium enolate of (*S*)-(+)-3-acetyl-4-isobutyl-2-thiazolidinone gave a diastereomeric mixture of alcohols **30**. Finally, a Wittig elongation led to the natural product **32**.

A number of natural products contain medium-sized lactone rings. Therefore, synthetic routes to these compounds



Scheme 3. Synthesis of laurencin **32**. TIPS = triisopropylsilyl; DIBALH = diisobutylaluminum hydride.

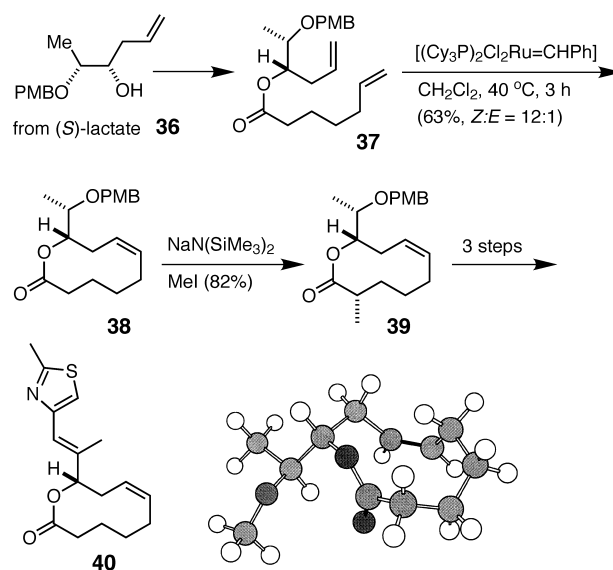
are highly desirable. Usually, macrolactonization reactions under highly dilute conditions or Baeyer–Villiger ring expansions are used to reach these targets. The group of Fürstner reported on the first successful synthesis of a ten-membered lactone by olefin metathesis (Scheme 4).^[37] Starting from cyclopentenone, a Michael addition–allylation sequence provided the cyclopentanone **33**. The silicon substituent makes the enolate a softer base and favors the 1,4-addition. The cyclization was effected by slowly combining solutions of **34** and **1** (10 mol%) and refluxing the mixture. While the cyclization was possible, the reaction led to double



Scheme 4. Olefin metathesis for the synthesis of unsaturated ten-membered lactone rings after Fürstner et al.

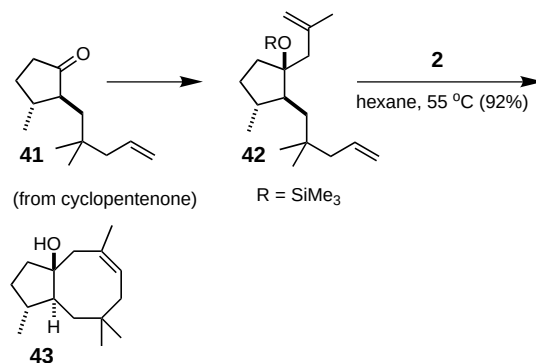
bond isomers in a moderate ratio. Compound (*Z*)-**35** is a component of the essential oil from jasmine. Most likely the constraint imposed by the five-membered ring favors cyclization over dimerization.

The Kalesse group subsequently described the synthesis of the ten-membered lactone **38** by an RCM reaction (Scheme 5).^[38] It is, however, not clear what is the role of the substituent during the cyclization. In any case, the stereochemical information of **38** was used to establish another stereocenter by a stereoselective alkylation of the medium-sized ring enolate. The cyclization also works with an acyclic precursor of **40**.^[39] The model illustrates the calculated conformation of **38** and explains the stereochemical situation for the alkylation of **38** to **39**.



Scheme 5. Olefin metathesis for the synthesis of unsaturated ten-membered lactone rings after Kalesse et al. PMB = *p*-methoxybenzyl. The model shows the calculated conformation of **38**.

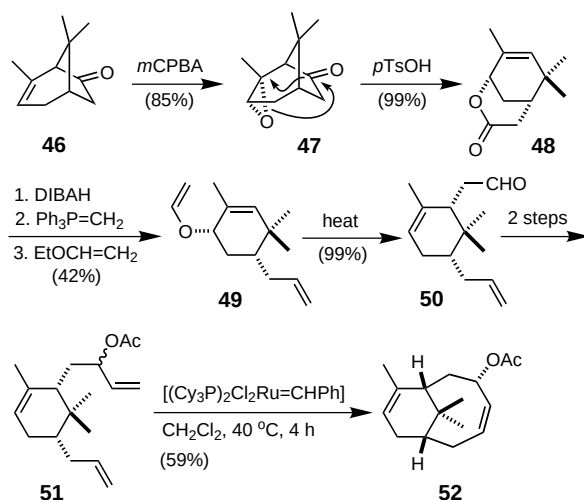
In principle, the same concept of conformational constraints can be used as a driving force in the preparation of medium-sized carbocycles (Scheme 6). An illustrative case is the synthesis of dactylol **43** using an RCM cyclization as a key step.^[40] The reaction catalyst was the Schrock molybdenum carbene complex **2** in hexane. The hydroxyl group of **42**



Scheme 6. Conformational constraints as a controlling force for the cyclization of medium-sized carbocycles.

needed to be protected as a silyl ether, otherwise the cyclization failed completely. Another instance of this kind is the annulation of eight-membered rings onto a sugar template, although moderate yields were reported for compounds **44** and **45**.^[41]

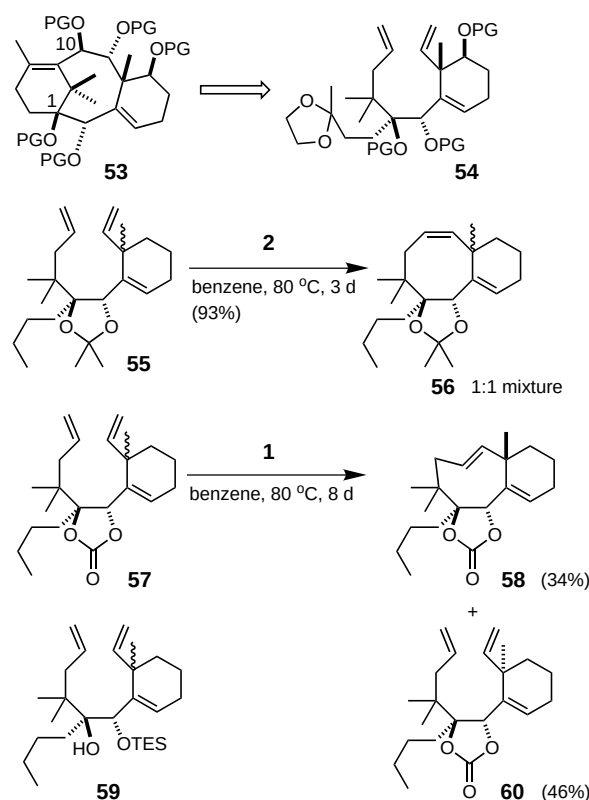
Clearly, bridged-ring systems, such as the one found in taxol, represent a formidable challenge for synthesis. Some of the difficulties in preparing the taxol skeleton are due to the geminal dimethyl groups. Nevertheless, an A,B-ring fragment could be synthesized by Blechert et al. by invoking an RCM reaction (Scheme 7).^[42] Starting from (–)- β -pinene, an iron-catalyzed CO insertion led to the ketone **46** and an isomeric



Scheme 7. Synthesis of a taxol A,B ring fragment by the RCM reaction after Blechert et al. *mCPBA* = 3-chloroperoxybenzoic acid; *pTs* = *p*-sulfonyl toluene.

ketone (not shown). Both compounds could be ultimately converted to the bridged lactone **48**: Epoxidation of **46** gave **47**, which rearranged in the presence of catalytic amounts of *pTsOH* in dichloromethane to the bridged lactone **47**. The *cis*-configuration of **48** was transformed to the aldehyde **50** through a Claisen rearrangement. Then **50** was converted to the cyclization substrate **51**. It was discovered that only one of the vinyl acetate diastereomers cyclized in the presence of the Grubbs catalyst. It is probable that the conformation required for the cyclization is unfavored for the β -acetate by a strong steric interaction with the geminal dimethyl groups of the cyclohexene.

The importance of conformational control on the outcome of RCM reactions was nicely demonstrated in a recent publication by Prunet et al.^[43] This group reported on the synthesis of the B,C ring systems of taxol by forming the central eight-membered ring. The overall synthetic plan is illustrated in Scheme 8. The eight-membered ring is envisioned to originate from an RCM reaction and a final intramolecular aldol condensation should complete the synthesis of the core structure **53**. During the cyclization to form the cyclooctene, not only a substantial enthalpic barrier must be overcome but the cyclization might also be impaired by steric hindrance from the olefin which is in a neopentilic position. In fact, the taxol model system **59**, when treated



Scheme 8. Synthesis of the taxol B,C ring fragments after Prunet et al. PG = protective group; TES = triethylsilyl.

with **1** (0.02 M in benzene, 80 °C, 8 d), gave only a low yield of cyclized products. However, a cyclic protecting group as in **55** brought about a positive effect. While **1** failed to produce macrocyclic products, the Schrock catalyst **2** delivered the cyclooctene **56** (as a separable mixture of diastereomers) in high yield. Most likely, the conformational constraint places the two double bonds at a suitable distance for the metathesis reaction. Based on these results, one would expect a similar result using the substrate **57**, in which a cyclic carbonate functions as a constraint. Surprisingly, the RCM cyclization gave the *trans*-cyclooctene **58**, whose structure was confirmed by X-ray analysis. This compound was formed only from one of the two isomers; the other diastereomer **57** was recovered unchanged. This result is more or less independent of the catalyst used (10 mol % **1**, benzene, 80 °C, 8 days; 10 mol % **2**, benzene, 80 °C, 3 days). Since it is generally believed that the metathesis occurs under thermodynamic control, this result is quite unusual.

In summary, the examples mentioned above illustrate the broad applicability of the RCM cyclization for the synthesis of medium-sized carbocycles and heterocycles. It is clear that this reaction will become—or even already is—a classical transformation in organic chemistry. For medium-sized rings, it is beneficial to use conformational constraints such as rings or stereoelectronic effects.

[1] K. C. Nicolaou, D. Vourloumis, N. Winssinger, P. Baran, *Angew. Chem.* **2000**, *112*, 46–126; *Angew. Chem. Int. Ed.* **2000**, *39*, 44–122.

[2] R. H. Grubbs, S. J. Miller, G. C. Fu, *Acc. Chem. Res.* **1995**, *28*, 446–452.

- [3] M. Schuster, S. Blechert, *Angew. Chem.* **1997**, *109*, 2124–2145; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2036–2055.
- [4] A. Fürstner, *Top. Catal.* **1997**, *4*, 285–299.
- [5] S. K. Armstrong, *J. Chem. Soc. Perkin Trans. 1* **1998**, 371–388.
- [6] R. H. Grubbs, S. Chang, *Tetrahedron* **1998**, *54*, 4413–4450.
- [7] A. Fürstner, *Synlett* **1999**, 1523–1533.
- [8] E. L. Dias, S. T. Nguyen, R. H. Grubbs, *J. Am. Chem. Soc.* **1997**, *119*, 3887–3897.
- [9] R. R. Schrock, J. S. Murdzek, G. C. Bazan, J. Robbins, M. DiMare, M. O'Regan, *J. Am. Chem. Soc.* **1990**, *112*, 3875–3886.
- [10] M. Scholl, T. M. Trnka, J. P. Morgan, R. H. Grubbs, *Tetrahedron Lett.* **1999**, *40*, 2247–2250.
- [11] L. Ackermann, A. Fürstner, T. Weskamp, F. J. Kohl, W. A. Herrmann, *Tetrahedron Lett.* **1999**, *40*, 4787–4790.
- [12] S. H. Pine, *Org. React.* **1993**, *43*, 1–91.
- [13] A. Fürstner, B. Bogdanović, *Angew. Chem.* **1996**, *108*, 2582–2609; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2442–2469.
- [14] P. Bertinato, E. J. Sorensen, D. Meng, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 8000–8001.
- [15] K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* **1996**, *108*, 2554–2556; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2399–2401.
- [16] D. Meng, D.-S. Su, A. Balog, P. Bertinato, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. Horwitz, *J. Am. Chem. Soc.* **1997**, *119*, 2733–2734.
- [17] K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, F. Roschangar, F. Sarabia, S. Ninkovic, Z. Yang, J. I. Trujillo, *J. Am. Chem. Soc.* **1997**, *119*, 7960–7973.
- [18] Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.* **1997**, *109*, 170–172; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166–168.
- [19] K. C. Nicolaou, D. Vourloumis, T. Li, J. Pastor, N. Winssinger, Y. He, S. Ninkovic, F. Sarabia, H. Vallberg, F. Roschangar, P. N. King, M. R. V. Finlay, P. Giannakakou, P. Verdier-Pinard, E. Hamel, *Angew. Chem.* **1997**, *109*, 2181–2187; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097–2102.
- [20] D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1997**, *109*, 543–544; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 523–524.
- [21] S. H. Kim, I. Figueroa, P. L. Fuchs, *Tetrahedron Lett.* **1997**, *38*, 2601–2604.
- [22] A. Fürstner, T. Gastner, H. Weintritt, *J. Org. Chem.* **1999**, *64*, 2361–2366.
- [23] P. E. Harrington, M. A. Tius, *Org. Lett.* **1999**, *1*, 649–651.
- [24] S. F. Martin, J. M. Humphrey, A. Ali, M. C. Hillier, *J. Am. Chem. Soc.* **1999**, *121*, 866–867.
- [25] C. A. Dvorak, W. D. Schmitz, D. J. Poon, D. C. Pryde, J. P. Lawson, R. A. Amos, A. I. Meyers, *Angew. Chem.* **2000**, *112*; *Angew. Chem. Int. Ed.* **2000**, *29*, in press.
- [26] S. J. Miller, S.-H. Kim, Z.-R. Chen, R. H. Grubbs, *J. Am. Chem. Soc.* **1995**, *117*, 2108–2109.
- [27] N. Diedrichs, B. Westermann, *Synlett* **1999**, 1127–1129.
- [28] C. A. Tarling, A. B. Holmes, R. E. Markwell, N. D. Pearson, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1695–1701.
- [29] C. E. Grossmith, F. Senia, J. Wagner, *Synlett* **1999**, 1660–1662.
- [30] G. R. Cook, P. S. Shanker, S. L. Peterson, *Org. Lett.* **1999**, *1*, 615–617.
- [31] M. Delgado, J. D. Martín, *J. Org. Chem.* **1999**, *64*, 4798–4816.
- [32] H. Oguri, S.-Y. Sasaki, T. Oishi, M. Hirama, *Tetrahedron Lett.* **1999**, *40*, 5405–5408.
- [33] P. A. V. van Hooft, M. A. Leeuwenburgh, H. S. Overkleef, G. A. van der Marel, C. A. A. van Boeckel, J. H. van Boom, *Tetrahedron Lett.* **1998**, *39*, 6061–6064.
- [34] M. T. Crimmins, A. L. Choy, *J. Am. Chem. Soc.* **1999**, *121*, 5653–5660.
- [35] S. D. Edwards, T. Lewis, R. J. K. Taylor, *Tetrahedron Lett.* **1999**, *40*, 4267–4270.
- [36] M. T. Crimmins, K. A. Emmitte, *Org. Lett.* **1999**, *1*, 2029–2032.
- [37] A. Fürstner, T. Müller, *Synlett* **1997**, 1010–1012.
- [38] K. Gerlach, M. Quitschalle, M. Kalesse, *Tetrahedron Lett.* **1999**, *40*, 3553–3556.
- [39] M. Quitschalle, M. Kalesse, *Tetrahedron Lett.* **1999**, *40*, 7765–7768.
- [40] A. Fürstner, K. Langemann, *J. Org. Chem.* **1996**, *61*, 8746–8749.
- [41] D. J. Holt, W. D. Barker, P. R. Jenkins, D. L. Davies, S. Garratt, J. Fawcett, D. R. Russell, S. Ghosh, *Angew. Chem.* **1998**, *110*, 3486–3488; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 3298–3300.
- [42] M. Wenz, D. Grossbach, M. Beitzel, S. Blechert, *Synthesis* **1999**, 607–614.
- [43] D. Bourgeois, A. Pancrazi, L. Ricard, J. Prunet, *Angew. Chem.* **2000**, *112*, 741–744; *Angew. Chem. Int. Ed.* **2000**, *39*, 726–728.

New Sulfur- and Selenium-Based Traceless Linkers—More than just Linkers?

Florencio Zaragoza*

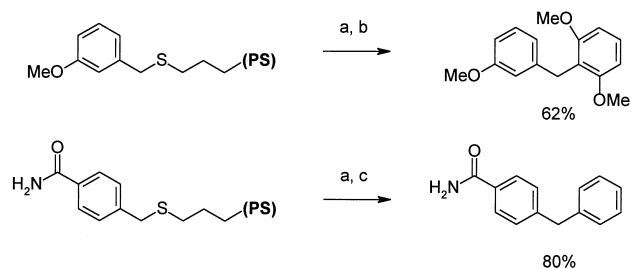
Linkers that enable the release of unfunctionalized hydrocarbons (alkanes, alkenes, arenes) from insoluble supports are attracting considerable interest.^[1] Such “traceless” linkers can give access to compound libraries devoid of a common functional group that was required for covalent attachment of the intermediates to the support. The production of highly diverse libraries by solid-phase synthesis should, therefore, be possible with such linkers. Herein some new strategies for traceless linking based on the cleavage of C–S or C–Se bonds are presented.

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Sulfur-Based Linkers

The carbon–sulfur bond in thioethers can be cleaved by photolysis^[2] or with reducing agents such as Raney nickel or tin hydrides.^[3] These cleavage conditions are not easily adapted to parallel synthesis, and only a few examples of thioether-based traceless linking have been reported.^[2, 3] Sulfones, available by oxidation of thioethers with *m*-chloroperbenzoic acid (MCPBA), are generally too stable to undergo homolytic or heterolytic C–S bond cleavage under suitably mild conditions to yield unfunctionalized hydrocarbons.^[4] More reactive than thioethers or sulfones, however, are trialkylsulfonium salts, which can be prepared on cross-linked polystyrene by the S-alkylation of thioethers with trialkyloxonium salts. Polystyrene-bound trialkylsulfonium salts have recently been introduced by Wagner and Mioskowski et al.^[5] as new traceless linkers for the preparation of

diarylmethanes (Scheme 1). These salts undergo reaction with soft nucleophiles such as iodide,^[6] or, upon catalysis by palladium(0), with arylboronic acids.^[5]

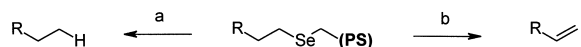


Scheme 1. Synthesis of diarylmethanes from benzyl thioethers. a) Et_3OBF_4 (5 equiv), CH_2Cl_2 , 0–20 °C, 8 h; b) 2,6-dimethoxyphenylboronic acid (1–2 equiv), $[\text{Pd}(\text{dppf})\text{Cl}_2]$ (0.2 equiv), K_2CO_3 (3 equiv), THF, 60 °C, 14 h; c) phenylboronic acid (1–2 equiv), $[\text{Pd}(\text{dppf})\text{Cl}_2]$ (0.2 equiv), K_2CO_3 (3 equiv), THF, 60 °C, 14 h. (PS) = cross-linked polystyrene; dppf = 1,1'-diphenylphosphanylferrocene.

Unfortunately, this new linking strategy is hampered by some problems. These include the formation of biaryls by homocoupling of the arylboronic acid and the contamination of the crude products with nonvolatile byproducts (various salts, excess boronic acid). Furthermore, it remains to be evaluated which functional groups will undergo irreversible alkylation during treatment with the trialkyloxonium salt. The concept of using a thioether as a linker is, however, highly attractive because thioethers are chemically inert under a broad selection of reaction conditions. If the S-alkylation required for activating the linker proceeds with high chemoselectivity, further nucleophiles or cleavage strategies based on trialkylsulfonium salts might offer interesting possibilities.

Selenium-Based Linkers

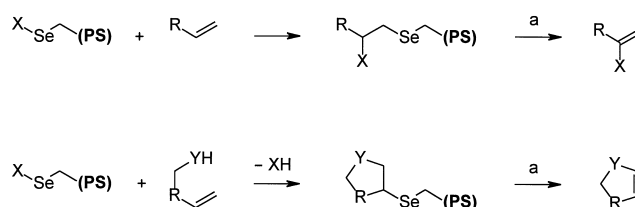
Polystyrene-bound selenides have proven to be versatile traceless linkers, which give access to alkanes by homolytic C–Se bond cleavage, and to alkenes by oxidation followed by β -elimination (Scheme 2).^[7] Oxidative cleavage of selenides



Scheme 2. Selenides as linkers for alkanes and alkenes. a) Bu_3SnH , AIBN, heat; b) oxidant, heat. (PS) = cross-linked polystyrene; AIBN = azobisisobutyronitrile.

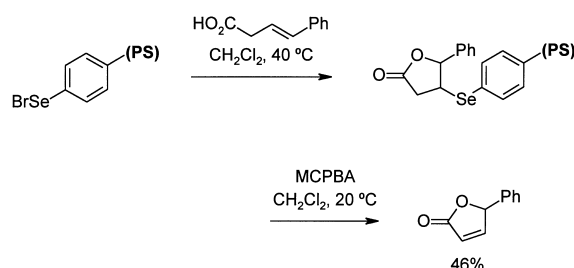
proceeds more readily than the cleavage of sulfoxides or sulfones. Since such oxidative cleavage is feasible with volatile reagents (for example, H_2O_2), it appears to be well suited for parallel synthesis.

Strategies for preparing selenides on insoluble supports include aliphatic nucleophilic substitution with anionic selenium reagents and the oxidative addition of support-bound electrophilic selenium reagents to alkenes.^[7] If alkenes containing a nucleophilic functionality are treated with support-bound electrophilic selenium reagents, cyclization can occur during the attachment to the support (Scheme 3).

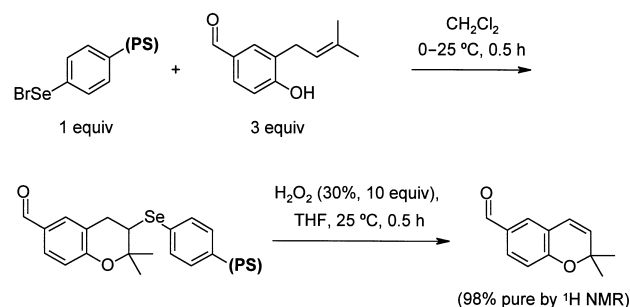
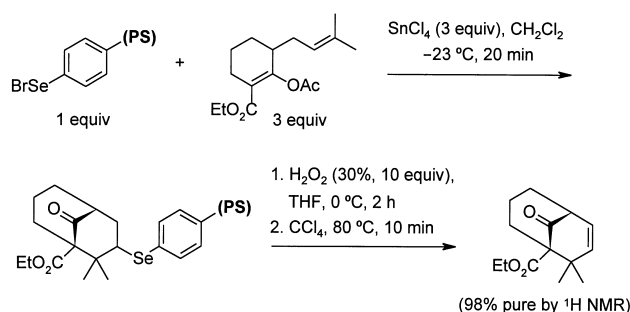


Scheme 3. Electrophilic selenium reagents as linkers for alkenes. a) Oxidant, heat. X = Br, CN, phthalimidy; (PS) = cross-linked polystyrene.

Some of the few examples reported for oxidative cyclizations with polymer-bound selenium reagents are shown in Schemes 4 and 5. Fujita et al.^[8] recently used polystyrene-bound selenyl cyanides and selenyl bromides to prepare γ -lactones (Scheme 4). The selenolactonization of 4-phenyl-3-butenic acid with selenyl cyanides required high reaction temperatures and catalysis by copper(II) chloride. Selenyl bromides, however, underwent smooth addition in dichloromethane without the need of any catalyst. Cleavage was effected by treatment with MCPBA in dichloromethane.^[8]



Scheme 4. Preparation of γ -lactones by selenolactonization with an insoluble selenyl bromide. (PS) = cross-linked polystyrene with a spacer; MCPBA = *m*-chloroperoxybenzoic acid.



Scheme 5. Synthesis of bicyclo[3.3.1]nonan-9-ones and 2,2-dimethyl-2H-chromenes by oxidative cyclization with support-bound selenyl bromide. (PS) = cross-linked polystyrene.

Nicolaou et al. utilized a similar strategy to construct carbon frameworks relevant to the synthesis of natural products on insoluble supports. Treatment of suitable precursors with polystyrene-bound selenyl bromide led to the formation of bicyclo[3.3.1]nonan-9-ones^[9] and 2,2-dimethyl-2H-chromanes^[10] (Scheme 5).

Selenides are inert towards a broad range of reaction conditions, such as the treatment with strong bases, organolithium reagents, and alkylating reagents. Also prolonged heating (for example, 140 °C, 48 h) does not lead to the cleavage of C–Se bonds. This stability was exploited in a multitude of transformations carried out with polystyrene-bound 2,2-dimethyl-2H-chromane-derived selenides (Scheme 5).^[11]

Conclusion

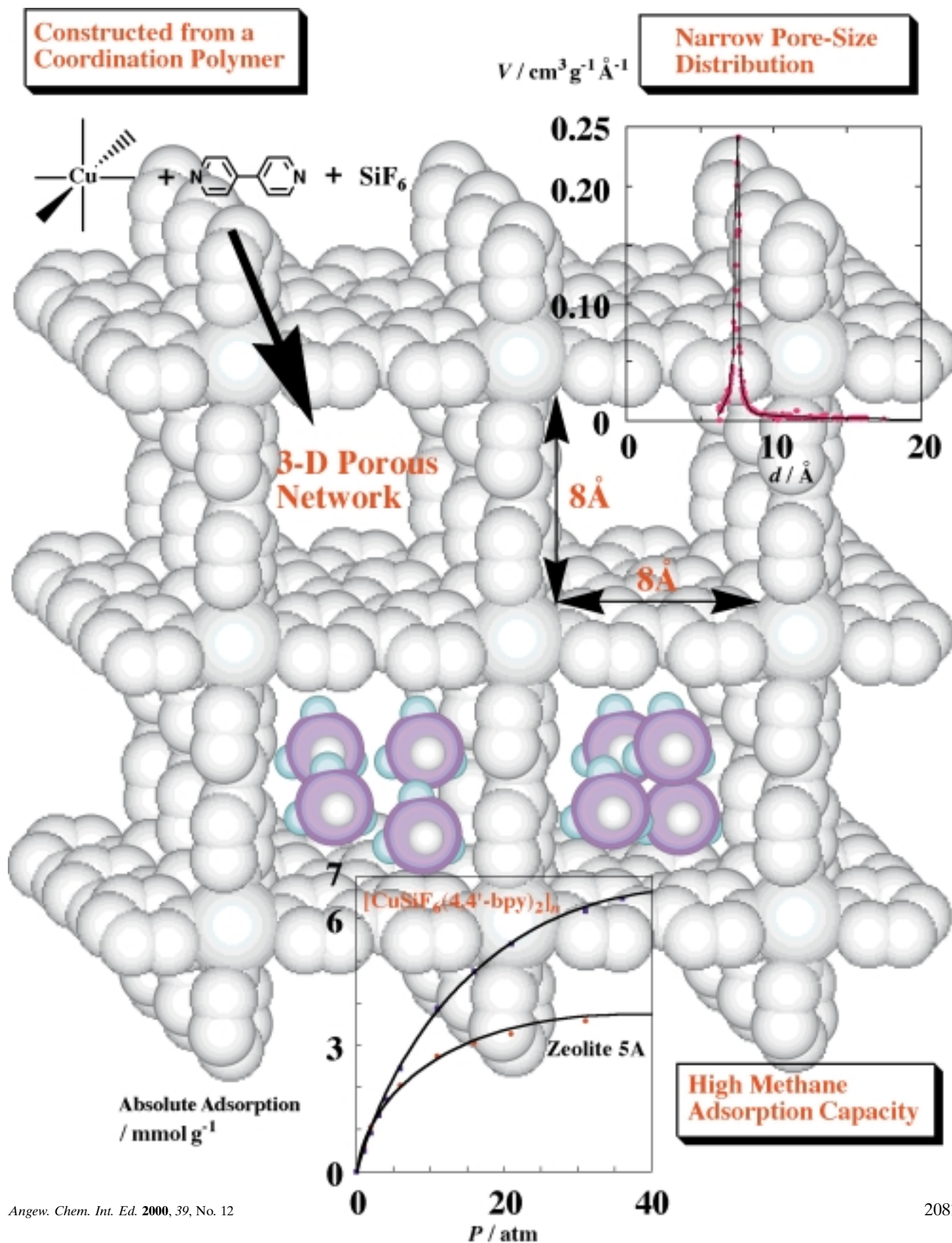
Chalcogen-based traceless linkers enable the solid-phase synthesis of products which were formerly only accessible by tedious, multistep solution-phase chemistry. Some of the newer linkers can be cleaved with volatile reagents and tolerate a broad range of reaction conditions, which gives the chemist plenty of freedom in the design of new synthetic sequences. Interestingly, support-bound electrophilic selenium reagents can also mediate useful chemical transformations of substrates during their attachment to the support, and thereby function as reagents as well as linkers.

Future developments should further focus on cleavage protocols that yield crude products devoid of nonvolatile by-products. Such cleavage strategies enable the preparation of essentially pure crude products, as required for the parallel synthesis of compound libraries. New traceless linkers cleav-

able with volatile reagents would be a useful supplement to existing methodologies, and would have the potential of finding widespread application.

- [1] a) I. W. James, *Tetrahedron* **1999**, 55, 4855–4946; b) C. J. Andres, D. L. Whitehouse, M. S. Deshpande, *Curr. Opin. Chem. Biol.* **1998**, 2, 353–362; c) F. Zaragoza Dörwald, *Organic Synthesis on Solid Phase*, Wiley-VCH, Weinheim, **2000**.
- [2] a) I. Sucholeiki, *Tetrahedron Lett.* **1994**, 35, 7307–7310; b) F. W. Forman, I. Sucholeiki, *J. Org. Chem.* **1995**, 60, 523–528.
- [3] a) K. W. Jung, X. Y. Zhao, K. D. Janda, *Tetrahedron Lett.* **1996**, 37, 6491–6494; b) K. W. Jung, X. Y. Zhao, K. D. Janda, *Tetrahedron* **1997**, 53, 6645–6652.
- [4] X. Y. Zhao, K. W. Jung, K. D. Janda, *Tetrahedron Lett.* **1997**, 38, 977–980. 2-Aminoacrylates have been prepared by oxidation and base-induced β -elimination of cysteine derivatives linked as thioethers to a support (M. Yamada, T. Miyajima, H. Horikawa, *Tetrahedron Lett.* **1998**, 39, 289–292). This cleavage strategy is only applicable to the preparation of alkenes substituted with strongly electron-withdrawing groups.
- [5] C. Vanier, F. Lorgé, A. Wagner, C. Mioskowski, *Angew. Chem.* **2000**, 112, 1745–1749; *Angew. Chem. Int. Ed.* **2000**, 39, 1679–1683.
- [6] G. A. Crosby, M. Kato, *J. Am. Chem. Soc.* **1977**, 99, 278–280.
- [7] a) T. Ruhland, K. Andersen, H. Pedersen, *J. Org. Chem.* **1998**, 63, 9204–9211; b) K. C. Nicolaou, J. Pastor, S. Barluenga, N. Winssinger, *Chem. Commun.* **1998**, 1947–1948; c) R. Michels, M. Kato, W. Heitz, *Makromol. Chem.* **1976**, 177, 2311–2320.
- [8] K. Fujita, K. Watanabe, A. Oishi, Y. Ikeda, Y. Taguchi, *Synlett* **1999**, 1760–1762.
- [9] K. C. Nicolaou, J. A. Pfefferkorn, G.-Q. Cao, S. Kim, J. Kessabi, *Org. Lett.* **1999**, 1, 807–810.
- [10] K. C. Nicolaou, J. A. Pfefferkorn, G.-Q. Cao, *Angew. Chem.* **2000**, 112, 750–755; *Angew. Chem. Int. Ed.* **2000**, 39, 734–739.
- [11] K. C. Nicolaou, J. A. Pfefferkorn, G.-Q. Cao, *Angew. Chem.* **2000**, 112, 755–759; *Angew. Chem. Int. Ed.* **2000**, 39, 739–743.

A new methane adsorbent was synthesized from a coordination polymer. This material adsorbs the gas to a remarkable extent and opens up new dimensions for the study of inorganic–organic hybrid adsorbents based on coordination polymers. Find out more on the following pages.



A New, Methane Adsorbent, Porous Coordination Polymer $[\{\text{CuSiF}_6(4,4'\text{-bipyridine})_2\}_n]$

Shin-ichiro Noro, Susumu Kitagawa,* Mitsuru Kondo, and Kenji Seki

The chemistry of microporous compounds has been studied extensively because of their unique applications, which includes size-selective separation, gas adsorption, heterogeneous catalysis. The creation of porous materials as adsorbents is an attractive research field for the storage and transport of natural gas. So far, porous inorganic materials such as zeolites, aluminum phosphates, and their derivatives have been studied in great detail.^[1–3] More recently, coordination polymers constructed from transition metal ions and bridging organic ligands have afforded new types of robust crystals with high degree of porosity.^[4–7] Although a large number of these crystals have been hitherto synthesized, still lacking are examples of coordination polymers in which the pores are usefully large and which the coordination framework is sufficiently robust and is maintained over a wide pressure region at ambient temperature. In addition, since nature dislikes a vacuum, the target of a large channels is fraught with difficulties during synthesis, which include 1) the occupation of counteranions in the channels; 2) the need to readily form an interpenetrating network; and that 3) the channel network may be disrupted upon removal of the guest molecules.

Therefore, a rational synthetic strategy is a most significant point for the creation of porous coordination polymers. Generally, when water or other solvents coordinate to metal ions, the resulting network is of low dimensionality and often forms no channels. In this paper, we report a novel 3D coordination polymer, $[\{\text{CuSiF}_6(4,4'\text{-bpy})_2\}_n \cdot 8\text{H}_2\text{O}]_n$ (**1**; 4,4'-bpy = 4,4'-bipyridine). For this compound, we selected Cu^{II} and SiF_6^{2-} as the building blocks for the construction of a coordination framework with high dimensionality. The Cu^{II} ion (d^9) is liable to undergo Jahn–Teller distortion and results in weakly coordinated bond sites in the axial positions. Consequently, SiF_6^{2-} can coordinate to Cu^{II} more easily than water or other solvents. Since the SiF_6^{2-} anion can link the copper atoms located in each nearest-neighbor square-grid layer, the resulting 3D framework is expected to be robust and free from the occupation of channels by uncoordinated

counteranions. We report herein on the successful synthesis of a porous coordination polymer using Cu^{II} and SiF_6^{2-} .

Single crystals were obtained by mixing $\text{Cu}(\text{BF}_4)_2 \cdot 6\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SiF}_6$, and 4,4'-bpy in a water/ethylene glycol (1:3) solvent. X-ray analysis demonstrates the 3D network is based on a square grid of $[\{\text{Cu}(4,4'\text{-bpy})_2\}_n]$ and pillars of SiF_6^{2-} ions. Figure 1 shows the coordination of the Cu^{II} ion in

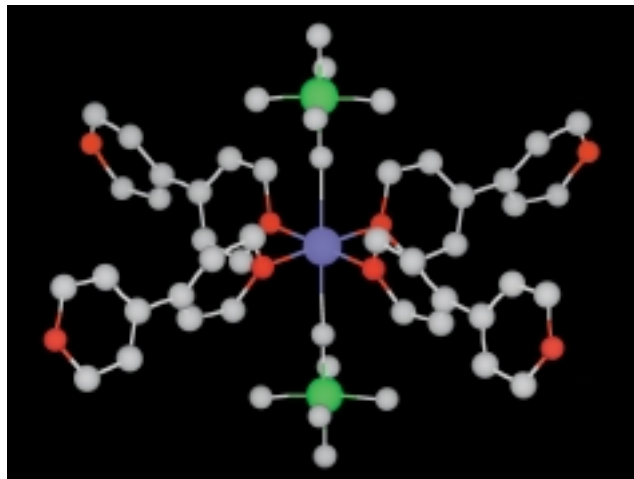


Figure 1. ORTEP representation of the structure around a Cu^{II} center (atomic colors: Cu blue; N red; Si green; C and F gray). Hydrogen atoms and one pyridine ring of the disordered 4,4'-bpy ligand are omitted for clarity. Selected bond lengths [Å]: Cu–F 2.355(5), Cu–N 2.011(5).

the crystal. The pyridine rings of the 4,4'-bpy ligand are disordered over two positions and four (pyridine) nitrogen atoms are coordinated to the Cu^{II} center. Association of two F atoms of the SiF_6 anions provides a (4+2) environment. Bridging the Cu^{II} ions with 4,4'-bpy affords a 2D square grid network. The layers are linked with coordinating SiF_6^{2-} ions to give a 3D structure without interpenetration (Figure 2c). This network yields channels with dimensions of about $8 \times 8 \text{ Å}^2$ along the *c* axis and about $6 \times 2 \text{ Å}^2$ along the *a* and *b* axes, as shown in Figure 2a and 2b, respectively. The channel is filled with eight crystallized water molecules per Cu^{II} ion. A similar network has been obtained for Zn^{II} the compound $[\{\text{Zn}(4,4'\text{-bpy})_2(\text{SiF}_6)_2\}_n]$ (**2**)^[8] was prepared in dimethyl formamide/dioxane (5:2). It has been previously reported that $[\{\text{Zn}(4,4'\text{-bpy})_2(\text{H}_2\text{O})_2\}_n(\text{SiF}_6)_n]$, which contains interpenetrating 2D $[\{\text{Zn}(4,4'\text{-bpy})_2(\text{H}_2\text{O})_2\}_n]$ sheets and no channels, was isolated from an aqueous media containing ZnSiF_6 and 4,4'-bpy.^[9] The framework in **2** is sensitive to the solvent used. In the case of **1**, the choice of dianion was essential and in the preliminary experiments we observed that a use of GeF_6^{2-} instead of SiF_6^{2-} forms a similar porous 3D network, $[\{\text{CuGeF}_6(4,4'\text{-bpy})_2\}_n]$, under the same reaction conditions.

The surface area, pore size distribution, and micropore volume of **1** were calculated from argon gas adsorption at 87.3 K measured with a volumetric adsorption apparatus (Micromeritics ASAP 2001M); the data was fitted to the BET equation and to a Horvath–Kawazoe differential pore volume (HK) plot.^[10] The HK plot shows only one sharp peak at about 7.8 Å (Figure 2d) to reveal that this material does have a square pore ($8 \times 8 \text{ Å}^2$) and agrees quantitatively

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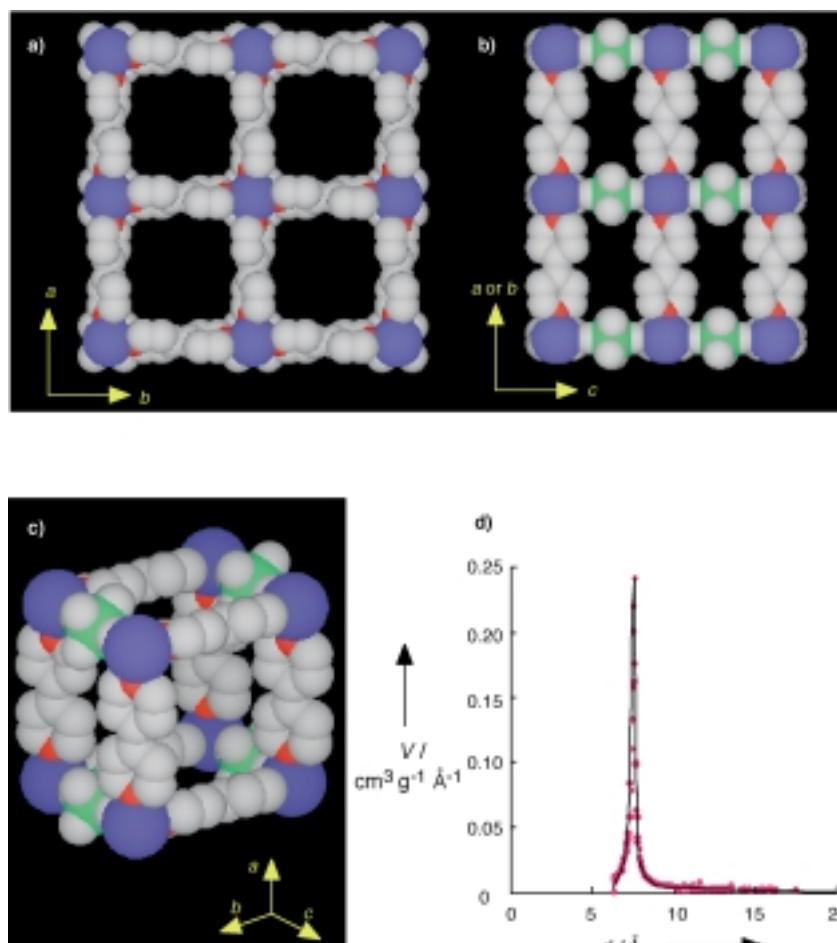


Figure 2. a) Space filling model of the channel structures along the *c* axis. b) Along the *a* or *b* axes. c) The 3D network. Atomic colors are as per Figure 1. The hydrogen atoms, one pyridine ring of the disordered 4,4'-bpy ligand, and crystallized water molecules are omitted for clarity. The pore size given in the text was estimated from this Figure when all the hydrogen atoms are added to the 4,4'-bpy molecules in a space-filling model. d) Horvath-Kawazoe differential pore volume plot. Only one sharp peak at approximately 7.8 Å was observed, which indicates that the size of microporous channels are regular.

with the result of the X-ray analysis. The argon molecules cannot pass the rectangular pore ($6 \times 2 \text{ Å}^2$) because of the larger diameter of argon. The micropore volume and specific surface area, on the basis of argon adsorption, are calculated to be 0.56 mL g^{-1} and $1337 \text{ m}^2 \text{ g}^{-1}$, respectively.

The stability of this network was studied by X-ray powder diffraction (XRPD) and thermal gravimetric (TG) analysis. Figure 3 shows the XRPD patterns at 373 K under high vacuum (approximately 0.1 mm Hg) with a simulated pattern based of the single-crystal data without crystallized water molecules. The TG analysis reveals that the crystallized solvent molecules are absent under the measurement conditions.^[11] The good agreement between the peaks in both diagrams demonstrates that the porous network is retained in this phase and indicates that the network is retained in the absence of any guest molecules in the channels.

Methane adsorption experiments were also carried out on **1** and on zeolite 5A, which has the highest known methane adsorption capacity.^[12–14] Figure 4 shows the isotherms for

methane adsorption in the pressure range 0–36 atm at 298 K. Desorption/readsorption experiments for **1** trace the same isotherm, which indicates that the channel structures are retained throughout this process. At 36 atm, the density of methane adsorbed in the micropores of **1** is 0.21 g mL^{-1} . The density of the compressed methane at 300 K and 280 atm is almost the same as that in **1** at 298 K and 36 atm ($=0.16 \text{ g mL}^{-1}$), indicative of a concentration effect from strong micropore filling in the cavities. The high pressure adsorption of supercritical methane in micropores has been studied with the following extended Dubinin–Radsushkevich (DR) equation.^[15]

$$\left[\ln\left(\frac{W_L}{W}\right)\right]^{1/2} = \left(\frac{RT}{\beta E_0}\right)(\ln P_{0q} - \ln P) \quad (1)$$

Here, the parameters W , βE_0 , and P_{0q} are the degree of adsorption at a pressure P , the adsorption energy, and the saturated vapor pressure of the quasi-vaporized supercritical methane, respectively. The large inherent micropore capacity, W_L , of 10.0 mmol g^{-1} is obtained from the Langmuir plot. This isotherm matches the DR equation well. The methane adsorption quantity at high pressure (about 6.5 mmol g^{-1} at 36 atm) is much larger than that of zeolite 5A (about 3.7 mmol g^{-1} at 36 atm). The obtained parameters are $\beta E_0 = 8 \text{ kJ mol}^{-1}$ and $P_{0q} = 284 \text{ atm}$. In conclusion, this material

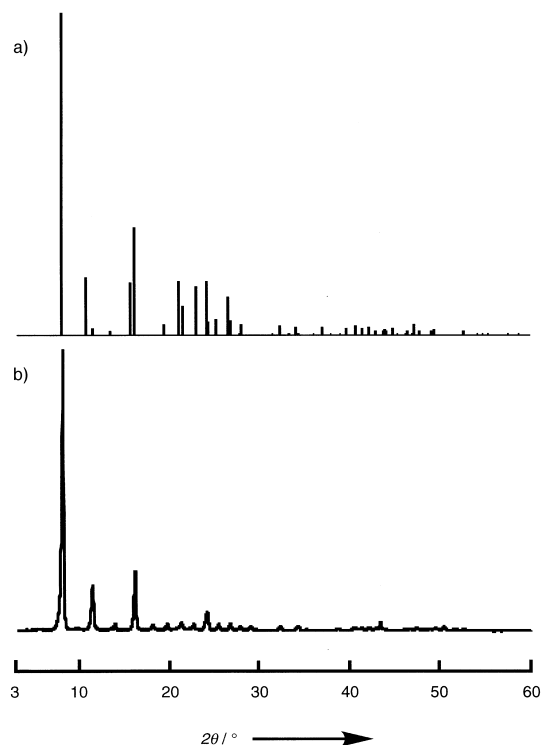


Figure 3. a) Simulated XRPD pattern upon removal of the water molecules from the single crystal and b) the observed pattern under reduced pressure at 373 K.

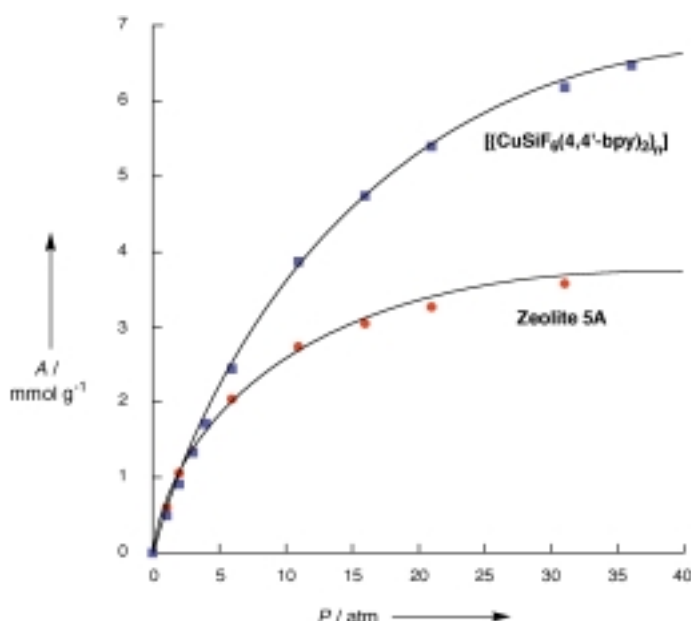


Figure 4. Methane adsorption isotherms at 298 K over a pressure range 0–36 atm (A = absolute adsorption).

has the high adsorption ability and opens up a new dimension for the study of adsorbents of inorganic–organic hybrid materials based on coordination polymers.

Experimental Section

Crystals of **1** suitable for single crystal X-ray analysis were prepared by diffusing an aqueous ethylene glycol solution containing a mixture of $\text{Cu}(\text{BF}_4)_2 \cdot 6\text{H}_2\text{O}$ (1.00 mmol) and $(\text{NH}_4)_2\text{SiF}_6$ (1.00 mmol) into an ethylene glycol solution of 4,4'-bpy (2.00 mmol) using a straight-type glass tube at room temperature. After one week, purple block-shaped crystals had precipitated. Four water molecules per unit cell evaporated in air to give a partly dehydrated compound (yield: 88%). Elemental anal. calcd for $[\text{CuSiF}_6(4,4'\text{-bpy})_2] \cdot 4\text{H}_2\text{O}_n$: C 40.71, H 4.10, N 9.50; found: C 40.81, H 3.50, N 9.27. IR (KBr pellet): $\tilde{\nu} = 3412$ (br), 3098 (w), 3045 (w), 1610 (m), 1535 (w), 1493 (w), 1412 (w), 1221 (w), 1078 (w), 995 (w), 812 (s), 742 (s), 644 (m), 482 cm^{-1} (s).

For the X-ray diffraction, a crystal was mounted on a glass fiber coated with epoxy resin. Measurements were made on a Rigaku Mercury charge-coupled device (CCD) system with graphite monochromated $\text{MoK}\alpha$ radiation. Crystal data: $\text{C}_{20}\text{H}_{32}\text{CuF}_6\text{N}_4\text{O}_5\text{Si}$, $M = 662.12$, tetragonal, space group $P4/mmm$ (No. 123), $a = 11.108(1)$, $c = 8.1107(9)$ Å, $V = 1000.8(1)$ Å³, $Z = 1$, $\rho_{\text{calcd}} = 1.098$ g cm⁻³, $\lambda(\text{MoK}\alpha) = 0.71069$ Å, $F(000) = 341.00$, $\mu(\text{MoK}\alpha) = 6.38$ cm⁻¹, $T = 233$ K, $2\theta_{\text{max}} = 54.9^\circ$, 7187 reflections measured, 937 observed ($I > 2\sigma(I)$), 50 parameters; $R = 0.056$, $R_w = 0.050$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-142080. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

The adsorption isotherms of methane gas on the sample were measured according to the reported procedure.^[12, 13] The apparatus was equipped with a Cahn R-100 electrobalance contained within a SUS steel pressure chamber connected with two separate lines for evacuation and adsorbate gas pressurization. After the sample was placed in the apparatus, the solvated molecules in the channels were removed under reduced pressure at 298 K. The removal process was monitored by the reduction in weight. The methane was admitted into the adsorption chamber and then the change in weight of the sample was monitored. The entire adsorption

isotherms at 298 K were determined by increasing the adsorbate gas pressure to a maximum of 36 atm. All changes in weight were corrected for buoyancy.

Zeolite 5A was purchased from Bayer. The adsorption isotherms of methane gas were measured with the same method as for the sample. The reported methane adsorption of zeolite 5A by Mentastý et al.^[14] (2.9 mmol g⁻¹ at 36 atm) was smaller than our result (3.7 mmol g⁻¹ at 36 atm). Therefore, we used the higher methane adsorption quantity as comparison data with **1**.

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- [1] A. Corma, *Chem. Rev.* **1997**, 97, 2373–2419.
- [2] G. A. Ozin, A. Kuperman, A. Stein, *Angew. Chem.* **1989**, 101, 373–390; *Angew. Chem. Int. Ed. Engl.* **1989**, 28, 359–376.
- [3] W. Hölderich, M. Hesse, F. Naumann, *Angew. Chem.* **1988**, 100, 232–251; *Angew. Chem. Int. Ed. Engl.* **1988**, 27, 226–246.
- [4] S. S.-Y. Chui, S. M.-F. Lo, J. P. H. Charmant, A. G. Orpen, I. D. Williams, *Science* **1999**, 283, 1148–1150.
- [5] H. Li, M. Eddaoudi, M. O’Keeffe, O. M. Yaghi, *Nature* **1999**, 402, 276–279.
- [6] H. Li, M. Eddaoudi, T. L. Groy, O. M. Yaghi, *J. Am. Chem. Soc.* **1998**, 120, 8571–8572.
- [7] M. Kondo, T. Okubo, A. Asami, S. Noro, T. Yoshitomi, S. Kitagawa, T. Ishii, H. Matsuzaka, K. Seki, *Angew. Chem.* **1999**, 111, 190–193; *Angew. Chem. Int. Ed.* **1999**, 38, 140–143.
- [8] S. Subramanian, M. J. Zaworotko, *Angew. Chem.* **1995**, 107, 2295–2297; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 2127–2129.
- [9] R. W. Gable, B. F. Hoskins, R. Robson, *J. Chem. Soc. Chem. Commun.* **1990**, 1667–1668.
- [10] G. Horvath, K. Kawazoe, *J. Chem. Eng. Jpn.* **1983**, 16, 470–475.
- [11] The TG curve shows the release of crystallized water molecules to 373 K, which is followed by decomposition of the framework at 423 K.
- [12] S.-Y. Zhang, O. Talu, D. T. Hayhurst, *J. Phys. Chem.* **1991**, 95, 1722–1726.
- [13] J. L. Zuech, A. L. Hines, E. D. Sloan, *Ind. Eng. Chem. Process Des. Dev.* **1983**, 22, 172–174.
- [14] L. Mentastý, A. M. Woestyn, G. Zgrablich, *Adsorpt. Sci. Technol.* **1994**, 11, 123–133.
- [15] K. Kaneko, K. Murata, K. Shimizu, S. Camara, T. Suzuki, *Langmuir* **1993**, 9, 1165–1167.

Structure and Mechanism in *Cinchona* Alkaloid Chemistry: Aqueous Hydrolysis Proceeds with Complete Inversion or Complete Retention of Configuration**

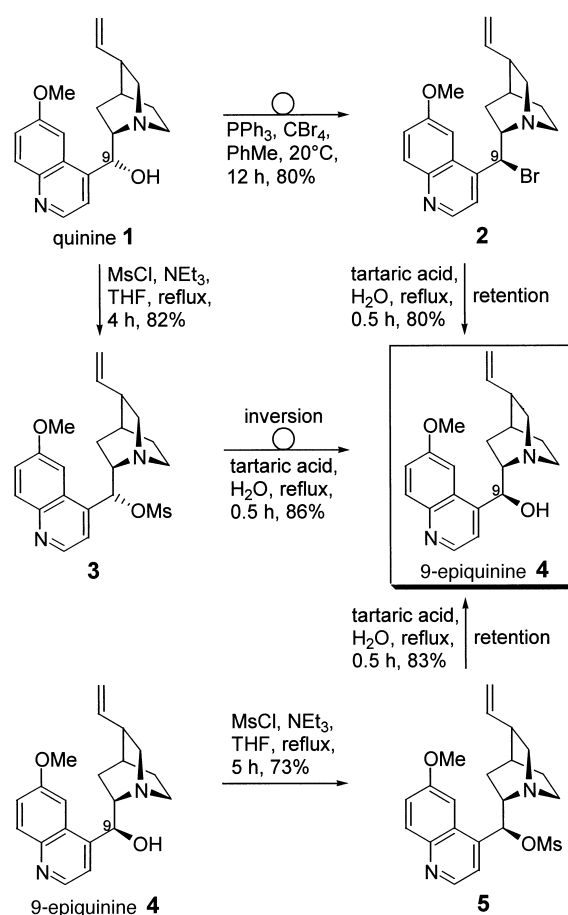
Wilfried. M. Braje, Jens Holzgreffe, Rudolf Wartchow, and H. Martin R. Hoffmann*

Solvolytic displacement reactions at saturated carbon centers are among the best known and most investigated reactions in organic chemistry. It is generally accepted that the steric course of solvolysis ranges from inversion of configuration to substantial and even complete racemization, dependent upon the stability and lifetime of any ion-pair and carbocation intermediates.^[1]

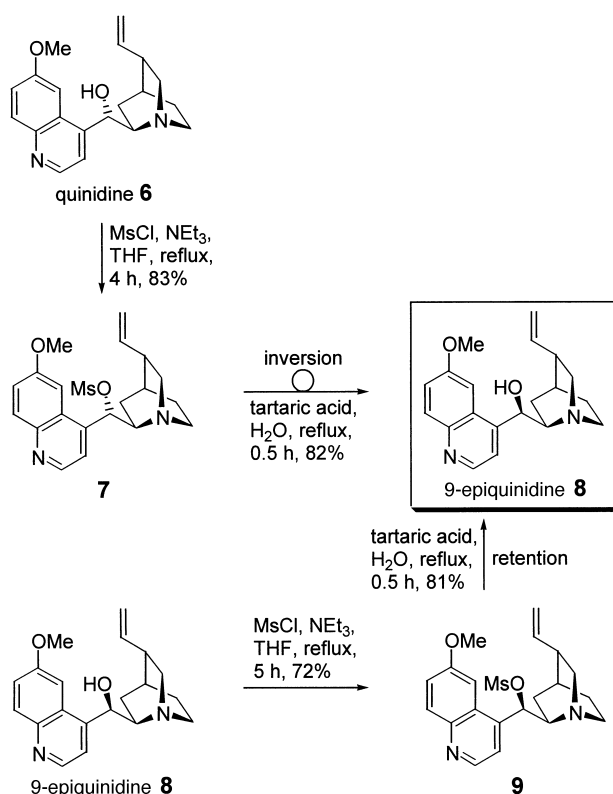
In the course of our work on *Cinchona* alkaloids we have investigated the hydrolysis of derivatives with a leaving group at carbon C9. Thus, quinine (1) was converted into the mesylate 3, which dissolved readily in water in the presence of tartaric acid (1 equivalent) as a proton donor. On heating to 100 °C, the completely *inverted* 9-epiquinine (4) was obtained in good yield as the only product in a spot-to-spot reaction (Scheme 1).^[2–4] Similarly, 9-epiquinine mesylate (5) was submitted to aqueous hydrolysis in the presence of two equivalents of tartaric acid at reflux. To our surprise, 9-epiquinine (4) was the only product and it was formed in a clean spot-to-spot reaction with complete *retention* of configuration.^[4] In an analogous sequence, pseudo-enantiomeric quinidine (6) was converted into the mesylate 7 and hydrolysed, furnishing 9-epiquinidine (8) with complete *inversion* of configuration (Scheme 2). Hydrolysis of O-mesylated epiquinidine (9) proceeded with complete *retention* of configuration. The results from the quinidine series confirm the quinine work.

Is the retentive hydrolysis of mesylates 5 and 9 triggered by an unusual fission of the sulfur–oxygen bond, perhaps assisted by intramolecular participation of the basic bridge-head nitrogen? N,O-Acyl and N,O-sulfonyl shifts have previously been encountered in *Cinchona* alkaloid chemistry.^[5] We therefore changed the leaving group from methanesulfonate to bromide. 9-Epibromoquinine (2) was subjected to the usual hydrolysis conditions and, again, the only product formed was 9-epiquinine (4) (Scheme 1).

Does retention of configuration involve an aziridinium ion intermediate and hence double inversion? Examples of neighboring nitrogen participation in 1,2-amino halides and



Scheme 1. Hydrolyses which lead to 9-epiquinine (4) through inversion or retention of configuration. Ms = Methanesulfonyl.



Scheme 2. Analogous hydrolysis reactions in the quinidine series.

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mesylates are well known.^[6] However, aziridinium ion participation has previously been ruled out in the silver benzoate mediated reaction of 9-epibromoquinine (**2**) with methanol as the solvent.^[7, 8]

We assume that the contrasting stereochemical results are due to conformational control in the ground and transition states,^[7] as well as hydrogen bonding in the solvent water. A clear picture of the preferred conformation of our starting material in the ground state was provided by NOE studies and X-ray crystal diffraction. The mesylate **3** is in an *anti, closed* conformation (Figure 1 a).^[9] Attack of water proceeds in S_N2-like fashion and is thought to be assisted by local hydrogen bonding involving the (protonated) bridgehead nitrogen atom.

In contrast, 9-epiquinine (**2**) and also mesylated 9-epiquinine (**5**) are structurally and conformationally very similar and adopt the *syn, open* conformation.^[11] Note that this conformation has a hydrophobic face (Figure 1 b), which probably disfavors attack by water on this side. Reaction with water is thought to proceed with a change of mechanism.^[12, 13]

In conclusion, there is little doubt that simple secondary aliphatic halides and sulfonates, which have usually been studied as model substrates and discussed in textbooks, are

solvolysed with overall inversion of configuration, accompanied by varying amounts of racemization. In the case of the *Cinchona* alkaloids and especially with water as a solvent, the well-defined rotamer population of the natural product, combined with molecular recognition of the hydrophilic binding pocket, is thought to play a critical role.^[14] Two distinct reactions are observed with water as the solvent. The duality of the steric course highlights the special role of *Cinchona* alkaloids in asymmetric synthesis.^[15] The transformations reported for the three substrates **2**, **5**, and **9** are to our knowledge the first examples of hydrolysis reactions which proceed with complete retention of configuration. After the puzzle of the hetero-*Cinchona* rearrangement,^[7] a further facet of quinine – quinidine chemistry^[16] has now been elucidated.

Experimental Section

Illustrative hydrolysis procedure: Quinine mesylate **3** (350 mg, 0.87 mmol) and tartaric acid (136 mg, 0.90 mmol) were dissolved in water (4 mL) and refluxed at 100 °C for 30 minutes. The solution was cooled to RT and sat. NaHCO₃ solution (10 mL) was added. The mixture was extracted several times with CHCl₃. The organic phase was dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by column chromato-

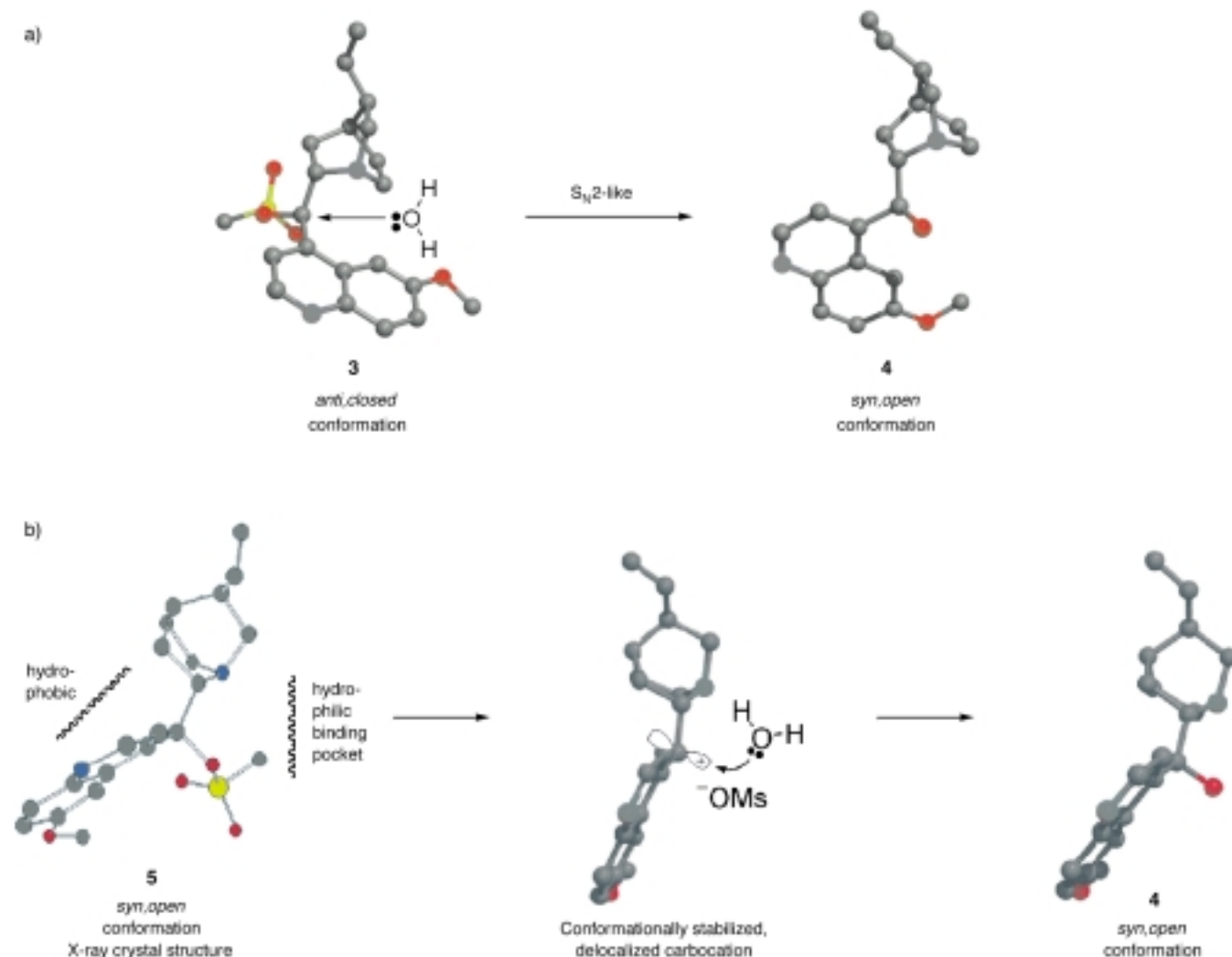


Figure 1. a) Postulated course^[10] of the hydrolysis of quinine mesylate (**3**) with natural configuration at C9: Inversion of configuration. b) Postulated course of hydrolysis of 9-epiquinine mesylate (**5**). Retention of configuration and front-side hydration are favoured by the *syn, open* conformation.

graphy (EtOAc/MeOH 4/1, then MeOH) to give **4** as a colorless solid (243 mg, 0.75 mmol) in 86% yield.

Preparation of 9-*epi*-bromoquinine (**2**): Quinine **1**, PPh₃ (2 equiv) CBr₄ (1.1 equiv) were stirred in toluene at RT for 12 h. Workup by extraction with dilute hydrochloric acid, neutralization of aqueous extract (sat. NaHCO₃ solution) and back-extraction with CHCl₃ (ca. 5 ×). The organic phase was dried (MgSO₄) and concentrated under reduced pressure. Column chromatography (EtOAc, then EtOAc/MeOH 4/1) gave **2**, colorless solid, yield 80% (X-ray structure data in ref. [7]).

Crystal structure analysis of 9-epiquinine mesylate (**5**):^[17] C₂₁H₂₆N₂O₄S₁, *M_r* = 402.51, orthorhombic, space group *P*₂₁₂₁₂₁ (No. 19), *a* = 8.408(1), *b* = 9.950(2), *c* = 23.954(3) Å, *V* = 2004.0(5) Å³, *Z* = 4, ρ_{calc} = 1.334 g cm⁻³, *F*(000) = 856, crystal size 0.48 × 0.14 × 0.20 mm, *T* = 300 K, $\mu(\text{MoK}\alpha)$ = 1.9 cm⁻¹. Data collection: Stoe IPDS diffractometer, graphite monochromated MoK α radiation (λ = 0.71073 Å), 2- θ range = 3.4–41.7°, data set *h, k, l* –8:8; –9:9; –23:23, 7204 measured reflections, 2084 independent reflections, 1351 observed reflections with *I* > 2 σ (*I*), *R*_{int} = 0.0945. Structure solution with SHELXS-86 and refinement with SHELXL-93, hydrogen atoms in geometrically calculated positions, max./min. residual electron density 0.22/–0.23 e Å⁻³, *R*(*F*) = 0.0482 based on 1351 reflections with *F*_o > 4 σ (*F*_o), *wR*2 = 0.0814, *wR*2 based on *F*² with 2084 reflections, Flack parameter –0.1(2).

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- [1] a) J. March, *Advanced Organic Chemistry*, 4th ed., Wiley, New York, **1992**, pp. 292–307; b) F. A. Carey, R. J. Sundberg, *Advanced Organic Chemistry*, 4th ed., Plenum Press, New York, **1993**; c) A. Streitwieser *Chem. Rev.* **1956**, 56, 571; d) A. Streitwieser, *Solvolytic Displacement Reactions*, McGraw–Hill, New York, **1962**; d) R. Brückner, *Reaktionsmechanismen*, Spektrum, Heidelberg, **1996**, p. 56.
- [2] Naturally occurring carboxylic acids other than tartaric acid were also investigated, but gave some minor side products. The order of decreasing selectivity was: tartaric acid > malic acid, succinic acid, citric acid > mandelic acid > lactic acid, *p*-toluenesulfonic acid, sulfuric acid.
- [3] See also: J. Susko, F. Szelag, *Bull. Int. Acad. Pol. Sci. Lett. Cl. Sci. Math. Nat. Ser. A* **1936**, 403.
- [4] Thin layer chromatography was performed on silica gel plates (60 F₂₅₄, Merck). Detection with UV light and bromocresol green and purification by column chromatography on silica gel showed no product other than 9-epiquinine (**4**). Diastereomeric quinine (**1**) was never observed.
- [5] a) T. G. Waddell, T. Rambalagos, K. R. Christie, *J. Org. Chem.* **1990**, 55, 4765; b) H. M. R. Hoffmann, O. Schrage, *Tetrahedron: Asymmetry* **1998**, 9, 1051.
- [6] a) J. Cossy, C. Dumas, D. G. Pardo, *Eur. J. Org. Chem.* **1999**, 1693; further recent examples: b) J. Wilken, M. Kossenjans, W. Saak, D. Haase, S. Pohl, J. Martens, *Liebigs Ann.* **1997**, 573.
- [7] W. M. Braje, R. Wartchow, H. M. R. Hoffmann, *Angew. Chem.* **1999**, 111, 2698; *Angew. Chem. Int. Ed.* **1999**, 38, 2540.
- [8] With neutral water as a solvent, 9-epibromoquinine (**2**) and silver benzoate also yield 9-epiquinine (**4**) as the only product with complete retention of configuration. However, the 1-azabicyclo[3.2.2] skeletal rearrangement^[7] is strongly solvent dependent. This ring expansion involves fission of two σ -bonds and takes place, for example, with silver benzoate in methanol or with silver benzoate in anhydrous acetone.
- [9] For a definition of *syn*, *anti* and *open*, *closed* conformations, see: a) G. D. H. Dijkstra, R. M. Kellogg, H. Wynberg, J. S. Svendsen, I. Markó, K. B. Sharpless, *J. Am. Chem. Soc.* **1989**, 111, 8069; b) G. D. H. Dijkstra, R. M. Kellogg, H. Wynberg, *J. Org. Chem.* **1990**, 55, 6121.
- [10] See also: H. B. Bürgi, J. D. Dunitz, J. M. Lehn, G. Wipff, *Tetrahedron* **1974**, 30, 1563.
- [11] The conformation of 9-epiquinine mesylate (**5**) and 9-epibromoquinine (**2**)^[7] was determined in the solid state (X-ray crystallography^[17]) as well as in solution (NOE studies, solvent CDCl₃) as *syn*, *open* (Figure 1b).
- [12] Generation of a stabilized intermediate carbocation seems facile, because the π electron cloud of the quinoline moiety is oriented

correctly for overlap with the vacant p orbital at C9 (Figure 1b). Hydration of the (protonated) bridgehead nitrogen and of the leaving group in the *syn*, *open* conformation is thought to favor nucleophilic attack of the carbocation from the front. Nonetheless, since the intermediate cation is delocalized and comparatively long-lived, the stereochemical outcome, that is complete retention of configuration, is remarkable.

- [13] 1-Phenylethyl halides (PhCH(Me)–X; X = Cl, Br), which may be considered as models for our O-mesyl cinchonanes and **2**, have been reported to solvolyse in aqueous ethanol with substantial racemization and up to 27% inversion of configuration. See: C. K. Ingold, *Structure and Mechanism in Organic Chemistry*, 2nd ed., Cornell University Press, Ithaca, **1969**, p. 521, Table 33–1.
- [14] X-ray crystal structure of the protonated quinine tartrate hemihydrate and conformation of the hydrogen tartrate ion: C. Ryttersgaard, S. Larsen, *Acta Crystallogr. Sect. C* **1998**, 54, 1698.
- [15] Selected recent examples for applications in asymmetric catalysis: Asymmetric dihydroxylation: a) H. C. Kolb, M. S. VanNieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, 94, 2483; b) ion-pairing and asymmetric phase transfer catalysis: E. J. Corey, F.-Y. Zhang, *Angew. Chem.* **1999**, 111, 2057; *Angew. Chem. Int. Ed.* **1999**, 38, 1931; c) B. Lygo, J. Crosby, J. A. Peterson, *Tetrahedron Lett.* **1999**, 40, 8671; d) M. J. O'Donnell, F. Delgado, R. S. Pottorf, *Tetrahedron* **1999**, 55, 6347; e) D. Martyres, *Synlett* **1999**, 9, 1508, and references therein.
- [16] Further recent works in the field of *Cinchona* alkaloids: a) W. M. Braje, J. Frackenhohl, O. Schrage, R. Wartchow, W. Beil, H. M. R. Hoffmann, *Helv. Chim. Acta* **2000**, 83, 777; b) S. Röper, J. Frackenhohl, O. Schrage, R. Wartchow, H. M. R. Hoffmann, *Org. Lett.* **2000**, 2, in press; c) J. Frackenhohl, H. M. R. Hoffmann, *J. Org. Chem.* **2000**, 65, in press.
- [17] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-115799 (**5**) and -115802 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

P₆ Manganocene and P₃ Cymantrene: Consequences of the Inclusion of Phosphorus Atoms in Mn-Coordinated Cyclopentadienyl Ligands**

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Dedicated to Professor Karl-Heinz Thiele on the occasion of his 70th birthday.

The phosphorus-rich analogue of the cyclopentadienyl ligand 3,5-di(*tert*-butyl)-1,2,4-triphospholyl (**1**) can either be

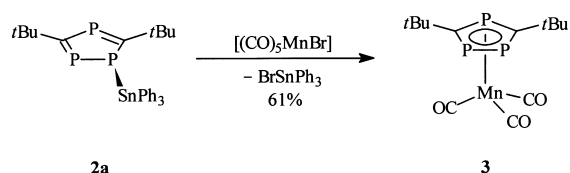
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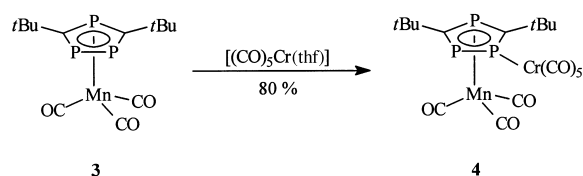
prepared from *tert*-butylphosphaalkyne units coordinated to a transition metal^[1] or transferred to a metal center as the triphospholyl ion **1**.^[2] Both synthetic routes, however, lead to only poor yields of sandwich complexes with this ligand, so we prepared 1-triorganylstannyl-3,5-di(*tert*-butyl)-1,2,4-triphosphole (**2**) as an alternative reagent for the transfer of **1** to a metal center.^[3] As preliminary experiments, in which **2** was treated with cobalt(i) and tin(ii) compounds, were very successful we implemented a systematic investigation of **2** as a reagent for the synthesis of sandwich and half-sandwich complexes with **1** as a ligand. The aim of the project is to determine both experimentally and theoretically the limits of simple concepts based on the analogy between phosphorus and carbon chemistry for the bonding characteristics of π ligands derived from unsaturated P-heterocycles. According to these principles, which include the diagonal relationship between the two elements^[4] or the isolobal principle,^[5] C–R fragments and phosphorus atoms should be freely exchangeable without significantly changing the properties of the substance in question.^[4] We regard the analogy between phosphorus and carbon to be no longer valid if the formally analogous compounds differ significantly in their reactivity, spectroscopic properties, or geometric structure.

We now report the synthesis and the properties of manganese complexes with **1** as ligands. We are interested in this class of substances because we hope to obtain direct information about the singly occupied molecular orbitals (SOMOs) of the paramagnetic oligophosphamanganocenes, especially by EPR spectroscopy. The EPR parameters should provide us with information about the degree of similarity between the different manganocene derivatives.

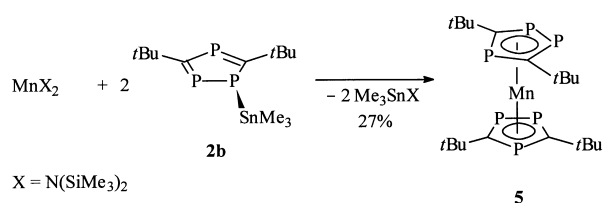
If, for instance, triphenylstannyltriphosphole **2a** is treated with $[(\text{CO})_5\text{MnBr}]$, triphosphacymantrene **3** is obtained in good yield.^[6] As was also found for other triphospholyl



complexes,^[7] **3** can also be used as a σ ligand because of its phosphorus lone pairs. When it is treated with $[(\text{CO})_5\text{Cr}(\text{thf})]$, the binuclear Mn/Cr complex **4** forms, also



in good yield. However, MnCl_2 proved to be unsuited for the synthesis of the hexaphosphamanganocene derivative **5**. The more soluble $[\text{Mn}[\text{N}(\text{SiMe}_3)_2]]$, however, can be treated with trimethylstannyltriphosphole **2b** to give **5**.



The triphosphacymantrene derivatives are diamagnetic and do not show any unexpected properties when compared with the prototype carbon compounds. The IR spectra of cymantrene and **3**, for example, each exhibit two $\nu(\text{CO})$ peaks, that differ by at most 17 cm^{-1} .^[8]

An X-ray crystal structure analysis^[9] of **5** (Figure 1) showed, on first sight, that it is placed in a homologous series with other hexaphosphametallocenes.^[10]

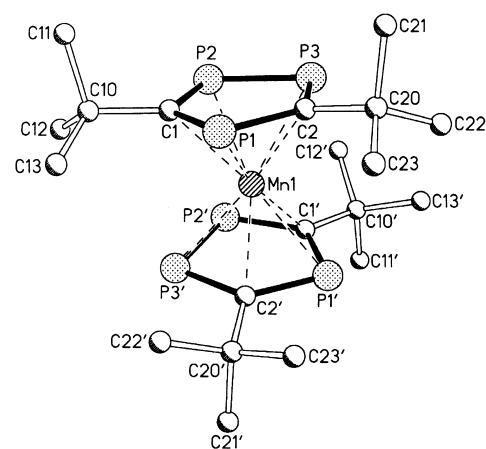


Figure 1. Molecular structure of **5** in the solid state. Hydrogen atoms are omitted for clarity.

Compound **5**, which is axially chiral because the ligands are twisted relative to each other, undergoes spontaneous separation of the enantiomers on crystallization. The angle between the planes of the two five-membered rings is $171.0(2)^\circ$ and the distance between Mn and the ring centers (171 pm) lies between those of the analogous iron and chromium compounds.^[10] Superconducting quantum interference device (SQUID) measurements showed **5** to be a low-spin compound.^[11] The EPR spectra of **5** as liquid and frozen solutions (Figure 2) are far better resolved than those of manganocenes with purely carbocyclic ligands^[12] and show the

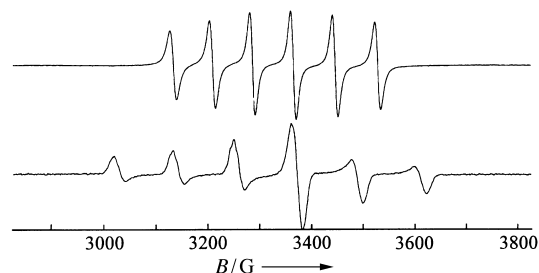


Figure 2. EPR spectra of **5** in *n*-hexane in the liquid phase at 293 K (above) and in the glassy, frozen solution at 100 K (below). For EPR parameters, see Experimental Section.

characteristics of axially symmetric g - and a (^{55}Mn)-hyperfine tensors that are consistent with a ^2A ground state.^[13] The lowering of the symmetry of the π ligand orbitals of **1**, caused by the P atoms, does not allow exactly degenerate frontier orbitals in **5** and thereby an exact axial symmetry for its SOMO is ruled out. However, the tensor components $g_{\perp}$ and $A_{\perp}(^{55}\text{Mn})$ could not be resolved and no hyperfine structure, due to the phosphorus or hydrogen nuclei, could be observed. This suggests a very small spin density on the ligands. The narrow linewidth of the spectra, the ^{55}Mn hyperfine structure, and the small g anisotropy indicate a nondegenerate spin state, spin density essentially centered on the metal, and a small spin–orbit coupling.^[12] Compound **5** therefore has very different properties from the high-spin manganocene and also from the low-spin decamethylmanganocene, for which a Jahn–Teller active, degenerate $^2\text{E}_{2g}$ ground state was found and which is incompatible with the EPR properties of **5**.^[12]

In order to characterize the electronic structure of **5** more completely, we calculated the structure and spin-density distribution with density functional theory. The calculations used Becke's three-parameter hybrid functional^[14] combined with the Lee, Yang, and Parr's nonlocal correlation functional (B3LYP),^[15] as implemented in Gaussian 98.^[16] The Schäfer-Horn-Ahlrichs split-valence basis set^[17] (SHAsv) was used for geometry optimizations and the subsequent frequency calculations. Additionally, we performed single-point calculations on the optimized geometries using the Schäfer-Horn-Ahlrichs triple-zeta basis set^[17] (SHATz) in order to obtain a more exact description of the electronic structure of **5**. Both basis sets were extended with a set of d functions with exponents of 0.6 and 0.34 on C and P, respectively, and of p and f functions with exponents of 0.092 and 0.96 on Mn.^[18]

Starting from the crystal structure of **5**, two C_2 -symmetrical minima were obtained for two different electronic states. One was the ^2A state expected on the basis of the EPR spectra and the other a ^2B state only 0.6 kcal mol^{−1} less stable. The energetic ordering of the two states can therefore not be determined clearly. Both wavefunctions proved to be stable.

Table 1 shows some selected bond lengths and angles for **5**. For the calculated ^2A state, the distance from the ring center to the metal (174 pm) and the torsional angle between the rings (81°) are in better accordance with the experimental values (171 pm, 76°) than for the ^2B (184 pm, 86°). When the calculated structures are superimposed on the crystal struc-

Table 1. Bond lengths in **5** [pm].

	exp.	$^2\text{A}^{\text{[a]}}$	$^2\text{B}^{\text{[a]}}$
Mn–P1	236.3(2)	240.6	252.1
Mn–P2	238.70(14)	242.8	244.1
Mn–P3	239.06(14)	243.0	246.8
Mn–C1	221.4(5)	222.9	230.1
Mn–C2	221.9(5)	227.4	239.3
P1–C1	176.9(5)	179.0	177.6
P1–C2	176.2(6)	178.6	177.0
P2–C1	177.9(5)	179.8	178.8
P3–C2	177.4(5)	179.2	178.5
P2–P3	213.1(2)	217.2	217.9

[a] B3LYP/SHAsv.

ture with the program VMD 1.2,^[19] the ^2A structure thus gives a smaller root-mean-square distance^[20] (RMSD) of 21 pm than the 34 pm RMSD of the ^2B structure. The calculated spin-density distributions for the two possible ground states of **5** are shown in Figure 3.^[21] Both are clearly metal centered. The shape of the spin-density distribution of the ^2A state corresponds largely to a d_{z^2} orbital; in contrast, that for the ^2B state resembles a d_{xy} or $d_{x^2-y^2}$ orbital.

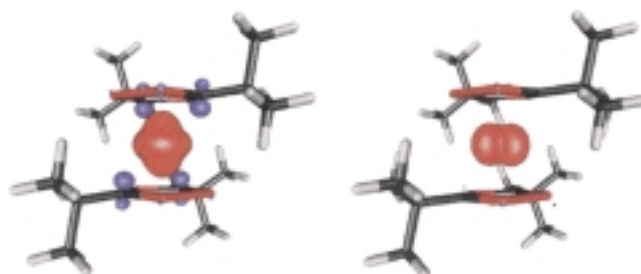


Figure 3. Calculated spin-density distribution of **5** for the ^2A (above) and ^2B (below) ground states. Isodensity contours are 0.006 e[−] b^{−3} (b = atomic unit bohr).

For **5**, the calculated isotropic and anisotropic ^{55}Mn hyperfine structures^[22] are shown in Table 2. Taking the experimental error limits and the likely accuracy of the calculated results into account, there is reasonable agreement between

Table 2. Isotropic and anisotropic hyperfine coupling constants of **5** [G].

	exp.	$^2\text{A}^{\text{[a]}}$	$^2\text{B}^{\text{[a]}}$
$\langle a(^{55}\text{Mn}) \rangle$	78.9 ± 3.6	− 63.2	− 44.8
A_{zz}	not resolved	6.0	− 133.7
A_{xx}	115.7 ± 5.1	− 92.9	− 0.8
A_{yy}	115.7 ± 5.1	− 102.7	0.1

[a] B3LYP/SHATz//B3LYP/SHAsv.

the calculated and experimental values for the ^2A state. However, the deviation from axial symmetry found in the calculations is larger than the error limits of the experimental values allow. The ^2B state, on the other hand, is calculated to have a significantly smaller $\langle a(^{55}\text{Mn}) \rangle$ coupling constant and an inverted splitting pattern compared with experimental results. Such a splitting pattern is found for decamethylmanganocene, which has a $^2\text{E}_{2g}$ ground state that corresponds to the ^2B state for **5**.^[12, 23] (Figure 4).

There are two possible explanations of the reversal of the order of the frontier orbitals observed here. Cyclopentadienyl

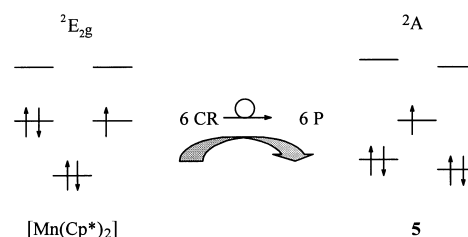


Figure 4. Schematic representation of the frontier orbitals of decamethylmanganocene and hexaphosphamanganocene **5**.

and triphospholyl ligands differ because of the lone pairs on phosphorus, which provide the possibility of interaction with the metal center. The calculations, however, showed no significant interaction with the d orbitals of the manganese center.^[25] On the other hand, the size of the phosphorus atoms alone causes significant changes in the electronic properties of the ligands. Not only the spatial extent of the phosphorus p orbitals but also the ring diameter play a role. These two effects can change the interaction between the d orbitals of the metal and the orbitals of the ligand significantly and thereby change the nature of the frontier orbitals. Our data does not yet allow a clear assignment of these effects.

In summary, by introducing six phosphorus atoms into the manganocene framework in [bis(3,5-di(*tert*-butyl)-1,2,4-triphospholyl)manganese], **5** leads to a ²A ground state, unprecedented for manganocene derivatives. Introducing three phosphorus atoms into each ligand thus results in a change in the energetic ordering of the frontier orbitals. The properties of hexaphosphamanganocene **5** can no longer be derived from simple P–C analogies. In contrast, triphosphacymantrene derivatives do not differ significantly from the prototype cymantrene. The π ligand 1,2,4-triphospholyl **1** thus represents a well suited system for determining the limits of the applicability of qualitative analogies between phosphorus- and carbon-based organic chemistry.

Experimental Section

3: A solution of 1-triphenylstannyl-3,5-di(*tert*-butyl)-1,2,4-triphosphole **2a** (0.894 g, 2.26 mmol) in THF (6 mL) was treated at room temperature with [Mn(CO)₅Br] (1.314 g, 3.25 mmol) in THF (10 mL). The mixture was refluxed for 2 h. After the volatile components were removed in vacuo, the red residue was distilled in a Kugelrohr apparatus. The fraction that distilled between 90 and 130 °C at 0.01 mbar was recrystallized from *n*-hexane to give of analytically pure **3** (0.50 g, 1.35 mmol, 61 %) in the form of yellow crystals; ¹H NMR (300.1 MHz, C₆D₆, 25 °C): δ = 1.24 (s, 18 H, C(CH₃)₃); ¹³C NMR (75.5 MHz, C₆D₆, 25 °C): δ = 222.2 (s, CO), 161.9 (ddd, ¹J(³¹P,¹³C) = 79.2 Hz, ¹J(³¹P,¹³C) + ²J(³¹P,¹³C) = 101 Hz, C_{ring}), 39.7 (ddd, ²J(³¹P,¹³C) = 17.4 Hz, ²J(³¹P,¹³C) + ³J(³¹P,¹³C) = 13.1 Hz, C(CH₃)₃), 36.9 (ddd, ³J(³¹P,¹³C) = 8.0 Hz, ³J(³¹P,¹³C) + ⁴J(³¹P,¹³C) = 8.3 Hz, C(CH₃)₃); ³¹P NMR (121.5 MHz, C₆D₆, 25 °C): A₂B system, δ = 114.10 (pd, ²J(³¹P,³¹P) = 42.7 Hz, 2 P, P(A)), 110.60 (pt, ²J(³¹P,³¹P) = 42.7 Hz, 1 P, P(B)); IR (THF): $\tilde{\nu}$ = 2019, 1952 cm⁻¹ (C=O); MS (FD): *m/z* (%): 370 (100) [*M*]⁺; MS (EI, 70 eV): *m/z* (%): 370 (15) [*M*]⁺, 314 (20) [*M* – 2 CO]⁺, 286 (100) [*M* – 3 CO]⁺; elemental analysis (%): calcd. for C₁₃H₁₈MnO₃P₃: C 42.18, H 4.90; found: C 42.50, H 4.86.

4: For the preparation of in situ [Cr(CO)₅(thf)], [Cr(CO)₆] (0.077 g, 0.35 mmol) was irradiated in THF (20 mL) for 3 h. A solution of **3** (0.114 g, 0.3 mmol) in THF (10 mL) was added at room temperature. The reaction mixture was stirred for 2 h at room temperature. The volatile components were removed in vacuo and the residue suspended in *n*-pentane and filtered. Crystallization from *n*-pentane yielded **4** (0.135 g, 0.24 mmol, 80 %) in the form of yellow-orange crystals; ¹H NMR (269.7 MHz, CD₂Cl₂, 25 °C): δ = 1.44 (s, 9 H, C(CH₃)₃), 1.32 (s, 9 H, C(CH₃)₃); ³¹P NMR (109.4 MHz, CD₂Cl₂, 25 °C): ABC system, δ = 77.6 (dd, ¹J(³¹P,³¹P) = 438.5 Hz, ²J(³¹P,³¹P) = 36 Hz, 1 P, P(A)), 100.4 (pt, ²J(³¹P,³¹P) = 36 Hz, ²J(³¹P,³¹P) = 48.6 Hz, 1 P, P(B)), 165.4 (dd, ¹J(³¹P,³¹P) = 438.5 Hz, ²J(³¹P,³¹P) = 48.6 Hz, 1 P, P(C)); IR (THF): $\tilde{\nu}$ = 2072 (s), 2023 (vs), 1998 (s), 1964 (vs, br), 1953 cm⁻¹ (vs, br) (C=O); MS (FD): *m/z* (%): 562 (100) [*M*]⁺.

5: [Mn[N(SiMe₃)₂]₂], analogously with ref. [25], was prepared in situ from dry MnCl₂ (0.193 g, 1.53 mmol) and 5 mL of a 0.6 molar (3.07 mmol) toluene solution of Na[N(SiMe₃)₂] in THF (20 mL). The solvents were removed in vacuo after completion of the reaction. A solution of 1-trimethylstannyl-3,5-di(*tert*-butyl)-1,2,4-triphosphole **2b** (0.943 g, 2.39 mmol) in 25 mL of *n*-hexane was added to the manganese

amide at –40 °C. The mixture was warmed to 20 °C. A red solution was formed and colorless [(Me₃Si)₂NSnMe₃] precipitated. After stirring for 2 h and cooling slowly to –30 °C, **5** was obtained in the form of black crystals, which were washed several times at –50 °C with *n*-hexane, to give **5** (0.170 g, 0.328 mmol, 27.5 %). EPR (9.34 GHz, *n*-hexane, 298 K): $\langle g \rangle$ = 2.008 ± 0.005, $\langle a(^{55}\text{Mn}) \rangle$ = 78.9 ± 3.6 G (⁵⁵Mn (100 %): I = 5/2); EPR (9.42 GHz, *n*-hexane, 100 K): $g_{\parallel}$ = 2.00 ± 0.02, $A_{\parallel}(^{55}\text{Mn})$ = unresolved, $g_{\perp}$ = 2.03 ± 0.01, $A_{\perp}$ = 115.7 ± 5.1 G (⁵⁵Mn (100 %): I = 5/2); the error limits given represent the maximum possible deviation from the mean value; MS (FD): *m/z* (%): 517 (100) [*M*]⁺.

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- [1] M. Driess, D. Hu, H. Pritzkow, H. Schauele, U. Zenneck, M. Regitz, W. Rosch, *J. Organometal. Chem.* **1987**, 334, C35.
- [2] R. Bartsch, P. B. Hitchcock, J. F. Nixon, *J. Chem. Soc. Chem. Commun.* **1987**, 1146.
- [3] A. Elvers, F. W. Heinemann, B. Wrackmeyer, U. Zenneck, *Chem. Eur. J.* **1999**, 5, 3143.
- [4] K. B. Dillon, F. Mathey, J. F. Nixon, *Phosphorus, The Carbon Copy*, Wiley, Chichester, **1998**.
- [5] R. Hoffmann, *Angew. Chem.* **1982**, 94, 225; *Angew. Chem. Int. Ed. Engl.* **1982**, 21, 711.
- [6] Mass spectroscopy hints on the existence of **4** was reported in ref. [2].
- [7] R. Bartsch, P. B. Hitchcock, J. F. Nixon, *J. Organomet. Chem.* **1988**, 344, C37.
- [8] L. M. C. Shen, G. G. Long, C. G. Moreland, *J. Organomet. Chem.* **1966**, 5, 362–369.
- [9] X-ray structure of **5** (C₂₀H₃₆MnP₆): Single crystals were obtained from *n*-hexane at –30 °C. Crystal dimensions: 0.50 × 0.35 × 0.25 mm. Data were collected at 200 K on a four-circle diffractometer (Siemens P4, graphite monochromatized MoK α radiation, λ = 0.71073 Å). The structure was solved by direct methods and refined on *F*² using full-matrix least-squares procedures (SHELXTL 5.03^[24]). Orthorhombic; space group C22₁ (no. 20); a = 11.368(2), b = 16.299(3), c = 13.680(3) Å; V = 2534.8(9) Å³; Z = 4; ρ_{calcd} = 1.355 g cm⁻³; μ = 0.905 mm⁻¹; $F(000)$ = 1084. A total of 3654 reflections were measured in the 2θ range 4.3–52.0°, of which 2351 symmetry-independent reflections were used for the refinement. The final *R* factors were R_1 = 0.0456 (for 1870 reflections with $F_0 \geq 4.0\sigma(F)$), wR_2 = 0.0987 (all data), GOF = 1.059; max./min. residual electron density 0.509/–0.309 e Å⁻³. The crystal investigated proved to be an inversion twin. A twin refinement gave a twinning ratio of 3:1. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-137320. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [10] a) R. Bartsch, P. B. Hitchcock, J. F. Nixon, *J. Organomet. Chem.* **1988**, 356, C1–C4; b) R. Bartsch, P. B. Hitchcock, J. F. Nixon, *J. Chem. Soc. Chem. Commun.* **1987**, 1146; c) R. P. B. Hitchcock, R. M. Matos, J. F. Nixon, *J. Organomet. Chem.* **1995**, 490, 155–162.
- [11] O. Waldmann, Physical Institute, University of Erlangen–Nuremberg, unpublished results. Measurements were performed with a commercial SQUID magnetometer (VL Quantum Design). The magnetic susceptibility declines markedly at temperatures lower than 50 K. This unexplained effect is being investigated.
- [12] a) J. L. Robbins, N. M. Edelstein, S. R. Copper, J. C. Smart, *J. Am. Chem. Soc.* **1979**, 101, 3853–3857; b) J. H. Ammeter, *J. Magn. Reson.* **1978**, 30, 299.
- [13] J. A. Weil, J. R. Bolton, J. E. Wertz, *Electron Paramagnetic Resonance, Elementary Theory and Practical Applications*, Wiley, New York, **1994**.
- [14] a) A. D. Becke, *J. Chem. Phys.* **1993**, 98, 1372–1377, 5648–5652; b) P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch, *J. Phys. Chem.* **1994**, 98, 11623–11627.
- [15] C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, 37, 785–789.
- [16] Gaussian 98, Revision A.5, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi,

- V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **1998**.
- [17] A. Schaefer, H. Horn, R. Ahlrichs, *J. Chem. Phys.* **1992**, 97, 2571–2577.
- [18] a) S. Huzinaga, *Gaussian Basis Sets for Molecular Calculations*, Elsevier, Amsterdam, **1984**; b) K. Raghavachari, G. W. Trucks, *J. Chem. Phys.* **1989**, 91, 1062–1065.
- [19] a) A. Dalke, W. Humphrey, S. Izrail, J. Stone, J. Ulrich, VMD für IRIX5, Version 1.2, **1998**; b) W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graphics* **1996**, 14.1, 33–38.
- [20] $RMSD = [\sum_{i=1}^N d_i^2/N]^{1/2}$ (N = number of the atom, d = deviation): A. R. Leach, *Molecular Modelling*, Addison Wesley Longman Ltd., Essex, **1996**, p. 445.
- [21] Visualization was performed with gOpenMol: L. Laakson, gOpenMol for Linux, Version 1.3, Centre of Scientific Computing, Espoo, Finland, **1999**.
- [22] a) V. Barone in *Recent Advances in Density Functional Methods*, Part I (Ed.: D. P. Chong), World Scientific Publishing, Singapore, **1996**; b) N. Rega, M. Cossi, V. Barone, *J. Chem. Phys.* **1996**, 105, 11060–11067; c) V. Barone, *Chem. Phys. Lett.* **1996**, 262, 201.
- [23] B. Horvath, R. Mössler, E. G. Horvath, *Z. Anorg. Allg. Chem.* **1979**, 450, 165.
- [24] SHELXTL 5.03 for Siemens Crystallographic Research Systems, Siemens Analytical X-Ray Instruments Inc., Madison, WI, USA, **1995**.
- [25] The natural bond orbital (NBO) and natural localized molecular orbital (NLMO) analyses^[26] showed no significant interaction between the lone pairs of the phosphorus atom and the manganese d orbitals.
- [26] a) J. P. Foster, F. Weinhold, *J. Am. Chem. Soc.* **1980**, 102, 7211–7218; b) A. E. Reed, F. Weinhold, *J. Chem. Phys.* **1985**, 83, 1736–1740.

Triple-Decker Type Coordination of a Fullerene Trianion in $[K([18]\text{crown-6})]_3[\eta^6, \eta^6\text{-C}_{60}](\eta^3\text{-C}_6\text{H}_5\text{CH}_3)_2$ —Single Crystal Structure and Magnetic Properties**

Thomas F. Fässler,* Rudolf Hoffmann, Stefan Hoffmann und Michael Wörle

Dedicated to Professor Hans-Herbert Brintzinger on the occasion of his 65th birthday

Molecular conductors are promising candidates for high-temperature superconductors because large phonon frequency bands with little dispersion and hence a large density of

state at the Fermi level (E_F) are possible. The alkali metal–fullerides A_3C_{60} (A is the alkali metal) are a fascinating class of compounds showing superconductivity up to 40 K.^[1,2] The trianions of the C_{60} fullerenes play a deciding role in understanding the superconductivity, and, therefore, the nature of the electronic ground state of $[C_{60}]^{3-}$ and its intermolecular interactions are of particular interest. The LUMO of a C_{60} molecule with I_h -symmetry is threefold degenerate, has t_{1u} -symmetry, and can be occupied by up to six electrons. In A_3C_{60} compounds these orbitals form a small electronic band which is half filled with electrons and is responsible for the metallic conductivity (Figure 1). The

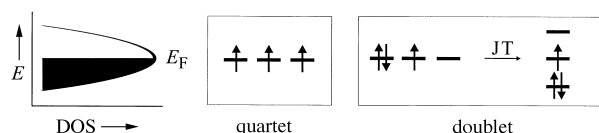
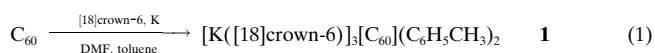


Figure 1. The three fold degenerate HOMO of the $[C_{60}]^{3-}$ ions with I_h symmetry (center) and the resulting density state (DOS) of the corresponding half-filled band in K_3C_{60} (left). In the doublet states, Jahn–Teller (JT) distortion is possible (right).

occupation of the molecular LUMOs of C_{60} by a further three electrons is expected to result in energetically low-lying quartet (^4A) and doublet states (^2H and ^2T). The doublet states are Jahn–Teller-unstable systems and structural distortion is possible (Figure 1).^[3–5] In spite of these interesting findings, there is still not a single crystal structure determination of a binary superconducting phase. Overlapping reflections, in refinements based upon X-ray and neutron powder diffraction studies, give plenty of scope for discussion as to the nature of the conformational disorder found in the K_3C_{60} fullerene molecules.^[6–8]

In the course of our investigations of soluble Zintl ions of the carbon group,^[9,10] we recently reported the reduction of C_{60} with potassium in DMF and the crystallization of a fullerene dianion with cryptands. In $[K([2.2.2]\text{crypt})]_2[C_{60}](C_6H_5CH_3)_4$ (**2**),^[11] following the principle of spherical close packing, the $[C_{60}]^{2-}$ units form layers which are separated from each other by bulky $[K^+([2.2.2]\text{crypt})]$ ions (Figure 2a and b).^[12] In attempting to prepare homoatomic anions of the heavier homologues of the carbon group by a one-pot synthesis, we chose to try the reduction of the elements tin and lead with alkali earth metals in the molten crown ether [18]crown-6.^[13–16] Using fullerenes in this synthetic procedure has now enabled us to perform a single crystal structure determination of a salt containing two ordered C_{60} trianions. We now report the synthesis, and structural and magnetic properties of the complex $[K([18]\text{crown-6})]_3[C_{60}](C_6H_5CH_3)_2$ (**1**).

Potassium dissolves in the molten crown ether [18]crown-6 at 313 K to give a deep blue solution. Carbon is added to the melt in the form of C_{60} . After the blue coloration disappears, DMF is added to the reaction mixture. Crystals of the title compound are then obtained by layering extracts of the reaction mixture with toluene [Eq. (1)]. The crystals are black cuboids which when crushed have a red hue.



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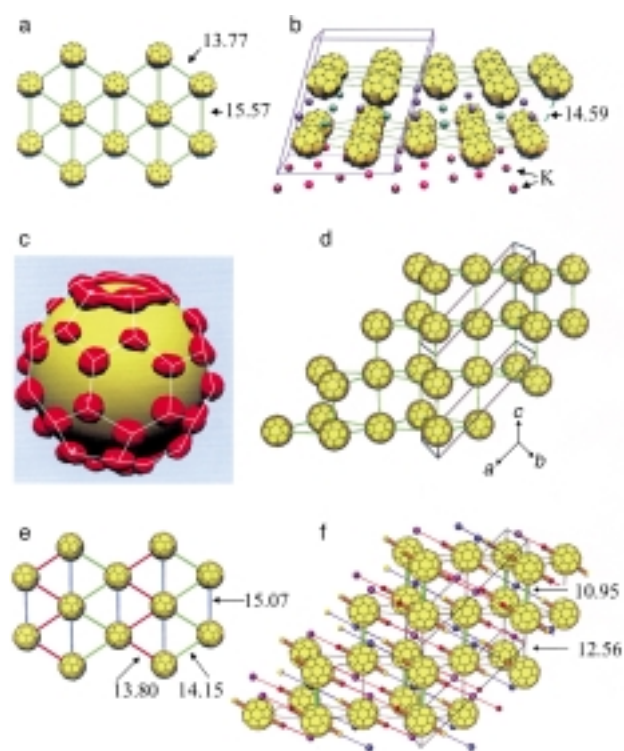


Figure 2. a) View of the distorted hexagonal arrangement of the fullerene molecules in the crystal structure of **2**. b) Arrangement of the fullerene molecules in **2**; instead of the $[K([2.2.2]crypt)]$ units only the K atoms (spheres) are shown. c) Three dimensional view of an equal electron density surface in the area of the fullerene molecule in **1** from Fourier calculations. The white lines show the C_{60} polyhedron. The uppermost six atoms coordinate to the K atom (see also Figure 3 a). d) Distorted primitive hexagonal arrangement of the fullerene units in **1**. Angles between the centers of the fullerene molecules in the plane perpendicular to the c -axis: 57.8° , 56.9° , and 65.3° . e) A layer of fullerene molecules in **1** in a plane perpendicular to the c -axis. f) Crystal structure of **2**. Instead of the $[K([18]crown-6)]$ units only the K atoms (spheres) are shown (K1 = blue, K2 = violet, K3 = red, and K4 = yellow). The K–K separations are shown as thin lines to show the packing more clearly.

The results of the single crystal structure analysis at 110 K are shown in Figures 2c–f and 3.^[17] The asymmetric unit contains one half of the C_{60} molecule and four K atoms. Consideration of the site symmetry at special positions comes to three K atoms per C_{60} molecule and, thus, a threefold negative charge on the C_{60} anion. The potassium atoms are each bound to the six O atoms of the crown ethers. Two of the potassium atoms additionally coordinate to two opposing hexagonal faces of the C_{60} polyhedron, the other two potassium atoms each coordinate to two toluene molecules (Figure 3 a).

Of the K atoms coordinated to the fullerene anion, K3 has six bonds to the C atoms which are of almost equal length (two of 3.279, two of 3.257, and two of 3.238 Å). K4 is shifted from the axis running through the center of the hexagon in the direction of the 5:6-ring connection (two bonds of 3.386, two of 3.261 and two of 3.138 Å; the shortest bonds are shown in Figure 3 a as solid lines). The asymmetric coordination of the K atoms is also shown by the angle between the hexagonal face of the fullerene and the plane through the six O atoms of the crown ether; the angles for K3 and K4 are 1.3° and 9.7° , respectively. The toluene molecules which complete the

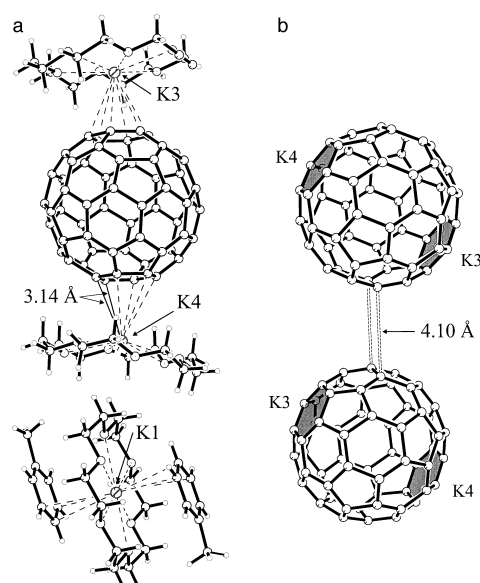


Figure 3. a) Relative arrangements of a $[K([18]crown-6)]_2[\eta^6,\eta^6-C_{60}]$ (above) and a $[K([18]crown-6)](\eta^3-C_6H_5CH_3)_2$ unit (below; see also the crystal packing diagram in Figure 2 f). b) Arrangement of two fullerene molecules along the c -axis. The broken lines indicate the shortest intermolecular C–C separations (see also the separations marked in green in Figure 2 f).

‘sandwich’ coordination of the other two potassium atoms, K1 and K2, bind as η^3 -ligands with three noticeably shorter K–C bond lengths (3.230(8)–3.517(5) Å compared to the three longer ones, 3.738–4.054 Å). The coordination of potassium by aromatic systems is known.^[18] The shortest K–C bond length in **1** is shorter than the K–C bonds in potassium complexes with η^6 -coordinated neutral aromatic molecules. In $[K^+(\text{dibenzo}[18]crown-6)](\eta^6-C_6H_6)$, the K–C bond lengths are in the range 3.29–3.50 Å^[18] and, in potassium complexes with cyclophane-like fullerene (dibenzo[18]crown-6) ligands, which have well defined distances between the C_{60} hexagon and the oxygens of the crown ether after covalent connection of the crown ether unit to the fullerene molecule, the shortest distance is 3.42 Å.^[19] The shorter K–C bond lengths in **1** arise from the greater charge on the fullerene and from steric factors. The convex surface of the fullerene allows a closer approach of the C_{60} and the K atom of the $[K^+([18]crown-6)]$ unit, relative to planar aromatic hydrocarbons.

A comparison of the coordination of the potassium atoms in **1** and in the superconducting phase K_3C_{60} , a cubic face-centered lattice of fullerene molecules in which the potassium atoms occupy the octahedral and tetrahedral holes, is of particular interest. In a model of K_3C_{60} , in which there are two orientational disorders of the C_{60} molecule, the potassium atom in the tetrahedral hole is surrounded by four hexagonal faces belonging to the four nearest neighboring C_{60} molecules. The K–C bond lengths for this potassium (3.27 Å) are similar to those in **1**. The K–C bond lengths for the K atoms in the octahedral holes are 3.69 Å.^[6, 20, 21]

The packing diagrams in Figures 2d–f show a situation analogous to the close packing of spheres, with layers of C_{60} molecules lying parallel to the b -axis and perpendicular to the c -axis. The distances between the centers of the fulleride anions in one layer are 13.80 (2 ×), 14.15 (2 ×), and 15.07 Å

(2 ×) and these correspond approximately to the separations in the layers of compound **2**. In **2** the stacking of the layers of fulleride ions resembles a hexagonal closest-packed array (Figure 2a and b), while in **1** the layers lie directly above each other, building a slightly distorted primitive hexagonal array (figure 2d and f). The layers are slightly puckered, resulting in shorter and longer distances between the ions. The shortest distance between fulleride centers (10.95 Å) is noticeably shorter than that in **2** (Figure 2b), but clearly longer than those of the cubic face-centered frameworks of the superconducting alkali metal intercalation complexes A_3C_{60} (10.07 (K₃C₆₀) to 10.25 Å (Rb₂CsC₆₀)).^[2]

The C₆₀ anions of the title complex are ordered; this can be seen in the three-dimensional distribution of the electron density around the centers of the atom positions in the fullerene molecules (Figure 2c). The two different C–C bond lengths (6:6 and 5:6 ring connections) in C₆₀ were, in each case, refined together and they converge to 1.380(5) and 1.460(5) Å, respectively. These bond lengths almost correspond to the values determined for a neutral C₆₀ molecule.^[22–24] A ‘leveling out’ of the two different bond lengths, as is the tendency in other ions,^[25] is not seen in this case.^[17]

The EPR spectra of freshly prepared microcrystalline powders show a broad isotrope signal at $g = 2.001$ (line width 4.08 mT) at room temperature, which indicates localization of the electrons.^[26] A simulation of the signal^[27, 28] allows identification of a second small line of lower intensity at $g = 2.000$ (line width 0.80 mT). Similar spectra are also obtained from binary phases from nominal compositions of A_3C_{60} ($A = K - Cs$)^[29] and from electrochemically formed anions.^[30, 31] The EPR spectra of C₆₀^{*n*−} ions ($n = 1 - 3$) are the subject of much discussion.^[32] There are other cases in which an obscured, sharp signal of lower intensity is found next to a broad line. Up to now it was not clear if this line is from a second spin state or from a fullerene carrying a different charge. Recently, it was suggested, for the monoanion C₆₀[−], that this small line arises from the formation of a fullerene dioxo radical through oxidation by oxygen.^[33, 34] In the spectrum of **1** we observe that the intensity of the sharp line increases when the probe is exposed to air.

The paramagnetic properties were confirmed by temperature-dependent susceptibility measurements. The magnetic behavior follows the Curie–Weiss law; analysis of the data in the temperature range 80–280 K afforded the parameters $C = 0.373(3)$ emu K mol^{−1}, $\theta = -4.0(5)$ K, and $\chi_0 = 200 \times 10^{-6}$ emu mol^{−1} (Figure 4).^[35] The calculated magnetic mo-

ment of $1.73 \mu_B$ corresponds to an isolated spin doublet ($S = \frac{1}{2}$). In agreement with these results the shortest intermolecular C–C separations indicate that only very weak interactions between the ions can be expected and show that the title complex has a diluted magnetic spin system. It is interesting to note that, in **1**, the carbon atoms of two different fullerene molecules lie only 4.10 Å apart (Figure 3b), the result of a type of [2+2] cycloaddition of the 5:6 ring connection. An analogous situation occurs in RbC₆₀, which can be described as a one dimensional polyfulleride formed by the [2+2] cycloadditions of the 6:6 ring connections of the fullerene molecules.^[36, 37]

Generally, investigations of the electronic states of C₆₀ trianions are difficult because, in solution the anions disproportionate and thus do not necessarily all have the same charge, and in the solid state either the exact composition or the complete structure is not known.^[30, 31, 38–40] The efficient synthesis of single crystal samples of the title complex, a molecular species containing [C₆₀]^{3−} ions, has enabled an extensive study of the structure–property relationships in fulleride trianions to be performed. The investigations of the magnetic properties show that, in this compound, the majority of the trianions are in the doublet state. A partial disproportionation into anions carrying two and four negative charges, as demonstrated for the [K([2.2.2]crypt)] salts of [Sn₉]^{3−} and [Pb₉]^{3−},^[41] can be ruled out.

Experimental Section

To perform the reduction in liquid [18]crown-6 at 313 K [18]crown-6 (800 mg, 3.00 mmol) and potassium (98 mg, 2.5 mmol) were weighed into a 20 mL Schlenk tube. The mixture was heated to 313–323 K so that the crown ether only just melted. C₆₀ (100 mg, 0.14 mmol) was added to the intense blue melt and the mixture was stirred for 1 h. After cooling, DMF (3.0 mL) was added and the mixture sonicated for 15 min. The resulting dark red solution was filtered and layered with toluene (4.5 mL). The product crystallized over the course of 2 weeks.

Alternatively, the reduction can be carried out in DMF. Potassium (21.6 mg, 0.56 mmol) and C₆₀ (100 mg, 0.14 mmol) were weighed into a 20 mL Schlenk tube. DMF (4 mL) was added and the mixture stirred at room temperature for approximately 14 h. During this time the color of the solution changed from green through violet to red. The red solution was filtered onto [18]crown-6 (202 mg, 0.75 mmol), sonicated for 30 min, and layered with toluene (5 mL). Over 2 weeks, the product precipitated as black rectangular cuboids. The yield from the second method is 145 mg (57.1% from C₆₀). Elemental analysis (C₁₁₀H₈₈O₁₈K₃, 1815.19 g mol^{−1}): calcd (found) [%]: C 72.79 (71.33), H 4.89 (5.18), K 6.46 (6.39).

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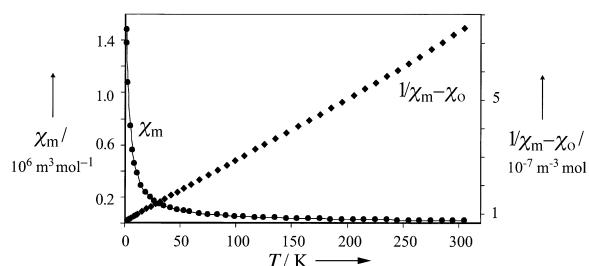


Figure 4. The temperature-dependence of the molar magnetic susceptibility χ_m (●) and the reciprocal temperature-dependent contribution $1/(\chi_m - \chi_0)$ (◆).

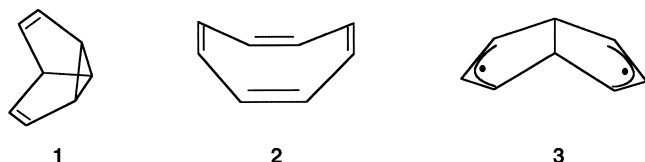
- [1] A. F. Hebard, M. J. Rosseinsky, R. C. Haddon, D. W. Murphy, S. H. Glarum, T. T. M. Palstra, A. P. Ramirez, A. R. Kortan, *Nature* **1991**, 350, 600.
- [2] R. M. Fleming, A. P. Ramirez, M. J. Rosseinsky, D. W. Murphy, R. C. Haddon, S. M. Zahurak, A. V. Makhija, *Nature* **1991**, 352, 787.
- [3] M. J. Rosseinsky, *Chem. Mater.* **1998**, 10, 2665.
- [4] W. H. Green, Jr., S. M. Gorun, G. Fitzgerald, P. W. Fowler, A. Ceulemans, B. C. Titeca, *J. Phys. Chem.* **1996**, 100, 14892.
- [5] F. Negri, G. Orlandi, F. Zerbetto, *J. Am. Chem. Soc.* **1992**, 114, 2909.

- [6] P. W. Stephens, L. Mihály, P. L. Lee, R. L. Whetten, S.-M. Huang, R. Kaner, F. Diederich, K. Holczner, *Nature* **1991**, 351, 632.
- [7] K. M. Allen, W. I. E. David, J. M. Fox, R. M. Ibberson, M. J. Rosseinsky, *Chem. Mater.* **1995**, 7, 764.
- [8] J. E. Fischer, G. Bendele, R. Dinnebier, P. W. Stephens, C. L. Lin, N. Bykovetz, Q. Zhu, *J. Phys. Chem. Solids* **1995**, 56, 1445.
- [9] T. F. Fässler in *Metal Clusters in Chemistry* (Eds.: P. Braunstein, L. A. Oro, P. R. Raithby), Wiley-VCH, Weinheim, **1999**, p. 1612.
- [10] T. F. Fässler, U. Schütz, *Inorg. Chem.* **1999**, 38, 1866.
- [11] [2.2.2]Crypt = [2.2.2]cryptand = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane.
- [12] T. F. Fässler, A. Spiekermann, M. Spahr, R. Nesper, *Angew. Chem.* **1997**, 109, 502; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 486.
- [13] [18]Crown-6 = 1,7,10,13,16-hexaoxacyclooctadecane.
- [14] T. F. Fässler, R. Hoffmann, *J. Chem. Soc. Dalton Trans.* **1999**, 3339.
- [15] T. F. Fässler, R. Hoffmann, *Angew. Chem.* **1999**, 111, 526; *Angew. Chem. Int. Ed.* **1999**, 38, 543.
- [16] T. F. Fässler, R. Hoffmann, *Chimia* **1998**, 52, 158.
- [17] X-Ray analysis of **1**: A crystal with dimensions $0.50 \times 0.30 \times 0.25 \text{ mm}^3$ was, after removal of solvent, washed with toluene and mounted in a glass capillary. Cell constants at 110 K: $a = 32.306(7)$, $b = 15.065(3)$, $c = 23.493(5) \text{ Å}$, $\beta = 133.44(3)^\circ$, $V = 8303 \text{ Å}^3$; space group $C2/m$ (Nr. 12), $Z = 4$, $\rho_{\text{calc}} = 1.452 \text{ g cm}^{-3}$. Data collection: STOE-IPDS diffractometer, $\text{MoK}\alpha$ radiation, $2\theta_{\text{max}} = 48.22^\circ$ (image plate separation 80 mm, angle of rotation 0.7° , 257 frames). 24011 Reflections, of which 6445 were independent ($R_{\text{int}} = 8.8\%$). $R_1 = 8.23\%$ and $wR_2 = 21.36\%$ for 4679 reflections with $I > 2\sigma(I)$; $R_1 = 11.21\%$ and $wR_2 = 23.83\%$ for all reflections; 626 parameters of which 49 were coupled. When all fullerene parameters were independently refined, six longer (1.62–1.64 Å, 5:6 ring connections) and twelve shorter C–C bond lengths (1.31–1.35 Å, 6:6 ring connections) were found. The parameters correlate with this model, thus the refinement with coupled bond length parameters was favored. Few residual electron density maxima ($< 1.47 \text{ e Å}^{-3}$) above the faces of the C_{60} polyhedra suggest that, besides the main orientation, a much smaller population of a second molecule, which is rotated by 90° about the K3–K4 axis of the main species, is also present. The molecule with this secondary orientation breaks the mirror plane. A refinement of two further fullerene molecules as well as the main species is not possible because of the correlation between the parameters. A twinned model fitting the data in an analogous manner does not lead to any improvement in the refinement. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-138621. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [18] J. L. Atwood, *J. Incl. Phenom.* **1985**, 3, 13.
- [19] J.-P. Bourgeois, P. Seiler, M. Fabbioni, E. Pretsch, F. Diederich, L. Echegoyen, *Helv. Chim. Acta* **1999**, 82, 1572.
- [20] C_{60}^- Trianions are also present in $\text{K}_3\text{C}_{60} \cdot 14 \text{ THF}$. The single crystal structure provides the arrangement of the ions but, because of the high disorder of the THF molecules, a stable refinement of the atom position of the fullerene molecule was not possible. Allowing for the highly distorted fullerene molecule, the K atoms are again seen to be coordinated to opposing hexagonal faces of the fullerene. A second type of K atom bridges two fullerene molecules across the 6:6 ring connections.^[21]
- [21] C. Janiak, S. Mühle, H. Hemling, *Polyhedron* **1995**, 15, 1559.
- [22] 1.355(9) and 1.47(2) Å, respectively: S. Liu, Y. Lu, M. M. Kappes, J. A. Ibers, *Science* **1991**, 254, 408.
- [23] 1.387(3) and 1.450(3) Å, respectively: H.-B. Bürgi, B. E. D. Schwarzenbach, S. Liu, Y. Lu, M. M. Kappes, J. A. Ibers, *Angew. Chem.* **1992**, 104, 667; *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 640.
- [24] 1.391 and 1.455 Å: W. I. E. David, R. M. Ibberson, J. C. Matthewman, K. Prassides, T. J. S. Dennis, J. P. Hare, H. W. Kroto, R. Taylor, D. R. M. Walton, *Nature* **1991**, 353, 147.
- [25] Average values for the bond lengths of the respective 6:6 and 5:6 ring connections in fullerenes. K_3C_{60} : 1.400(4) and 1.45(1),^[6] 1.400(6) and 1.453(5),^[7] and, for theoretical studies, 1.408 and 1.445,^[7] $[\text{K}^+][2.2.2]\text{crypt}]_2[\text{C}_{60}]^{2-}(\text{C}_6\text{H}_5\text{CH}_3)_4$: 1.39(1) and 1.45(1);^[12] $[\text{PPN}^+]_2[\text{C}_{60}]^{2-}$ (PPN⁺ = bis(triphenylphosphine)iminium): 1.399(2) and 1.449(3);^[42] $[\text{Ba}^{2+}(\text{NH}_3)_7][\text{C}_{60}]^{2-} \cdot \text{NH}_3$: 1.398(5) and 1.447(4);^[43] $[\text{Ni}(\text{C}_5\text{Me}_5)_2]^+[\text{C}_{60}]^- \cdot \text{CS}_2$: 1.389(3) and 1.449(3).^[44]
- [26] The ESR spectra were recorded on a Bruker EMX-080-spectrometer with diphenylpicrylhydrazyl as the external standard.
- [27] G. P. Lazos, B. M. Hoffmann, C. G. Franz, *POWFIT (QCPE no. 265), Program for Anisotropic EPR Spectra Simulation*, Quantum Chemistry Program Exchange, Indiana University, Bloomington (USA).
- [28] W. H. Press, B. P. Flannery, S. A. Teukolsky, W. T. Vetterling, *Numerical Recipes ESR*, Cambridge University Press, Cambridge, **1986**.
- [29] F. Bensebaa, B. Xiang, L. Kevan, *J. Phys. Chem.* **1992**, 96, 10258.
- [30] M. M. Khaled, R. T. Carlin, P. C. Trulove, G. R. Eaton, S. S. Eaton, *J. Am. Chem. Soc.* **1994**, 116, 3465.
- [31] S. S. Eaton, A. Kee, R. Konda, G. R. Eaton, P. C. Trulove, R. T. Carlin, *J. Phys. Chem.* **1996**, 100, 6910.
- [32] S. S. Eaton, G. R. Eaton, *Appl. Magn. Reson.* **1996**, 11, 155.
- [33] P. L. Boulas, R. Subramanian, M. T. Jones, K. M. Kadish, *Appl. Magn. Reson.* **1996**, 11, 239.
- [34] Y. L. Hwang, C. C. Yang, K. C. Hwang, *J. Phys. Chem.* **1997**, 101, 7971.
- [35] The magnetic susceptibility measurements were made with a Quantum Design MPMS5S SQUID magnetometer with a field strength of 2 T using freshly prepared samples of ground single crystals (weight 30 mg). A suprasil tube with a diameter of 4 mm was used as a sample holder, with its signal experimentally corrected. The parameters C (Curie Constant), θ (Curie temperature), and χ_0 (temperature independent contribution of the magnetic susceptibility) were calculated from the high temperature region with the appropriate formula: $\chi_m = C/(T - \theta) + \chi_0$. For three microcrystalline samples which were obtained from different reactions, the values for C , θ , and χ_0 were in the range $0.373\text{--}0.402 \text{ emu K mol}^{-1}$, $-3.3^\circ\text{--}-4.3^\circ$, and $200 \times 10^{-6}\text{--}670 \times 10^{-6} \text{ emu mol}^{-1}$, respectively.
- [36] S. Pekker, L. Forró, L. Mihály, A. Jánossy, *J. Solid State Commun.* **1994**, 90, 349.
- [37] P. W. Stephens, G. Bortel, G. Faigel, M. Tegze, A. Jánossy, S. Pekker, G. Oszlanyi, L. Forró, *Nature* **1994**, 370, 636.
- [38] M. S. Dresselhaus, G. Dresselhaus, P. C. Eklund, *Science of Fullerenes and Carbon Nanotube*, Academic Press, New York, **1996**.
- [39] P. C. Trulove, R. T. Carlin, G. R. Eaton, S. S. Eaton, *J. Am. Chem. Soc.* **1995**, 117, 6265.
- [40] P. Bhyrappa, P. Paul, J. Stinchcombe, D. W. Boyd, C. A. Reed, *J. Am. Chem. Soc.* **1993**, 115, 11004.
- [41] T. F. Fässler, M. Hunziker, M. Spahr, H. Lueken, *Z. Anorg. Allg. Chem.* **2000**, 626, 692.
- [42] P. Paul, Z. Xie, R. Bau, P. D. W. Boyd, C. A. Reed, *J. Am. Chem. Soc.* **1994**, 116, 5145.
- [43] K. Himmel, M. Jansen, *Inorg. Chem.* **1998**, 37, 3437.
- [44] W. C. Wan, X. Liu, G. M. Sweeney, W. E. Broderick, *J. Am. Chem. Soc.* **1995**, 117, 9580.

The Valence Isomerization of Cyclooctatetraene to Semibullvalene**

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Tricyclo[3.2.1.0^{2,8}]octa-2,6-diene or semibullvalene (SBV; **1**), an isomer of 1,3,5,7-cyclooctatetraene (COT; **2**) was isolated by Zimmerman and Grunewald.^[1] At -140°C , it has the lowest energy barrier of any presently known compound capable of undergoing the Cope rearrangement of the double bond system. This barrier to the thermal



isomerization has been determined experimentally to be $5.5 \pm 0.1 \text{ kcal mol}^{-1}$.^[2] High level theoretical studies of the Cope rearrangement have been recently reported.^[3, 4] Martin, Urbanek, and Walsh^[5] established that COT should be the principal product of thermal decomposition of SBV. Further, the heat of formation of SBV was estimated to be $\Delta_f H^0 = 73.6 \pm 1.0 \text{ kcal mol}^{-1}$ (298 K), a value close to the experimental value of COT $\Delta_f H^0 = 71.1 \pm 0.3 \text{ kcal mol}^{-1}$ (298 K). The experimental results clearly show that SBV and COT lie close in energy and that COT is involved in two reversible valence isomerization processes: The isomerization at about 100°C produces bicyclo[4.2.0]octa-2,4,7 triene^[6] and has been extensively studied.^[7, 8] The other isomerization is observed at 300°C and leads to SBV. Martin, Urbanek, and Walsh

postulated that the mechanism of the conversion of SBV to COT probably involves the intermediate bicyclo[3.3.0]octa-2,6-dien-4,8-diyl diradical **3**, which suggests a stepwise mechanism for the reaction. From a thermochemical estimate,^[9] they established a value of $\Delta_f H^0 = 95 \pm 4 \text{ kcal mol}^{-1}$ for this intermediate but, in their conclusions, the authors also stated its formation is not rate determining for the higher temperature COT rearrangement.

The participation of intermediate **3** in the valence isomerization of COT to SBV had been already suggested in an early theoretical study by Iwamura and Morio^[10] on the basis of an analysis of the symmetry of the reaction. The transition structure, starting from COT, belongs to the C_2 symmetry group, whereas the product SBV belongs to the C_s symmetry group. The authors concluded that the two structures had to be connected by a structure of C_{2v} symmetry that contained elements of both symmetry groups and thereby postulated the intermediate structure **3**. The limitations in the theory existing at that time prevented a full explanation of the dynamics of these fascinating, interrelated chemical processes, which are of fundamental importance in modern chemistry. In contrast, the introduced concept of bifurcation^[11, 12] leads to a logical and consistent explanation of both such chemical processes that are, at first glance, considered to be different.

Herein we report theoretical results supporting the Martin, Urbanek, and Walsh arguments but some corrections were introduced in the construction of the potential energy hypersurface (PES) of the interconversion **1**→**2**. High level quantum-chemical methods were used in our study: the G2 theory for the thermochemistry and Multiconfigurational CASSCF calculations for the equilibrium between SBV and COT. All the calculations were performed with the Gaussian 94 and Gaussian 98 suite of quantum-chemical programs.^[13]

To investigate the possibility of a thermodynamical equilibrium between SBV and COT, we carried out G2(MP2) and G2 calculations on the COT isomers involved in the VI process.^[14] To our knowledge, these computations are the second example of the application of such methods to a system of the size studied here.^[8] The enthalpies of formation for SBV, COT, and **3** are given in Table 1. For SBV and COT, the theoretical results are within the limits of the experimental error, while the calculated enthalpy of formation of the putative intermediate **3** clearly differs from the estimated value. This difference encourages us to search for an alternative reaction pathway. However, our calculated $\Delta_f H^0$ value situates this diradical **3** near to the transition state of the valence isomerization from COT to SBV (heat of formation $114 \text{ kcal mol}^{-1}$ [5]).

Table 1. Calculated (G2(MP2) and G2) and experimental heats of formation [kcal mol^{-1}] at 298 K for COT **2**^[a], SBV **1**, and the diradical **3**.

Method	COT 2		SBV 1		Diradical 3	
	G2(MP2)	G2	G2(MP2)	G2	G2(MP2)	G2
Atomization	77.2	75.8	77.6	76.1	116.3	115.4
Bond separation	72.0	71.4	72.9	72.2	–	–
Experimental	71.1 ± 0.3 ^[b]		73.6 ^[c]		95.0 ^[d]	

[a] Results from ref. [8]. [b] Value obtained from experimental $\Delta_f H_{\text{liq}}^0$ and $\Delta_f H_{\text{vap}}^0$ (see refs. [13] and [14] in ref. [8]). [c] Value taken from ref. [5]. [d] Estimated value, taken from ref. [12] in ref. [5].

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It is also important to highlight that this biradical is involved in another valence isomerization of COT: the interconversion to 1,5-dihydropentalene. This process, and the rearrangement of tricyclo[3.3.0.0^{2,6}]octa-3,7-diene to SBV, were also considered in the paper of Martin, Urbanek, and Walsh^[5] and will be the subject of a forthcoming paper from us.

The reaction pathways were studied at the CASSCF/6-31G(d) level. With an active space of eight electrons in eight orbitals, the stationary points were characterized by analytical frequency calculations at the same level and the energies were corrected with CASMP2 single-point calculations. Starting from COT, this method allowed us to locate the proposed C_2 -symmetric transition state (TS). An intrinsic reaction coordinate (IRC) calculation in the direction of SBV showed that the TS leads to a structure of C_{2v} symmetry which corresponds to the structure proposed by Iwamura and Morio.^[10] However, this structure was identical to that for the Cope rearrangement, which was previously found in a detailed investigation of the Cope rearrangement of SBV at several levels of theory (HF, CASSCF, CASPT2N, and DFT (Becke3LYP)).^[3, 4] The calculated energetic barrier between COT and SBV, shown in Figure 1, is similar to the estimation of Martin, Urbanek, and Walsh.^[5]

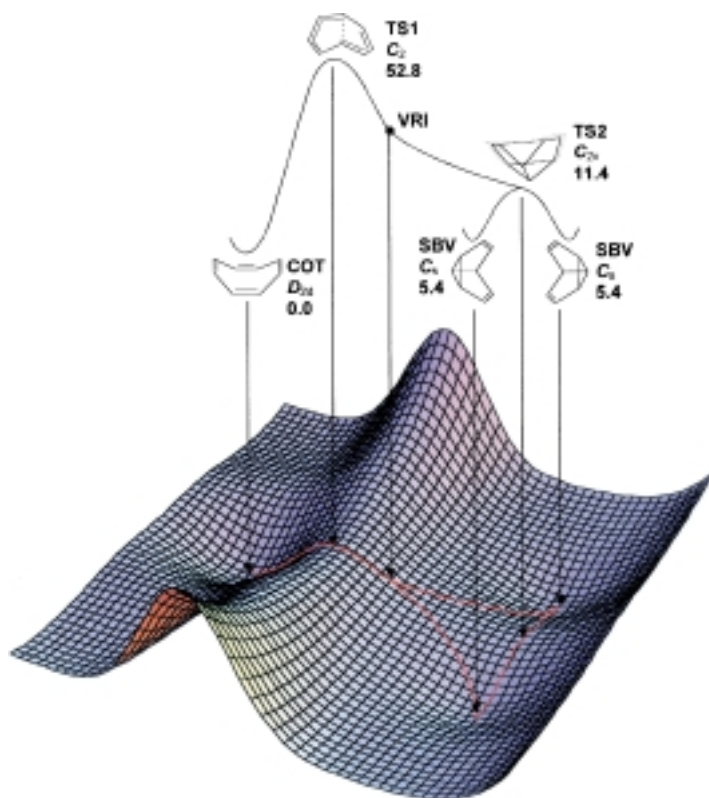


Figure 1. Potential energy hypersurface for the transition from COT to SBV through the transition states TS1 and TS2 and the bifurcation point VRI. The $\Delta_f H^\circ$ (0 K) values [kcal mol⁻¹] are relative to COT.

Therefore, our IRC calculation shows that the two transition states are connected along the C_2 symmetry coordinate. It can be shown mathematically that along the IRC there must lie a valley–ridge inflection (VRI) point or a bifurcation, a point where a) the energy Hessian projected onto the space orthogonal to the IRC has one eigenvector orthogonal to the

curve with its eigenvalue equal to zero, and b) the derivative of this eigenvalue along the direction of the curve is negative. This can be easily understood if one considers the sign change of the eigenvalues which belong to the eigenvectors of the projected Hessian orthogonal to the curve along the IRC. At the transition states, every eigenvector of the projected Hessian is an eigenvector of the Hessian. At TS1, the IRC curve starts along the transition vector and all the eigenvectors orthogonal to the curve of the projected Hessian have positive eigenvalues. At TS2, the IRC curve has a direction orthogonal to the transition vector. Therefore, this vector is a projected Hessian eigenvector orthogonal to the curve whose eigenvalue is negative. This eigenvalue changes from positive at TS1 to negative at TS2 and therefore it follows that the eigenvalue must have zero value at some point along the IRC. This is the simple idea that lies behind the Bolzano theorem.

To locate the bifurcation point, we have used a program developed by us^[17] in conjunction with the Gaussian 98 package.^[13] In this algorithm, the Hessian is projected to the subspace orthogonal to the IRC curve. To achieve sufficient accuracy, the Hessian at the first point of the IRC was calculated analytically every ten IRC points. By this way it was possible to locate the turning point of the eigenvector of interest. This eigenvector corresponds to the not totally symmetric vibrational coordinate of the Cope rearrangement.

The system runs along the IRC of C_2 symmetry from TS1, passes a VRI, and reaches a second transition state, TS2, of C_{2v} symmetry. From TS2, the molecule will decay to the C_s symmetry energy minimum of SBV (Figure 1). Therefore, with the help of the bifurcation, we have shown the existence of a “concerted pathway” for the interconversion of COT and SBV, and this pathway is connected to the degenerate Cope rearrangement of SBV. We have also established the intrinsic relationship between both processes, which until now were considered to be independent.

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- [1] H. E. Zimmerman, G. L. Grunewald, *J. Am. Chem. Soc.* **1966**, *88*, 183–186.
- [2] A. K. Cheng, F. A. L. Anet, J. Mioduski, J. Meinwald, *J. Am. Chem. Soc.* **1974**, *96*, 2887–2891.
- [3] H. Jiao, P. von R. Schleyer, *Angew. Chem.* **1993**, *105*, 1830–1833; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1760–1763.
- [4] H. Jiao, R. Nagelkerke, H. A. Kurtz, R. V. Williams, W. T. Borden, P. von R. Schleyer, *J. Am. Chem. Soc.* **1997**, *119*, 5921–5929.
- [5] H.-D. Martin, T. Urbanek, R. Walsh *J. Am. Chem. Soc.* **1985**, *107*, 5532–5534.
- [6] R. Huisgen, F. Mietzsch, *Angew. Chem.* **1964**, *76*, 36–38; *Angew. Chem. Int. Ed. Engl.* **1964**, *3*, 83–87.
- [7] J.-L. Andrés, O. Castaño, A. Morreale, R. Palmeiro, R. Gomperts, *J. Chem. Phys.* **1998**, *108*, 203–207.
- [8] O. Castaño, R. Notario, R. Gomperts, J.-L. M. Abboud, R. Palmeiro, J.-L. Andrés, *J. Phys. Chem. A* **1998**, *102*, 4949–4951.
- [9] S. W. Benson, *Thermochemical Kinetics*, Wiley, New York, **1976**.
- [10] H. Iwamura, K. Morio, *Bull. Chem. Soc. Jpn.* **1972**, *45*, 3599–3603.
- [11] M. V. Basilevsky, *Chem. Phys.* **1977**, *24*, 81–89.
- [12] P. Valtazanos, K. Ruedenberg, *Theor. Chem. Acta* **1986**, *69*, 281–307.
- [13] *Gaussian 98, Revision A.6*, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo,

- S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh PA, **1998**.
- [14] The G2(MP2) and G2 procedures were introduced by Pople and co-workers[15, 16] with the purpose of making reliable theoretical predictions. The two methods differ in their implementation of additivity approximations but each corresponds effectively to calculations at the QCISD(T)/6-311+G(3df,2p) level with MP2(full)/6-31G(d) optimized geometries. Scaled HF/6-31G(d) zero-point vibrational energies and higher level corrections accommodate the remaining deficiencies.
- [15] L. A. Curtiss, K. Raghavachari, J. A. Pople, *J. Chem. Phys.* **1993**, *98*, 1293–1298.
- [16] L. A. Curtiss, K. Raghavachari, G. W. Trucks, J. A. Pople, *J. Chem. Phys.* **1997**, *94*, 7221–7230.
- [17] This procedure will be published elsewhere with another examples of VRI from the (CH)₈ family of isomers.

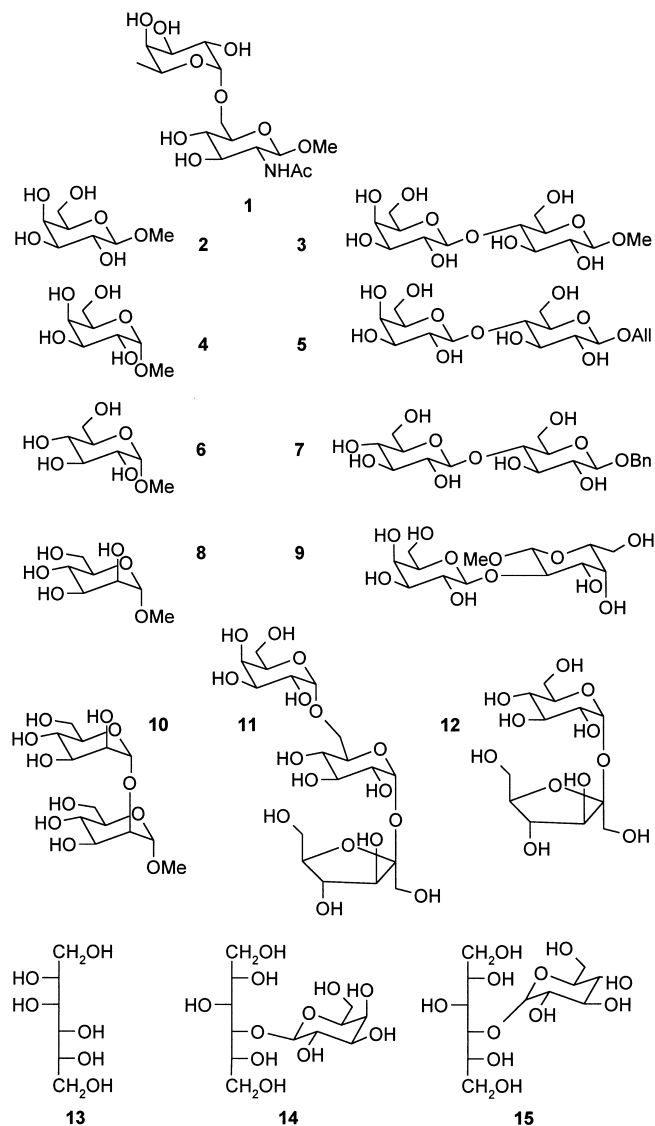
Application of 3D-TOCSY-trNOESY for the Assignment of Bioactive Ligands from Mixtures**

Lars Herfurth, Thomas Weimar, and Thomas Peters*

An increasing body of work demonstrates that NMR spectroscopy provides powerful protocols to screen compound mixtures such as combinatorial libraries for their binding activity towards receptor proteins.^[1–5] Compared to other screening techniques, NMR has specific advantages. One important feature is that NMR experiments allow parallel screening protocols without the need to subsequently separate the library components. In addition, NMR potentially delivers precise structural and topological information on the bioactive ligand, the protein binding site, or both. A comparison of NMR screening protocols published so far shows that two main classes of experiments may be distinguished. One category of experiments targets primarily the receptor protein and aims at identifying those amino acids involved in binding. For instance, specific absorption rate measurements, “SAR by NMR”, employs ¹⁵N labeling of the proteins to allow for fast heteronuclear single quantum coherence (HSQC) experiments.^[2] Another class of experi-

ments relies on changes of specific ligand properties upon binding to a receptor protein. In the main, altered relaxation, diffusion, or both have been utilized as the basis for such experiments.^[3] It has been recently shown that saturation transfer difference (STD) experiments are especially useful for identifying ligands with binding activity and, furthermore, deliver information on the binding epitope.^[4]

Herein, we report on a library consisting of 15 individual carbohydrates (Scheme 1) in the presence of the lectin *Aleuria aurantia* agglutinin (AAA). We have already shown



Scheme 1. Components of the library tested for the binding activity towards AAA. All components are present in approximately 10 mM concentration with a binding site:ligand ratio of 1:20 for each. Details of the sample preparation have been described previously.^[5a]

that transfer NOE spectroscopy (trNOESY) experiments deliver typical cross-peak patterns that allow assignment of the bioactive component if all individual compounds are known.^[5a] However, without this prior knowledge, an unambiguous assignment would be impossible. In the following, we present a strategy that closes this gap.

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Initially, a one-dimensional (1D) STD spectrum and a STD-TOCSY spectrum of the library in the presence of AAA were acquired (Figure 1; the 1D STD spectrum is not shown). Two individual spin systems were unambiguously identified. In comparison to trNOESY spectra, STD-TOCSY spectra have

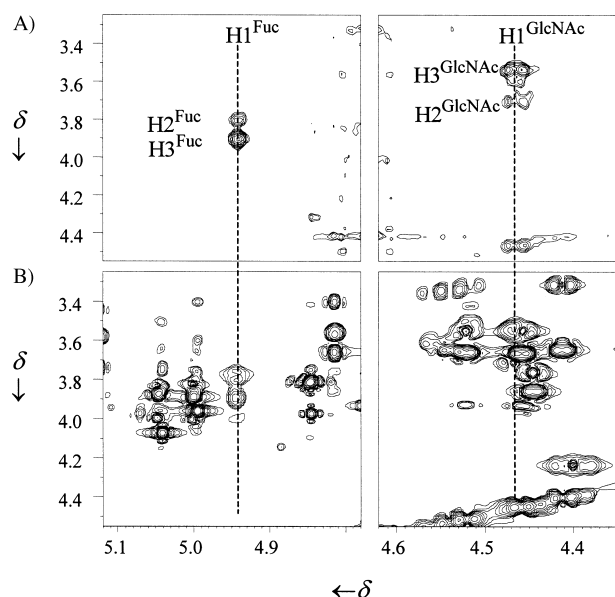
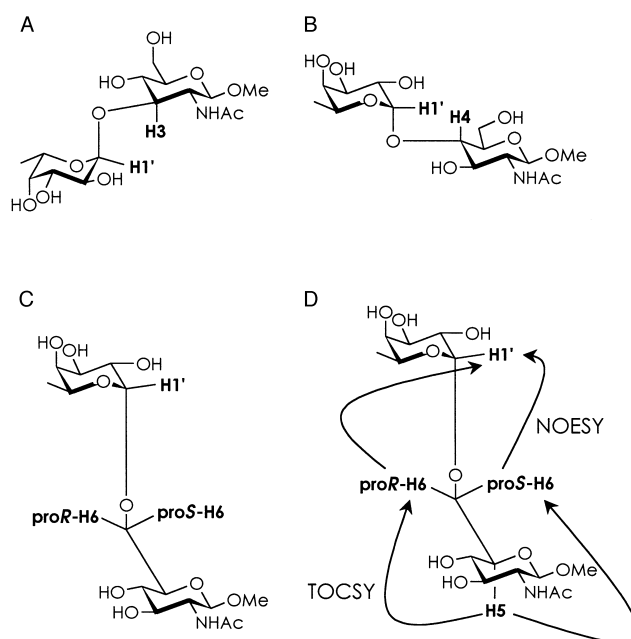


Figure 1. Comparison the region of anomeric protons $H1^{\text{Fuc}}$ (left) and $H1^{\text{GlcNAc}}$ (right) of a TOCSY (B) and a STD-TOCSY (A) spectrum of the library in the presence of AAA. In (B), only the cross peaks of compound 1 involved in binding remain.

the advantage that cancellation between negative and positive cross peaks does not occur. From the chemical shifts and coupling constants, it was easy to assign the two STD-TOCSY cross peak patterns to an α -L-fucose (Fuc) and a β -D-N-acetyl glucosamine (GlcNAc) residue. Important spectral characteristics comprised the C6-bound methyl group of Fuc, the O-methyl group, the N-acetyl group, and the anomeric protons. From previous trNOESY experiments, it was also clear that the GlcNAc residue was linked to an O-methyl group at the anomeric carbon atom. Only the linkage type between the Fuc and GlcNAc remained unknown. From a STD-TOCSY spectrum this information could not be obtained because scalar couplings between protons across glycosidic linkages are much too small to allow for an assignment of the linkage site. Fortunately, interglycosidic NOEs, or trNOEs, contain this information (Scheme 2). In principle, interglycosidic trNOEs are obtained from trNOESY spectra but, in this case, severe overlap between protons of the Fuc and the GlcNAc residue prohibited the discrimination of inter- and intraglycosidic trNOEs. For instance, the $H2^{\text{Fuc}}$ signal overlapped with that of $\text{proS-H6}^{\text{GlcNAc}}$, and the $H3^{\text{Fuc}}$ signal overlapped with $\text{proR-H6}^{\text{GlcNAc}}$, which prevented an unambiguous assignment of the trNOEs to $H1^{\text{Fuc}}$. It is well known that resonance overlap can be overcome by increasing the number of dimensions in a NMR experiment. Therefore, a 3D-TOCSY-trNOESY experiment^[6] was performed. In this experiment, protons are labeled according to their association



Scheme 2. Interglycosidic trNOESY pathways as a function of the glycosidic linkage type between Fuc and GlcNAc. Predominant interglycosidic NOEs are in bold face. A) (1 $\rightarrow$ 3) linkage. B) (1 $\rightarrow$ 4) linkage. C) (1 $\rightarrow$ 6) linkage. D) Combination of TOCSY and trNOESY pathways allows an unequivocal identification of the linkage site. Magnetization pathways are labeled as arrows and correspond to cross peaks in Figure 2.

with a particular spin system during the TOCSY step and the trNOESY step conveys the necessary interglycosidic trNOE information.

Inspection of the 3D TOCSY-trNOESY spectrum of the library (Scheme 1) in the presence of AAA showed that $H1^{\text{Fuc}}$ gave trNOEs to protons that had scalar coupling partners in the spectral region around $\delta \approx 3.5$. From the chemical shifts, it was clear that these protons did not belong to Fuc because this residue had no protons with $\delta < 3.6$, except for the C6-bound methyl group. In addition, two of the protons that gave trNOEs to $H1^{\text{Fuc}}$ were scalar coupled to each other. This information was sufficient to identify the linkage type between Fuc and GlcNAc. Figure 2 shows a ω_1/ω_2 slice at the frequency of $H1^{\text{Fuc}}$ ($\omega_3 = 4.95$). All cross peaks observed in

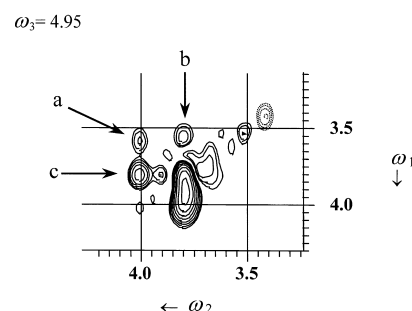


Figure 2. ω_1/ω_2 slice from a 3D-TOCSY-trNOESY experiment, in which ω_3 is set to the resonance frequency of the anomeric proton, $H1^{\text{Fuc}}$ ($\delta = 4.95$). Connectivities through scalar coupling (TOCSY transfer, first step) and through space cross relaxation (NOESY transfer, second step) are shown. All cross peaks indicate magnetization that has finally been transferred to $H1^{\text{Fuc}}$: a) $H5^{\text{GlcNAc}} \rightarrow \text{proS-H6}^{\text{GlcNAc}} \rightarrow H1^{\text{Fuc}}$, b) $H5^{\text{GlcNAc}} \rightarrow \text{proR-H6}^{\text{GlcNAc}} \rightarrow H1^{\text{Fuc}}$, c) $\text{proR-H6}^{\text{GlcNAc}} \rightarrow \text{proS-H6}^{\text{GlcNAc}} \rightarrow H1^{\text{Fuc}}$.

this slice had corresponding protons that, in common, gave trNOEs to H1^{Fuc}. In addition, scalar coupling partners were easily identified since the ω_1/ω_2 slice reflected TOCSY pathways. The scalar coupling pattern was attributed to protons proR-H6^{GlcNAc}, proS-H6^{GlcNAc}, and H5^{GlcNAc}, as indicated in Figure 2. It should be emphasized that the intraglycosidic trNOEs between H2^{Fuc} and H3^{Fuc} were also present in this spectrum and overlapped with the proR-H6^{GlcNAc}/proS-H6^{GlcNAc} pattern. However, the scalar coupling of the latter two protons to H5^{GlcNAc} at $\delta = 3.51$ allowed a clear discrimination. Inspection of Scheme 2 shows that only a (1→6) linkage between the two sugar residues explains the observed cross peak pattern.

In summary, a 3D-TOCSY-trNOESY experiment allowed unambiguous identification of the bioactive component of a carbohydrate library without prior knowledge of the individual components of the library. Carbohydrates certainly represent an important class of biological macromolecules^[7, 8] but, nonetheless, the experimental protocol described here is not limited to carbohydrate derivatives.

Experimental Section

All spectra were acquired on a Bruker DRX 500 MHz spectrometer with a 5 mm TXI probehead at 306 K. TOCSY and STD-TOCSY were measured with 512 increments and 16 transients using a 50 ms MLEV-17 spinlock field of 7.5 kHz. Saturation transfer was achieved by using 40 selective 270° Gaussian pulses of duration 50 ms and spacing 10 ms. The protein envelope was irradiated at 2.8 ppm (on-resonance) and 40 ppm (off-resonance). Subsequent subtraction of on- and off-resonance spectra was achieved via phase cycling.

For the 3D-TOCSY-trNOESY experiment, 124, 256, and 1014 data points in F1, F2, and F3, respectively, were acquired with eight transients each. The acquisition time was 127 ms, which results in a total relaxation delay of 1.6 s. The total measurement time was approximately five days. The mixing time for the NOESY step was 130 ms. Suppression of zero-quantum coherence^[9] was achieved by parallel application of a spinlock field (2.5 ms, 7.5 kHz) and a gradient pulse (2.5 ms, approximately 5 G cm⁻¹) prior to the NOE mixing time. Signals from HDO were suppressed by presaturation with a weak radio-frequency field.

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- [1] a) J. M. Moore, *Curr. Opin. Biotechnol.* **1999**, *10*, 54–58; b) P. Keifer, *Curr. Opin. Biotechnol.* **1999**, *10*, 43–41; c) B. J. Stockman, *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *33*, 109–151.
- [2] a) P. J. Hajduk, T. Gerfin, J.-M. Boehlen, M. Haerberli, D. Marek, S. W. Fesik, *J. Med. Chem.* **1999**, *42*, 2315–2317; b) S. B. Shuker, P. J. Hajduk, R. P. Meadows, S. W. Fesik, *Science* **1996**, *274*, 1531–1534.
- [3] a) P. J. Hajduk, E. T. Olejniczak, S. W. Fesik, *J. Am. Chem. Soc.* **1997**, *119*, 12257–12261; b) A. Chen, M. J. Shapiro, *J. Am. Chem. Soc.* **1998**, *120*, 10258–10259; c) M. Lin, M. J. Shapiro, J. R. Wareing, *J. Org. Chem.* **1997**, *62*, 8930–8931.
- [4] a) M. Mayer, B. Meyer, *Angew. Chem.* **1999**, *111*, 1902–1906; *Angew. Chem. Int. Ed.* **1999**, *38*, 1784–1788; b) J. Klein, R. Meinicke, M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **1999**, *121*, 5336–5337.
- [5] a) B. Meyer, T. Weimar, T. Peters, *Eur. J. Biochem.* **1997**, *246*, 705–709; b) T. Peters, B. Meyer, WO-B 19649359, **1996**; c) D. Henrichsen, B. Ernst, J. L. Magnani, W.-T. Wang, B. Meyer, T. Peters, *Angew. Chem.* **1999**, *111*, 106–110; *Angew. Chem. Int. Ed.* **1999**, *38*, 98–102.
- [6] a) C. Griesinger, O. W. Sørensen, R. R. Ernst, *J. Magn. Reson.* **1989**, *84*, 14–63; b) H. Oschkinat, C. Cieslar, C. Griesinger, *J. Magn. Reson.* **1990**, *86*, 453–469.

- [7] a) A. Varki, *Glycobiology* **1993**, *3*, 97–130; b) A. Varki, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390–7397.
- [8] J. Jiménez-Barbero, J.-L. Asensio, F.-J. Cañada, A. Poveda, *Curr. Opin. Struct. Biol.* **1999**, *9*, 549–555.
- [9] A. L. Davis, G. Estcourt, J. Keeler, E. D. Laue, J. J. Titman, *J. Magn. Reson. A* **1993**, *105*, 167–183; b) T. Weimar, *Magn. Reson. Chem.*, in press.

Natural-Product Hybrids: Design, Synthesis, and Biological Evaluation of Quinone – Annonaceous Acetogenins**

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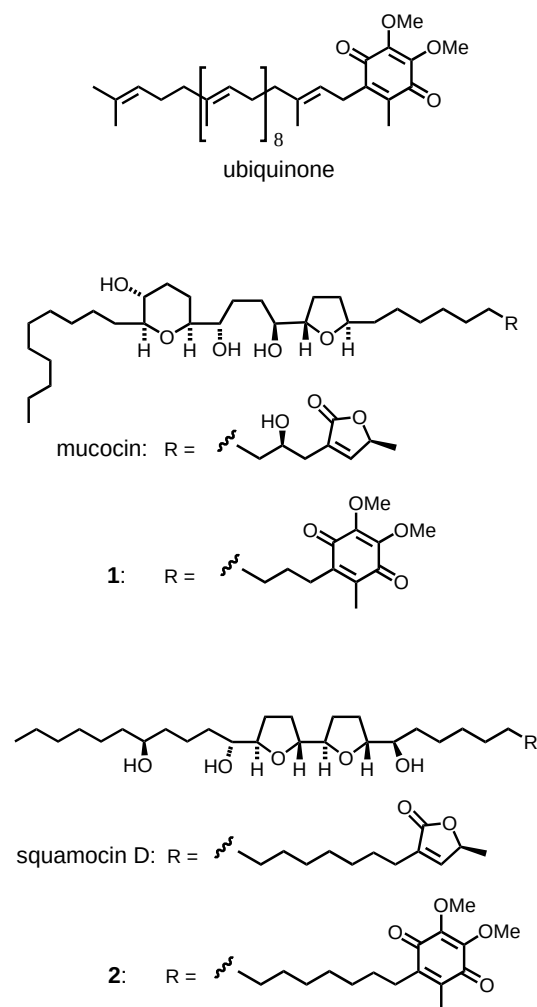
The acetogenins of *Annonaceae* are a class of natural products with interesting antitumor, immunosuppressive, pesticide, and antimicrobial activities.^[1] Their main mode of action is the inhibition of the mitochondrial complex I (NADH-ubiquinone oxidoreductase).^[2] Mucocin^[3] and squamocin D^[4] are two representative members of the *Annonaceae* acetogenins, which show their characteristic structural features: An ether core consisting of tetrahydrofuran (THF) and tetrahydropyran (THP) rings flanked with a left and a right side chain. At the end of the right side chain is located a butenolide unit. The long alkyl chains place at least parts of the compound in the lipophilic interior of the mitochondrial membrane.^[5]

It has been proposed that the annonaceous acetogenins act at the terminal electron-transfer step of complex I between the Fe–S cluster N2 and the ubiquinone pool.^[2] The butenolide subunit may bind at the quinone binding site of complex I. The reduction potential of an α -alkyl- α,β -unsaturated butyrolactone ($E_p^c = -2.69$ V (irrev.), CH₃CN, versus the saturated calomel electrode (SCE)) is much more negative than the reduction potentials of the quinone group ($E_p^{cl} = -0.75$ V, $E_p^{cl} = -1.48$ V, CH₃CN, versus SCE).^[6] Therefore an electron transfer from the Fe–S cluster N2 to the butenolide is unlikely to occur. With the goal to further elucidate the mechanism of action of the acetogenins and to provide molecular probes for complex I studies we designed quinone – mucocin **1** and quinone – squamocin D **2**. In these compounds the butenolide part of mucocin and squamocin D is exchanged against the quinone part of ubiquinone. Here we

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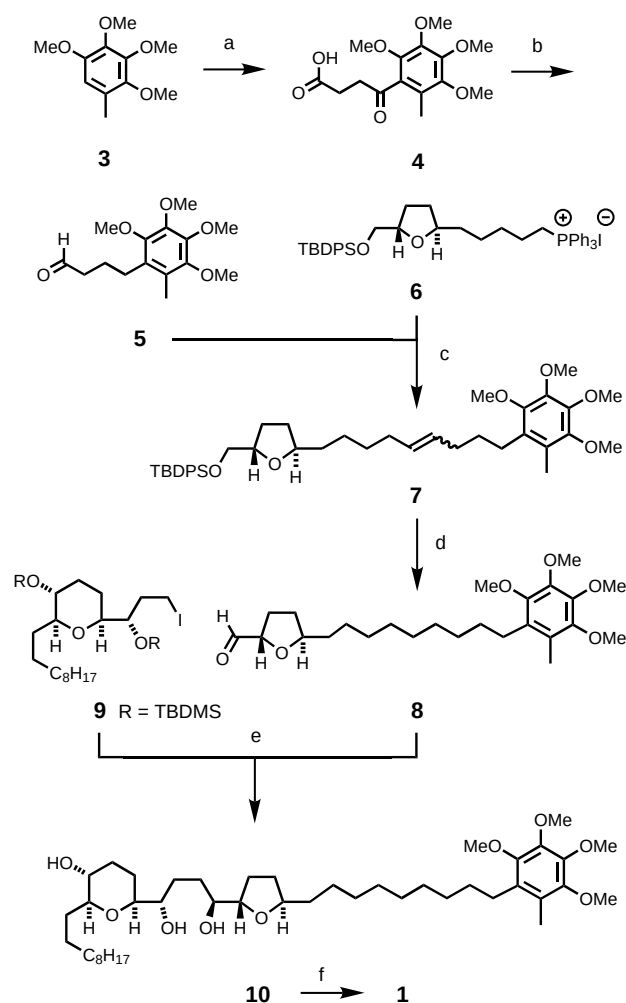
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



report on the first synthesis of this novel type of hybrid compounds as well as on their interaction with mitochondrial complex I.

The syntheses of **1** and **2** build upon the modular strategy developed for our total syntheses of mucocin^[7] and squamocin D.^[8] *ortho*-Lithiation of 2,3,4,5-tetramethoxytoluene (**3**)^[9] and reaction with succinic anhydride gave the oxocarboxylic acid **4** (Scheme 1). Reduction with LiAlH₄ provided a diol, whose benzyl alcohol function was removed by reduction with Et₃SiH/BF₃·OEt₂.^[10] A subsequent Swern oxidation to the aldehyde **5** followed by a Wittig reaction with the phosphonium salt **6**^[11] delivered the olefin **7**, which was transformed into the aldehyde **8** by a standard sequence. A chelation-controlled addition of an organomagnesium compound prepared from the iodide **9**^[7a,b] by iodine lithium exchange/transmetalation to the aldehyde **8** gave, after removal of the *tert*-butyldimethylsilyl (TBDMS) protecting groups, the triol **10**. Oxidation of the tetramethoxytolyl subunit with ceric ammonium nitrate (CAN)^[12] yielded the target compound quinone–mucocin **1** (Table 1).

The starting point for the synthesis of quinone–squamocin D (**2**) was the aldehyde **11** (Scheme 2). Its reaction with lithiated compound **3** gave **12**, which was converted into the iodide **13**. Iodine–metal exchange and addition to the aldehyde **14**^[13] gave after oxidation/*L*-selectride reduction



Scheme 1. a) *n*BuLi, TMEDA, *n*-hexane, 0 °C, 30 min, succinic anhydride, THF, 2 h, 63%; b) 1. LiAlH₄, THF, 3 h, 72%; 2. Et₃SiH, BF₃·OEt₂, CH₂Cl₂, 12 h, 88%; 3. (COCl)₂, DMSO, NEt₃, CH₂Cl₂, –40 °C, 2.5 h, 78%; c) 6. NaHMDS, THF, 0 °C, 20 min; 5, 0 °C, 1 h, 87%; d) 1. Pt/C, H₂, EtOAc, 6 h, 97%; 2. *n*Bu₄NF, THF, 2.5 h, 95%; 3. (COCl)₂, DMSO, NEt₃, CH₂Cl₂, –40 °C, 1.5 h, 89%; e) 1. **9** (1.3 equiv), *n*BuLi (2.4 equiv), Et₂O, –105 °C, 4 min, MgBr₂·OEt₂ (2.8 equiv), –100 → –40 °C (2 h) → –78 °C, **8** (1 equiv) → –10 °C, 2 h, 47%, diastereoselectivity 7:1, chromatographic separation of the epimers; 2. HF, CH₃CN/CH₂Cl₂, 1 h, 90%; f) 2,6-pyridinedicarboxylic acid, CAN, CH₃CN/H₂O, 0 °C, 4 h, 68%. TBDPS = *tert*-butyldiphenylsilyl, TMEDA = tetramethylethylenediamine, NaHMDS = sodium hexamethyldisilazide, CAN = Cer(IV) ammonium nitrate.

and removal of the TBDMS groups the triol **15**. A CAN oxidation of **15** yielded the target compound quinone–squamocin D (**2**, Table 1).

The synthetic compounds **1**, **2**, **10**, and **15** were tested as inhibitors of complex I (bovine-heart mitochondrial; Table 2).^[14–16] The known strong inhibitor rotenon (IC₅₀ = 1.0 nM) was used as a reference sample.^[2] It was found that quinone–mucocin (**1**, IC₅₀ = 3.6 nM) was ten times more active than mucocin itself (IC₅₀ = 33 nM). In contrast the hydroquinone–mucocin dimethyl ether (**10**) was a weaker inhibitor than the natural product. Quinone–squamocin D (**2**) and its hydroquinone-dimethyl ether (**15**) also displayed activities in the nanomolar range (IC₅₀ = 1.7 and IC₅₀ = 4.7 nM, respectively). These results show that natural-product hybrids **1** and **2** are very potent inhibitors of mitochondrial

Table 1. Selected analytical and spectroscopic data of compounds **1** and **2**.

1: $[\alpha]_D^{25} = -20.5$ ($c = 0.044$, CH_2Cl_2); $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta = 0.85$ (t, $J = 6.4$ Hz, 3H, 34- H_3), 1.13–1.88 (m, 42H, alkyl), 1.89–2.14 (m, 3H, 13, 14, 22- H_2), 1.99 (s, 3H, 35-Me), 2.42 (t, $J = 6.8$ Hz, 2H, 3- H_2), 2.69 (br s, 1H, OH), 2.82 (br s, 1H, OH), 3.02 (dt, $J = 8.8, 2.2$ Hz, 1H, 24-H), 3.08–3.18 (m, 1H, 20-H), 3.18–3.32 (m, 1H, 23-H), 3.34–3.52 (m, 2H, 16, 19-H), 3.71–3.90 (m, 2H, 12, 15-H), 3.96 (s, 6H, 37, 38-OMe); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta = 11.9$ (35-Me), 14.1 (C-34), 22.7, 25.5, 26.2, 26.4, 26.9, 28.3, 28.3, 28.7, 28.7, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 29.8, 31.9, 32.0, 32.4, 32.6, 35.6, (C-3 to C-11, C-13, C-14, C-17, C-18, C-21, C-22, C-25 to C-33), 61.1 (37, 38-OMe), 70.6 (C-23), 73.5 (C-19), 73.8 (C-16), 79.3 (C-12), 80.1 (C-20), 81.9 (C-15), 82.0 (C-24), 138.6, 143.1, 144.3 (C-2, C-35, C-37, C-38), 184.2, 184.7 (C-1, C-36); HR-MS (EI): calcd for $\text{C}_{41}\text{H}_{72}\text{O}_9$ ($[M^+ + 2\text{H}]$): 708.5176; found: 708.5166.

2: $[\alpha]_D^{25} = +3.1$ ($c = 0.13$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta = 0.86$ (t, $J = 6.6$ Hz, 3H, 34- H_3), 1.20–1.67 (m, 42H, 4- CH_2 to 14- H_2 , 17-H', 18-H', 21-H', 22-H', 25- H_2 to 27- H_2 , 29- H_2 to 33- H_2), 1.89–2.10 (m, 4H, 17-H'', 18-H'', 21-H'', 22-H''), 1.99 (s, 3H, 39- H_3), 2.42 (t, $J = 6.4$ Hz, 2H, 3- H_2), 3.37–3.53 (m, 2H, 15-H, 24-H) overlap with 3.53–3.62 (m, 1H, 28-H), 3.84–3.98 (m, 10H, 16-H, 19-H, 20-H, 23- H_2 , $2 \times \text{OCH}_3$); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta = 11.9$ (CH_3 –39), 14.1 (C-34), 21.4 (C-26), 22.6 (C-33), 25.4, 25.7, 26.4, 28.67, 28.74, 29.4, 29.5, 29.6, 29.7, 29.9, 31.9 (C-3 to C-13, C-17, C-18, C-21, C-22, C-30 to C-32), 32.6 (C-25), 33.1 (C-14), 36.8, 37.7 (C-27, C-29), 61.2 (OCH_3), 71.7 (C-28), 74.4, 74.7 (C-15, C-24), 81.7, 81.8, 82.9, 83.0 (C-16, C-19, C-20, C-23), 138.7, 143.1, 144.2 (C-2, C-35, C-37, C-38), 184.2, 184.7 (C-1, C-36); HR-MS: (EI): calcd: 708.5176 ($[M + 2\text{H}]^+$); found: 708.5177.

Table 2. Inhibition of mitochondrial complex I by natural acetogenins and synthetic analogues.

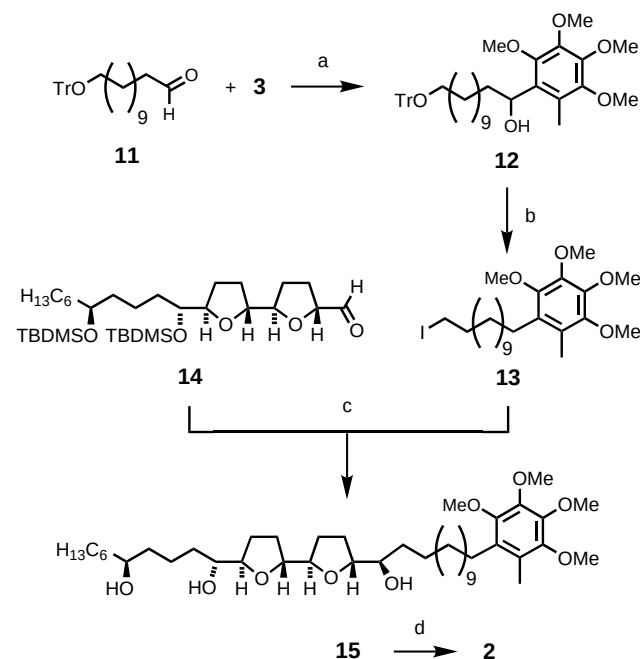
Compound	IC ₅₀ [nm] ^[a]	IC ₅₀ [μmol/ mg protein] ^[a]	IC ₅₀ [μmol/ mg protein] ^[b]
mucocin	34	45	33.3
1	3.6	4.9	
10	123	163	
squamocin D	–	–	8.7
squamocin A	1.0	1.3	
2	1.7	2.3	
15	4.7	6.2	
rotenone	1	1.3	

[a] Bovine-heart mitochondria were prepared as described before.^[15] The inhibition of oxygen uptake was measured. The respiratory activities were analyzed with a Clark-type oxygen electrode (100 mm sodium phosphate pH = 7.4, 1 mm N,N,N',N' -ethylenediaminetetraacetic acid (EDTA), 1 mm MgCl_2 , 0.5 mg mL^{-1} protein).^[16] [b] Literature data.^[14b]

none binding site of complex I.^[2c] Our data support this hypothesis, although other structural features may also contribute, since the IC₅₀ of quinone **2** and hydroquinone dimethyl ether **15** are in the same range. It has been demonstrated that the quinone-binding site in complex I is quite large, probably occupied by both acetogenin subunits, the butenolide as well as the THF/THP part.^[14a]

Natural-product hybrids of type **1** and **2** should be useful molecular probes for further investigations of the ubiquinone binding site of complex I. In particular a more detailed look at the redox properties of the system complex I/quinone annonaaceous acetogenin seems promising. The new modular synthetic approach presented here allows the efficient access to quinone–annonaaceous hybrids with different quinone moieties and different redox properties.

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Scheme 2. a) $n\text{BuLi}$, TMEDA, n -hexane, 0°C , 2.5 h, 54%; b) 1. Et_3SiH , $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , $-78 \rightarrow -20^\circ\text{C}$, 14 h, 81%; 2. I_2 , PPh_3 , imidazole, CH_2Cl_2 , 0°C , 4 h, 65%; c) 1. **13**, $t\text{BuLi}$, $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$, Et_2O , $-100 \rightarrow -40 \rightarrow -78^\circ\text{C}$, **14**, $-78 \rightarrow 0^\circ\text{C}$, 3.5 h, 52%; 2. $(\text{COCl})_2$, DMSO, NEt_3 , CH_2Cl_2 , $-60 \rightarrow -40^\circ\text{C}$, 1.5 h, 96%; 3. L-selectride, THF, $-100 \rightarrow -70^\circ\text{C}$, 1.5 h, 99%, diastereoselectivity 88:12, separation of the epimers by chromatography; 4. 5% HF in CH_3CN , THF, 20°C , 1.5 h, 77%; d) CAN, 2,6-pyridinedicarboxylic acid, 0°C , 4 h, 70%. Tr = triphenylmethyl.

complex I. The structural similarity between the butenolide and the quinone led to the assumption that both groups interact in a similar way with the ubiquinone reduction site in complex I^[2b] and previous studies postulated that the annonaaceous acetogenins are competitive inhibitors at the ubiqui-

- [1] a) F. Q. Alali, X.-X. Liu, J. L. McLaughlin, *J. Nat. Prod.* **1999**, 62, 504–540; b) M. C. Zafra-Polo, B. Figadère, T. Gallardo, J. R. Tormo, D. Cortes, *Phytochemistry* **1998**, 48, 1087–1117; c) A. Cavé, B. Figadère, A. Laurens, D. Cortes in *Progress in the Chemistry of Organic Natural Products* (Eds.: W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, C. Tamm), Springer, New York, **1997**, 70, pp. 81–288.
- [2] a) M. Degli Eposti, *Biochim. Biophys. Acta* **1998**, 1364, 222–235; b) H. Miyoshi, M. Ohshima, H. Shimada, T. Akagi, H. Iwamura, J. L. McLaughlin, *Biochim. Biophys. Acta* **1998**, 1365, 443–452; c) T. Friedrich, P. Van Heek, H. Leif, T. Ohnishi, E. Forche, B. Kunze, R. Jansen, W. Trowitzsch-Kienast, G. Höfle, H. Reichenbach, H. Weiss, *Eur. J. Biochem.* **1994**, 219, 691–698.
- [3] G. Shi, D. Alfonso, M. O. Fatope, L. Zeng, Z.-M. Gu, K. He, J. M. McDougal, J. L. McLaughlin, *J. Am. Chem. Soc.* **1995**, 117, 10409–10410.
- [4] a) M. Sahai, S. Singh, M. Singh, Y. K. Gupta, S. Akashi, R. Yuji, K. Hirayama, H. Asaki, H. Araya, N. Hara, T. Eguchi, K. Kakinuma, Y. Fujimoto, *Chem. Pharm. Bull.* **1994**, 42, 1163–1174; b) G.-X. Zhao, L. R. Miesbauer, D. L. Smith, J. L. McLaughlin, *J. Med. Chem.* **1994**, 37, 1971–1974.
- [5] H. Shimada, J. B. Grutzner, J. F. Kozlowski, J. L. McLaughlin, *Biochemistry* **1998**, 37, 854–866.
- [6] The cyclic voltammograms of various quinones and butenolide samples are given in the Supporting Information.
- [7] a) S. Bäurle, S. Hoppen, U. Koert, *Angew. Chem.* **1999**, 111, 1341–1344; *Angew. Chem. Int. Ed.* **1999**, 38, 1263–1266; b) S. Hoppen, S. Bäurle, U. Koert, *Chem. Eur. J.* **2000**, in press; c) S. Takahashi, T. Nakata, *Tetrahedron Lett.* **1999**, 40, 723–726; d) S. Takahashi, T. Nakata, *Tetrahedron Lett.* **1999**, 40, 727–730; e) P. Neogi, T. Doundoulakis, A. Yazbak, S. C. Sinha, S. C. Sinha, E. Keinan, *J. Am. Chem.*

- Soc. **1998**, 120, 11279–11284; f) P. A. Evans, V. S. Murthy, *Tetrahedron Lett.* **1999**, 40, 1253–1256; f) P. A. Evans, V. S. Murthy, *Tetrahedron Lett.* **1998**, 39, 9627–9628; g) P. A. Evans, J. D. Roseman, *Tetrahedron Lett.* **1997**, 38, 5249–5252.
- [8] a) U. Emde, U. Koert, *Tetrahedron Lett.* **1999**, 40, 5979–5982; b) U. Emde, U. Koert, *Eur. J. Org. Chem.* **2000**, in press.
- [9] E. Keinan, D. Eren, *J. Org. Chem.* **1987**, 52, 3872–3875.
- [10] a) J. L. Fry, M. Orfanopoulos, M. G. Atlington, W. R. Dittmann, S. B. Silverman, *J. Org. Chem.* **1978**, 43, 374–375; b) I. Fleming, A. Barbero, D. Walter, *Chem. Rev.* **1997**, 97, 2063–2192.
- [11] The phosphonium salt **6** was prepared from (2*S*,5*S*)-5-[(*tert*-butyldiphenylsiloxy)methyl]tetrahydrofuran-2-carbaldehyde (U. Koert, M. Stein, H. Wagner, *Liebigs Ann.* **1995**, 1415–1426) by side-chain elongation with a Wittig reaction.
- [12] L. Syper, K. Kloc, J. Mlochowski, Z. Szula, *Synthesis* **1979**, 521–522.
- [13] **14** was prepared by routes described for the synthesis of squamocin D see reference [8].
- [14] a) M. Oshima, H. Miyoshi, K. Sakamoto, K. Takegami, J. Iwata, K. Kuwabara, H. Iwamura, T. Yagi, *Biochemistry* **1998**, 37, 6436–6445; b) D. Alfonso, H. A. Johnson, T. Colman-Saizarbitoria, C. P. Presley, G. P. McCabe, J. L. McLaughlin, *Nat. Toxins* **1996**, 181–188.
- [15] A. L. Smith, *Methods Enzymol.* **1967**, 10, 81–86.
- [16] P. C. Hinkle, M. L. Yu, *J. Biol. Chem.* **1979**, 254, 2450–2455.

Synthesis of Trioxane Using Heteropolyacids as Catalyst

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Acetal resin is a term used to describe the high molecular weight polymers and the copolymers of formaldehyde. First commercialized as a homopolymer in 1960 by DuPont, acetal resins are engineering thermoplastics which have found broad use in areas where traditionally metals were applied.^[1] Shortly thereafter, researchers at Celanese developed an acetal resin based on the copolymerization of trioxane and cyclic ethers, such as ethylene oxide.^[2] In 1962 a commercial plant began producing this acetal copolymer. Since then, a rapid expansion of acetal resin production has occurred worldwide.

Up to 1971 DuPont and Celanese (alone or in joint ventures with other companies) were the sole producers of acetal

resins. In 1972 Asahi Chemical started to produce the acetal homopolymer utilizing the world's third type of polyacetal technology.^[3] Asahi Chemical also industrialized the acetal copolymer in 1985.^[4] At present, the annual demand of acetal resins in the world is about 400 000 t per year.

The biggest problem in the case of acetal resins is the energy consumption during its production. The main aspect is the energy requirement in the monomer process. For example, it requires a great deal of energy to get the monomeric trioxane that is needed for the acetal copolymer from aqueous formaldehyde. In the commercial process, trioxane is obtained by heating aqueous formaldehyde in the presence of an acid catalyst like sulfuric acid [Eq. (1)].



Even though the equilibrium concentration of trioxane is low in the reaction mixture, in the commercial production process trioxane is removed as the distillate from the reaction mixture in the distillation tower.^[5] The vapor–liquid equilibrium between trioxane and aqueous formaldehyde is such that when the trioxane concentration in the liquid phase is low, trioxane shows a very high volatility compared to formaldehyde and water in the vapor phase. Thus, almost all the trioxane contained in the vapor phase from the reaction mixture can be concentrated into the distillate in the distillation tower under the proper refluxing conditions. Since the heat of vaporization of the water–formaldehyde mixture is much higher than that of trioxane,^[6] most of the energy for trioxane synthesis is consumed in the vaporization of water and formaldehyde.^[7] From the viewpoint of the energy requirement, the key point of the trioxane synthesis is the high yield and high selectivity in a one-pass vaporization.

At Asahi Chemical, we developed the *tert*-butyl alcohol process, that is the selective hydration of isobutene using a highly concentrated heteropolyacid as the catalyst.^[8] We also developed a new process for producing polyoxytetramethylene glycol with a narrow molecular weight distribution using a heteropolyacid as a catalyst for the polymerization of tetrahydrofuran.^[9, 10] With this knowledge, we investigated the catalytic activity of heteropolyacids for the synthesis of trioxane. We observed some interesting phenomena and a superior advantage of heteropolyacids over conventional catalysts like sulfuric acid.

The results for the reaction at atmospheric pressure and 100 °C are shown in Table 1. The best features of using the heteropolyacid, instead of sulfuric acid, as a catalyst for trioxane synthesis were the higher conversion and selectivity. For example, for the same selectivity of 97 %, the conversion by sulfuric acid was 20 %, while the conversion by the heteropolyacid was 27 % (drawn from entries 10 and 6 respectively in Table 1). Heteropolyacids provided a 35 % higher yield (yield = conversion × selectivity) than sulfuric acid. A similar result was also obtained for the hydration of isobutene.^[8] In this case, high selectivity of heteropolyacids was reported to be related to the big size of their anions.

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Table 1. Results from the synthesis of trioxane with heteropolyacids and with sulfuric acid as the catalyst.^[a]

Entry	Catalyst ^[b]	<i>t</i> [min] ^[c]	Conv. [%] ^[d, e]	<i>S</i> [%] ^[e, f]
1	PW (30)	39	18.3	98.5
2	PW (100)	34	24.6	98.3
3	PW (100)	50	25.5	98.1
4	SiW (30)	23	22.0	99.3
5	SiW (30)	42	22.7	99.2
6	SiW (100)	27	26.7	97.1
7	SiW (100)	41	27.5	95.4
8 ^[g]	SiW (100)	19	28.0	97.1
9	H ₂ SO ₄ (2)	38	14.5	98.5
10	H ₂ SO ₄ (5)	52	17.4	97.5
11	H ₂ SO ₄ (10)	31	17.9	97.4
12	H ₂ SO ₄ (30)	12	21.0	96.5
13	H ₂ SO ₄ (30)	18	22.3	94.5
14	H ₂ SO ₄ (30)	27	23.6	93.5

[a] The reaction was carried out at 100 °C under atmospheric pressure; unless noted otherwise, 55 wt % formalin was used. [b] PW = phosphotungstic acid, SiW = silicotungstic acid; the number in parentheses indicates the amount of catalyst (in g) per initial 100 g of formalin. [c] The contact time *t* is defined here for simplicity as follows: *t* [min] = volume of the reaction mixture [mL]/rate of feed formalin [mL min⁻¹], assuming the density of the feed formalin is 1.0 g mL⁻¹. [d] Conversion [%] = $\{3[\text{trioxane}] + 2[\text{methyl formate}] + [\text{methylal}]\} / [\text{feed formalin}] \times 100$. Almost all the by-product is methyl formate, and only a small amount of methylal was observed. The amount of formic acid was negligibly small. Methyl formate is generated by the Cannizzaro reaction from two molecules of formaldehyde. Methylal is formed by the reaction of two molecules of methanol and one molecule of formaldehyde. [e] For the calculation of conversion and selectivity *S* concentrations in mol g⁻¹ were used. [f] *S* [%] = $\{[\text{trioxane}] / ([\text{trioxane}] + 2[\text{methyl formate}] + [\text{methylal}])\} \times 100$. [g] 61 wt % formalin was used.

With continuous feeding of 55 wt % formalin to the reaction mixture, no scale formation was observed for the heteropolyacids; that is, no paraformaldehyde was formed. However, for sulfuric acid, some whitening of the reaction mixture—that is, formation of paraformaldehyde—was observed. Thus the upper limit of the feed formaldehyde concentration using sulfuric acid as catalyst was considered to be 55 wt %. On the other hand, for the heteropolyacids, by increasing the amount of the heteropolyacid in the reaction mixture from 30 to 100 g (per initial 100 g of formalin), continuous feeding of 61 wt % formalin to the reaction mixture was possible without any problems of scale formation. This phenomenon meant an increase in the solubility of formalin in the presence of heteropolyacids, which might result from the interaction of the heteropolyacids with an ether linkage of the oligooxymethylene glycol.

Increasing the formaldehyde concentration from 55 to 61 wt % with the same conversion and same selectivity means an increase of 10% in the yield of trioxane per energy for trioxane synthesis.^[7] Thus, considering the increase in conversion and the increase in feed formalin concentration, an increase of 50% for the yield of trioxane per energy for trioxane synthesis may be possible.

We investigated the solubility of formalin in the heteropolyacids. Figure 1 shows that increasing the weight percent of heteropolyacid enhanced the solubility of formalin. The reaction temperature was changed by altering the vaporizing pressure. We thus determined the whitening temperature for the reaction mixtures. With an increase in the weight percent

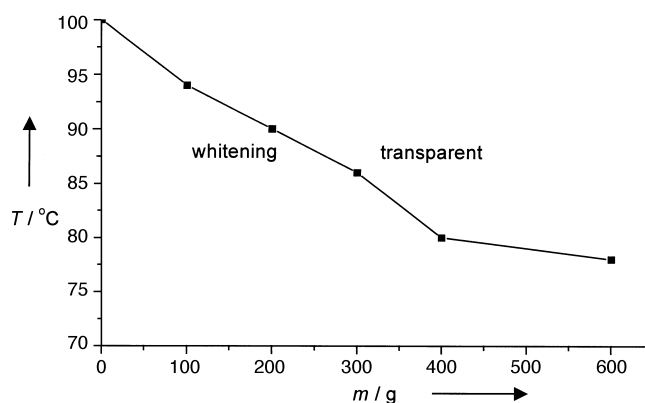


Figure 1. Plot of the lowest temperature *T* for which upon cooling no whitening (paraformaldehyde formation) occurs as a function of the amount *m* of heteropolyacid per 100 g of formalin. The catalyst was phosphotungstic acid; 55 wt % formalin was used; contact time 12–30 min.

of heteropolyacid, ever lower temperatures could be reached without whitening. In the case of a conventional catalyst like sulfuric acid, with feeding of 55 wt % formalin, the critical temperature was around 100 °C. However, through the use of heteropolyacids, we could work at lower reaction temperatures without whitening by increasing the amount of the heteropolyacids in the reaction mixtures. In Figure 1, we show the example of using phosphotungstic acid as catalyst. Similar results were obtained in the case of silicotungstic acid. These facts suggested that the heteropolyacids have a high solvation power for formalin.

The effect of temperature on the trioxane synthesis is shown in Table 2. An increase in temperature results in a decrease in conversion and selectivity of trioxane formation. Thus, high selectivity and high conversion were attained using highly concentrated heteropolyacids under reduced pressure (to achieve reaction temperatures of 81–84 °C; entries 7–10). These phenomena are thought to be characteristic for heteropolyacids and could not be achieved using a conventional catalyst like sulfuric acid, considering the fact that in the sulfuric acid catalyst system the critical temperature was 100 °C for the 55 wt % formalin feed.

In Figure 2, the relationships between the yield and selectivity obtained under various conditions are shown. With

Table 2. Effects of temperature *T* on conversion and selectivity *S* in the synthesis of trioxane.^[a]

Entry	Catalyst ^[b]	<i>T</i> [°C]	<i>t</i> [min] ^[c]	Conv. [%] ^[d, e]	<i>S</i> [%] ^[e, f]
1 ^[g]	PW (30)	110	15	17.7	98.6
2 ^[g]	PW (30)	110	20	18.3	98.1
3 ^[g]	PW (30)	110	48	22.9	97.3
4 ^[h]	PW (30)	100	39	18.3	98.5
5 ^[h]	PW (100)	100	34	24.6	98.3
6 ^[h]	PW (100)	100	50	25.5	98.1
7 ^[i]	PW (300)	84	25	30.1	98.4
8 ^[j]	PW (400)	83	15	27.5	99.2
9 ^[j]	PW (400)	83	37	28.9	98.6
10 ^[k]	PW (600)	81	36	31.2	99.2

[a] 55 wt % formalin was used. [b]–[f] See footnotes [b]–[f] of Table 1. [g] *p* = 2.65 atm. [h] *p* = 1 atm. [i] *p* = 0.44 atm. [j] *p* = 0.42 atm. [k] *p* = 0.33 atm.

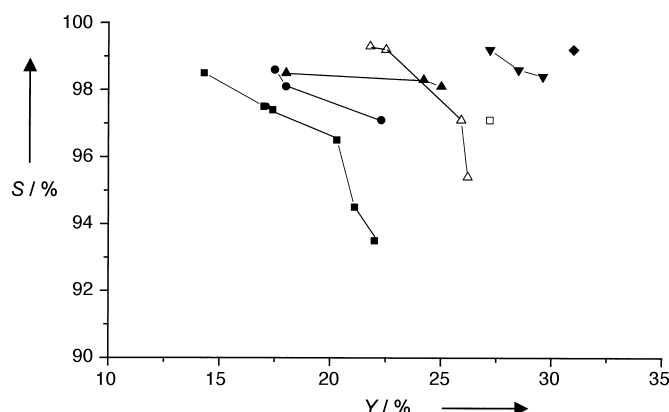


Figure 2. Relationships between yield Y and selectivity S under various conditions (see Tables 1 and 2). Unless noted otherwise, 55 wt % formalin was used. The catalyst and reaction temperature are as follows: ■ sulfuric acid at 100 °C, ● phosphotungstic acid at 110 °C, ▲ phosphotungstic acid at 100 °C, ▼ phosphotungstic acid at 83–84 °C, ◆ phosphotungstic acid at 81 °C, △ silicotungstic acid at 100 °C, □ silicotungstic acid at 100 °C using 61 wt % formalin.

an increase in yield, a decrease in selectivity was observed. However, a decrease in reaction temperature was accompanied by an increase in selectivity. Comparing the results obtained using heteropolyacids with those obtained using sulfuric acid, it is apparent that the heteropolyacid provides a higher yield and higher selectivity than sulfuric acid. Especially, by lowering the reaction temperature, a higher yield and higher selectivity can be attained.

In conclusion, considering the increase in conversion and selectivity as well as the increase in formalin solubility upon the use of heteropolyacids as a catalyst for trioxane synthesis, it might be possible to increase the yield of trioxane by over 50 % yield of trioxane per energy for trioxane synthesis.^[7] Therefore, heteropolyacids are worth developing as a catalyst for the commercial production of trioxane. This would open a new aspect for the industrial application of heteropolyacids as a catalyst in addition to the synthesis of *tert*-butyl alcohol (selective hydration of isobutene)^[8] and polymerization of tetrahydrofuran to polyoxytetramethylene glycol.^[9]

Experimental Section

We mainly used a 55 wt % aqueous formaldehyde solution, which was prepared by dissolving the 84 wt % paraformaldehyde flake (Sumitomo Chemical) in water at 100 °C. For the heteropolyacids, we used the commercial chemical grade of silicotungstic acid ($\text{H}_4\text{SiW}_{12}\text{O}_{40} \cdot 26\text{H}_2\text{O}$) and phosphotungstic acid ($\text{H}_3\text{PW}_{12}\text{O}_{40} \cdot 29\text{H}_2\text{O}$), supplied by Nippon Inorganic Color and Chemical, without further purification.

For the reactions, a 200-mL glass reactor was used. A thermal jacket, which was added to the top cover of the reactor, was heated with steam so that the vapor phase in the reactor did not condense on the inner face of the top cover. Thus, the reaction gas mixture was correctly carried (without change in composition) to the condenser without inner refluxing. (If partial condensation occurred on the inside surface of the top cover, the relative amount of trioxane was higher and thus the data measured gave the wrong results.) The reaction mixture (100 mL) consisting of the catalyst and 55 wt % formalin feed was heated in an oil bath. Formalin was continuously fed to the reactor, and the reaction product continuously vaporized from the homogenous liquid reaction mixture. The vapor phase condensed in the

condenser, and the condensate (water/formaldehyde/trioxane) was analyzed by gas chromatography. The level of the reaction mixture was controlled by a photo level controller, and the feed rate of formalin was adjusted to obtain a constant level of the reaction mixture. The contact time was regulated by changing the vaporizing speed, which was adjusted by changing the oil bath temperature. The measurement for the reaction was carried out after the stationary state was confirmed; the carbon balance (wt %) in feed formalin and distillate was equal.

For the trioxane synthesis above 100 °C (that is, under pressure, 110 °C), a stainless steel reactor was used, feeding 55 wt % formalin and drawing out the vapor phase into a condenser at low pressure until atmospheric pressure was reached. For the trioxane synthesis below 100 °C (that is, the reaction under reduced pressure), the same system was used as for reactions at atmospheric pressure.

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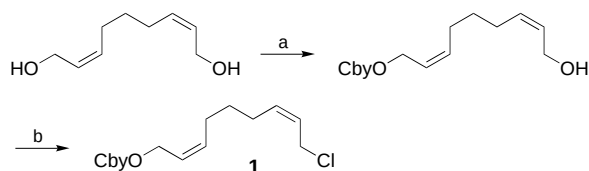
- [1] a) C. E. Schweitzer, R. N. MacDonald, J. O. Punderson, *J. Appl. Polym. Sci.* **1959**, *1*, 158; b) T. A. Koch, P. E. Lindvig, *J. Appl. Polym. Sci.* **1959**, *1*, 164; c) W. H. Linton, H. H. Goodman, *J. Appl. Polym. Sci.* **1959**, *1*, 179–184.
- [2] C. Walling, F. Brown, K. Bartz (Celanese), US-A 3 027 352, **1962**.
- [3] a) Y. Kobayashi, I. Suzuki, S. Ishida, *Hydrocarbon Process.* **1972**, *51*(11), 111; b) K. Matsuzaki, J. Masamoto, *Ind. Eng. Chem. Res.* **1998**, *37*, 1729.
- [4] J. Masamoto, T. Iwaisako, K. Yoshida, K. Matsuzaki, K. Kagawa, H. Nagahara, *Makromol. Chem. Macromol. Symp.* **1991**, *42/43*, 409.
- [5] J. S. T. Wall (Celanese), JP-A 73-11357, **1973**.
- [6] Heat of vaporization for an aqueous formaldehyde–trioxane mixture: water: 2.4, formaldehyde: 2.0, trioxane: 0.46 kJ g⁻¹.
- [7] If the trioxane concentration in the distillate of one pass vaporization is 10 wt %, the energy requirement of trioxane synthesis is about 10 g of steam per 1 g of trioxane, using steam as the heat source.
- [8] A. Aoshima, *Shokubai* **1987**, *29*, 378.
- [9] A. Aoshima, S. Tonomura, S. Yamamatsu, *Polym. Adv. Technol.* **1990**, *2*, 127.
- [10] A. Aoshima, *Shokubai* **1991**, *33*, 34.

Enantioselective Synthesis of Functionalized 1,5-Cyclononadienes by Intramolecular Cycloalkylation under α,α' -Diallyl Coupling**

Alexander Deiters, Roland Fröhlich, and Dieter Hoppe*

In spite of the biological importance of medium-size carbocycles, only a few ring-closing reactions are known for the synthesis of nine-membered monocarbocyclic rings.^[1] Classic examples are the acyloin condensation of nonanedioic acid dimethyl esters^[2a] and the McMurry reaction^[2b]; however, for reasonable yields, a high dilution of the substrate and a long reaction time are always necessary.^[2c]

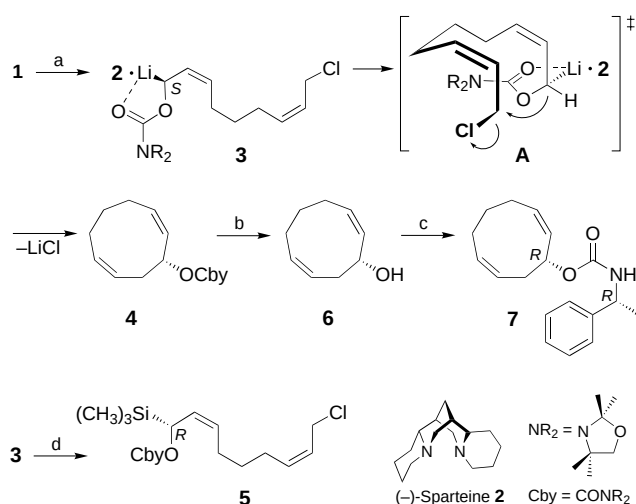
When we applied our protocol for an intramolecular lithium-ene reaction^[3] on the (2*Z*,7*Z*)-9-chloro-2,7-nona-dienyl carbamate **1**, we found a suprisingly efficient synthesis of a nine-membered carbocycle. The reaction proceeds to completion without any by-products within 2 h, even in 0.15 M solution. On the other hand, the (2*E*,7*E*)-isomer furnishes the expected *cis*-1,2-dialkenylcyclopentane **14**, as we recently reported (see Scheme 5).^[3] Compound **1** was synthesized from the known compound *cis,cis*-nona-2,7-diene-1,9-diol (Scheme 1).^[4]



Scheme 1. Synthesis of the dieny carbamate **1**. a) 0.3 equiv NaH, 0.3 equiv CbyCl, THF, Δ , 85 %; b) 1.0 equiv *n*BuLi, 1.3 equiv MsCl, 1.0 equiv LiCl, THF, $-78^\circ\text{C} \rightarrow \text{RT}$, 73 %.^[23] Cby = 2,2,4,4-tetramethyl-1,3-oxazolidin-3-ylcarbonyl, Ms = methanesulfonyl.

Treatment of **1** with *n*-butyllithium/(–)-sparteine (**2**)^[5] in toluene at -88°C provided the (1*R*,2*Z*,7*Z*)-2,7-cyclononadienyl carbamate **4** in 73 % yield and with an *ee* value of 88 % (e.r. = 94:6; Scheme 2).^[6,7] The *S* configuration of the intermediate lithium complex **3** was confirmed by conversion into the silane (*R*)-**5** (87 % *ee*).^[8,9] Thus, the α,α' -coupling^[10] of the allyl moieties in **3** proceeds under inversion of configuration at the metal-bearing carbon atom and, therefore, presumably passes through the transition state **A**.^[11]

The cleavage^[12] of the carbamate group led to the (*R*)-cyclononadienol **6** and addition of this to (*R*)-1-phenylethyl



Scheme 2. Cycloalkylation of the dieny carbamate **1** and synthesis of the silane **5**. a) 2.0 equiv *n*BuLi, 2.0 equiv **2**, toluene, -88°C , 73 %, e.r. = 94:6 (88 % *ee*.); b) 1.) 2.0 equiv $\text{CH}_3\text{SO}_3\text{H}$, CH_3OH , Δ ; 2.) 4.0 equiv KOH, CH_3OH , Δ , 96 %; c) 3.0 equiv (*R*)-(+)-1-phenylethyl isocyanate, CH_2Cl_2 , Δ , 63 %; d) 1.5 equiv *n*BuLi, 1.5 equiv (–)-sparteine, 2.7 equiv $(\text{CH}_3)_3\text{SiCl}$, toluene, -78°C , 61 %, 87 % *ee*.^[23]

isocyanate furnished the carbamate **7**. The X-ray analysis^[13] of **7** (Figure 1) clearly shows the relative configuration *l* and hence the *R* configuration for (+)-**6** and (+)-**4**.

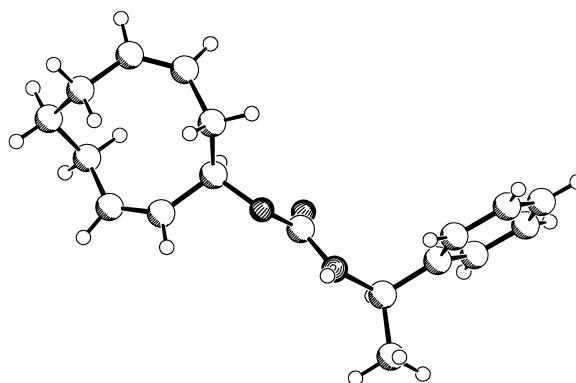


Figure 1. X-ray crystal structure analysis of **7**.^[13]

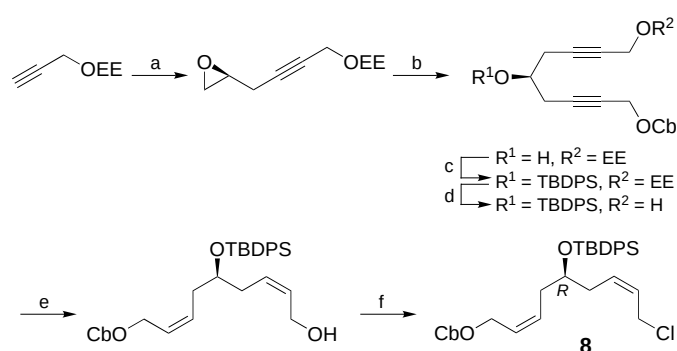
The analogous TBDPS-oxidiényl carbamate (*R*)-**8** has been synthesized starting from (*S*)-epichlorohydrin (Scheme 3). Cyclization of (*R*)-**8** (100 % *ee*) by means of *n*BuLi/**2** furnished the enantiomerically pure cyclononadiendiol derivatives (1*R*,5*S*)-**9** and (1*S*,5*S*)-**9**, in a diastereomeric ratio of 94:6, which are easily separated by chromatography.^[15] The stereogenic center at C-5 affects neither the deprotonation nor the cyclization step; by use of the achiral base *n*BuLi/TMEDA, (1*R*,5*S*)-**9** and (1*S*,5*S*)-**9** were obtained in the ratio 53:47. Corresponding to this, *rac*-**8** furnished, after treatment with *n*BuLi/**2**, the epimers (1*R*,5*S*)-**9** and (1*R*,5*R*)-**9** in 74 % yield with a diastereomeric ratio of 55:45 and each with an *ee* value of 92 % (Scheme 4). After deprotection to the alcohol (1*R*,5*R*)-**10** and X-ray analysis, the relative configuration *l* was confirmed (Figure 2).^[16]

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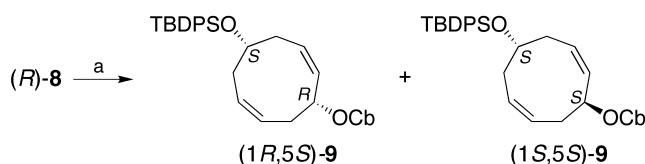
[+] X-ray analysis

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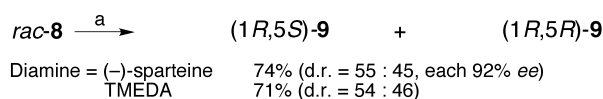
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



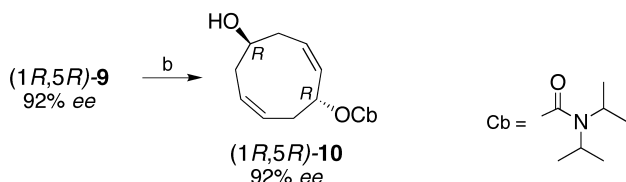
Scheme 3. Synthesis of the enantiomerically pure dienyl carbamate **8**. a) 1.) 1.0 equiv *n*BuLi, 0.5 equiv (*S*)-(+)-epichlorohydrin, 1.0 equiv $\text{BF}_3 \cdot \text{OEt}_2$, THF, $-78^\circ\text{C} \rightarrow \text{RT}$; 2.) 1.0 equiv NaH, THF, -10°C , 89%; b) 2.0 equiv HCCCH_2OCb ,^[14a] 2.0 equiv *n*BuLi, 2.0 equiv $\text{BF}_3 \cdot \text{OEt}_2$, THF, $-78^\circ\text{C} \rightarrow \text{RT}$, 98%;^[14b] c) 1.2 equiv TBDPSCI, 2.5 equiv imidazole, DMF, 83%; d) 0.07 mass % amberlyst 15, MeOH, 40°C , 95%; e) H_2 , 5.0 mass % Lindlar catalyst, 0.15 equiv quinoline, MeOH, 96%; f) 1.0 equiv *n*BuLi, 1.2 equiv MsCl, 1.0 equiv LiCl, THF, $-78^\circ\text{C} \rightarrow \text{RT}$, 76%.^[23] Cb = diisopropylaminocarbonyl, EE = ethoxyethyl, TBDPS = *tert*-butyldiphenylsilyl.



Diamine = (–)-sparteine
TMEDA 79% (d.r. = 94 : 6, each 100% ee)
70% (d.r. = 53 : 47, each 100% ee)



Diamine = (–)-sparteine
TMEDA 74% (d.r. = 55 : 45, each 92% ee)
71% (d.r. = 54 : 46)



Scheme 4. Cycloalkylation of the dienyl carbamate **8** and deprotection of the product **9**. a) 2.0 equiv *n*BuLi, 2.0 equiv diamine, toluene, -88°C , 2 h; b) 3.0 equiv tetrabutylammonium fluoride, THF, 74%.^[23] TMEDA = *N,N,N',N'*-tetramethylethylenediamine.

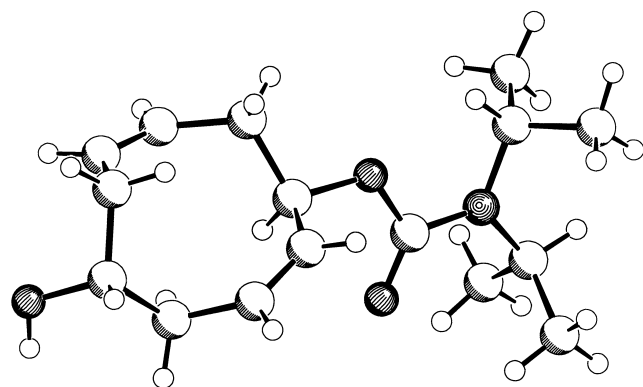
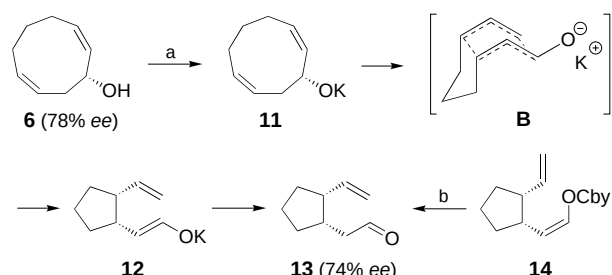


Figure 2. X-ray crystal structure analysis of (1*R*,5*R*)-**10**.^[16]

The (*Z,Z*)-cyclonona-1,5-diene structure is destined for Cope rearrangements which pass through boatlike transition states.^[17] After conversion of **6** into the potassium alcoholate **11** an anionic oxy-Cope rearrangement^[18a] takes place at room temperature. The aldehyde (+)-**13**, obtained after protonation of the potassium enolate **12**, shows 95% stereoselectivity for the rearrangement.^[18b] This seems to be the highest grade of chiral transmission in anionic oxy-Cope rearrangements of dienols which only contain a stereogenic center at the oxygen-bearing carbon atom.^[19] The absolute configuration of **13** was determined by its synthesis from **14**^[3] through deprotection of the enol.^[20] Together with the *R* configuration of **6**, a reaction pathway via the transition state **B** with an equatorial oxido group^[19c] takes place (Scheme 5).



Scheme 5. Enantioselective anionic oxy-Cope rearrangement. a) 1.3 equiv $\text{KN}(\text{Si}(\text{CH}_3)_3)_2$, THF, RT, 75%; b) 15 equiv $\text{Al}(\text{Bu}_2\text{H})$, toluene, RT, 65%.

The method reported herein provides simple, flexible, and also enantioselective access to highly functionalized nine-membered carbocycles. The application of the homoenolate chemistry^[5a, 21] on the configuratively stable secondary lithium species generated by deprotonation of **4** bears additional possibilities in the synthesis of nine-membered ring compounds.^[22]

Experimental Section

Under argon, compounds **1** (500 mg, 1.52 mmol) and **2** (713 mg, 3.04 mmol) were dissolved in toluene (10 mL). After cooling to -88°C , a 1.6 M solution of *n*-butyllithium in hexane (1.90 mL, 3.04 mmol) was slowly injected and the solution was stirred at this temperature for 2 h. Subsequently, CH_3OH (1 mL) and saturated aqueous NH_4Cl (1 mL) were added and the reaction mixture was allowed to warm to room temperature. Standard work up and purification by flash column chromatography over silica gel (Et_2O :pentane = 2:5) furnished **4** (324 mg, 73%, 88% ee) as a colorless solid (m.p. 72°C).

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- [1] The synthesis of nine-membered rings is particularly strongly disfavored because of the adverse entropic term, the Baeyer strain, and the Pitzer strain, as well as the transannular interactions: E. L. Eliel, S. H. Wilen in *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, pp. 675–685.
- [2] a) A. T. Bloomquist, Y. C. Meinwald, *J. Am. Chem. Soc.* **1958**, *80*, 630–632; b) T. Lecta in *Active Metals* (Ed.: A. Fürstner), VCH, Weinheim, **1995**, pp. 85–132; J. E. McMurry, K. L. Kees, *J. Org. Chem.* **1977**, *42*, 2655–2656; c) Other ring-closing reactions leading to monocarbocyclic nine-membered rings; intramolecular 1,4-addition (35% yield): P. Deslongchamps, B. L. Roy, *Can. J. Chem.* **1986**, *64*, 2068–2075; Thorpe–Ziegler condensation (3% yield): J. P. Schaefer, J. J. Bloomfield, *Org. React.* **1967**, *15*, 3–203.

- [3] A. Deiters, D. Hoppe, *Angew. Chem.* **1999**, *111*, 529–532; *Angew. Chem. Int. Ed.* **1999**, *38*, 546–548.
- [4] P. Lennon, M. Rosenblum, *J. Am. Chem. Soc.* **1983**, *105*, 1233–1241.
- [5] Reviews: a) D. Hoppe, T. Hense, *Angew. Chem.* **1997**, *109*, 2376–2410; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2282–2316; b) P. Beak, A. Basu, D. J. Gallagher, Y. S. Park, S. Thayumanavan, *Acc. Chem. Res.* **1996**, *29*, 552–560.
- [6] If the reaction is carried out at -78°C , **4** is obtained in 66% yield and 86% ee. By using the achiral base *n*BuLi/TMEDA in Et₂O at -78°C , *rac*-**4** is yielded in 76%.
- [7] Compound **4**: Mp 72°C ; $[\alpha]_{\text{D}}^{20} = +14.8$ ($c = 0.59$, CHCl₃; 88% ee); ¹H NMR (400 MHz, CDCl₃): $\delta = 1.34\text{--}1.56$ (m, 14H, CH₂, CH₃(Cby)), 1.85–2.03 (m, 4H, CH₂), 2.22 and 2.43 (both m, each with 1H, CH₂), 3.70 and 3.71 (s, 2H, CH₂(Cby)), 5.10 (dd, 1H, CH, ³J = 7.8, 7.8 Hz), 5.38 (dt, 1H, CH, ³J = 9.6, 9.6 Hz), 5.47 (dt, 1H, CH, ³J = 5.8, 10.1 Hz), 5.62 (m, 1H, CH), 5.79 (dt, 1H, CH, ³J = 10.1, 8.5 Hz). The enantiomeric ratio of **4** was determined by gas chromatography on a chiral stationary phase (Beta-Dex 120, Supelco, USA).
- [8] Compound **5**: $[\alpha]_{\text{D}}^{20} = +10.3$ ($c = 1.27$, CHCl₃; 87% ee); ¹H NMR (300 MHz, CDCl₃): $\delta = 0.05$ (s, 9H, Si(CH₃)₃), 1.30–1.51 (m, 14H, CH₂, CH₃(Cby)), 1.96–2.30 (m, 4H, CH₂), 3.69 (s, 2H, CH₂(Cby)), 4.07 (dd, 2H, CH₂Cl, ⁴J = 1.8, ³J = 5.1 Hz), 5.35–5.42 (m, 3H, CH, CHSi), 5.58–5.62 (m, 2H, CH). The enantiomeric ratio of the silane **5** was determined by ¹H NMR shift experiments in the presence of [Eu(hfc)₃] (hfc = 3-(heptafluoropropylhydroxymethylene)-D-camphorate). After hydrogenation of the double bonds and hydrogenolytic cleavage of the chlorine atom, the corresponding 1-trimethylsilylnonyl carbamate was obtained.^[5] Its enantiomer was synthesized by the *s*BuLi/(–)-sparteine method.^[5a] A correlation of the optical rotations assigned the R configuration for (+)-**5**.
- [9] Stereoconversion in the silylation of allyllithium-(–)-sparteine complexes: K. Behrens, R. Fröhlich, O. Meyer, D. Hoppe, *Eur. J. Org. Chem.* **1998**, 2397–2403; H. Paulsen, C. Graeve, D. Hoppe, *Synthesis* **1996**, 141–144.
- [10] a) H. Yamamoto et al. reported intermolecular α,α' -allyl couplings by the use of allylbarium reagents.^[10b] However, an intramolecular coupling reaction was unknown: A. Yanagisawa, K. Yasue, H. Yamamoto, *Synlett* **1996**, 842–844; A. Yanagisawa, H. Hibino, S. Habaue, Y. Hisada, H. Yamamoto, *J. Org. Chem.* **1992**, *57*, 6386–6387; b) A. Yanagisawa, H. Yamamoto in *Active Metals* (Ed.: A. Fürstner), VCH, Weinheim, **1995**, pp. 61–84.
- [11] Presumably, the topology of the transition state **A** is influenced by $\pi\text{--}\pi^*$ interactions of the allylic moieties by precoordination and, therefore, favor the entropic term of the cyclization. In principle a γ,γ' -coupling between C-3 and C-7 is also possible and would lead to the *cis* configured five-membered ring. However, this would cause an increase of transannular and diaxial interactions of the side chains with the ring system; for this reason, the formation of a nine-membered ring takes place.
- [12] F. Hintze, D. Hoppe, *Synthesis* **1992**, 1216–1218.
- [13] X-ray crystal structure analysis of **7**: C₁₈H₂₃NO₂, $M_r = 285.37$, light yellow crystal, $0.20 \times 0.10 \times 0.05$ mm, $a = 23.258(1)$, $c = 5.106(1)$ Å, $\gamma = 120^{\circ}$, $V = 2392.0(5)$ Å³, $\rho_{\text{calcd}} = 1.189$ g cm^{–3}, $\mu = 0.77$ cm^{–1}, absorption correction with SORTAV ($0.985 \leq T \leq 0.996$), $Z = 6$, trigonal, space group *P*3₁ (No. 144), $\lambda = 0.71073$ Å, $T = 198$ K, ω and φ scans, 20958 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda] = 0.65$ Å^{–1}, 7097 independent ($R_{\text{int}} = 0.056$) and 4457 observed reflections [$I \geq 2\sigma(I)$], 387 refined parameters, $R = 0.066$, $wR^2 = 0.131$, max./min. residual electron density $0.42/ -0.25$ e Å^{–3}, Flack parameter $-0.1(14)$, the asymmetric unit contains two independent, nearly identical molecules, hydrogens were calculated and refined as riding atoms.^[10b]
- [14] a) For 2-alkynyl carbamates, see: S. Dreier, M. Dyrbusch, D. Hoppe, *Synlett* **1991**, 397–400; b) For a similar reaction sequence, see: S. Hatakeyama, H. Irie, T. Shintani, Y. Noguchi, H. Yamada, M. Nishizawa, *Tetrahedron* **1994**, *50*, 13369–13376.
- [15] Compound (1*R*,5*S*)-**9**: $[\alpha]_{\text{D}}^{20} = -37.7$ ($c = 0.43$, CHCl₃, 100% ee); ¹H NMR (300 MHz, CDCl₃): $\delta = 1.07$ (s, 9H, *t*Bu), 1.22 (d, 12H, CH₃(Cb), ³J = 6.9 Hz), 1.93–2.00 (m, 1H, CH₂), 2.11 (dd, 2H, CH₂, ³J = 6.9, 3.6 Hz), 2.19–2.38 (m, 2H, CH₂, CH₂), 2.53 (ddd, 1H, CH₂, ³J = 8.7, 9.0, ²J = 12.9 Hz), 3.80–4.03 (m, 3H, CH(Cb), CHOSi), 5.03 (dd, 1H, CHOCb, ³J = 6.0, 6.9 Hz), 5.35 (dt, 1H, CH, ³J = 10.6, 5.7 Hz), 5.67–5.81 (m, 3H, CH, CH, CH), 7.32–7.70 (m, 10H, CH(phenyl)). After cleavage of the TBDPS group, the enantiomeric ratio of (1*R*,5*S*)-**10** was determined as described in reference [7].
- [16] a) X-ray crystal structure analysis of (1*R*,5*R*)-**10**: C₁₆H₂₇NO₃, $M_r = 281.39$, colorless crystal, $0.40 \times 0.25 \times 0.15$ mm, $a = 26.159(5)$, $b = 10.884(2)$, $c = 12.918(3)$ Å, $\beta = 113.71(2)^{\circ}$, $V = 3367.5(12)$ Å³, $\rho_{\text{calcd}} = 1.110$ g cm^{–3}, $\mu = 6.04$ cm^{–1}, absorption correction with ψ -scan data ($0.794 \leq T \leq 0.915$), $Z = 8$, monoclinic, space group *C*2 (No. 5), $\lambda = 1.54178$ Å, $T = 223$ K, $\omega/2\theta$ scans, 3697 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda] = 0.62$ Å^{–1}, 3611 independent ($R_{\text{int}} = 0.077$) and 3159 observed reflections [$I \geq 2\sigma(I)$], 372 refined parameters, $R = 0.044$, $wR^2 = 0.119$, max./min. residual electron density $0.24/ -0.17$ e Å^{–3}, Flack Parameter 0.3(2), the asymmetric unit contains two independent, nearly identical molecules, hydrogens were calculated and refined as riding atoms; b) The data sets were collected with Nonius CAD4 and Nonius KappaCCD diffractometers equipped with a Nonius FR590 sealed tube generator or a Nonius FR591 rotating anode generator. The following programs were used: For data collection, EXPRESS (Nonius B.V., **1994**) and COLLECT (Nonius B.V., **1998**); for data reduction, MolEN (K. Fair, Enraf–Nonius B.V., **1990**) and Denzo-SMN (Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307–326); for absorption corrections for CCD data, SORTAV (R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33–37; R. H. Blessing, *J. Appl. Crystallogr.* **1997**, *30*, 421–426); for structure solution, SHELXS-97 (G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467–473); for structure refinement, SHELXL-97 (G. M. Sheldrick, University of Göttingen, **1997**); for graphics, SCHAKAL (E. Keller, University of Freiburg, **1997**). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-139019 and -139020. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [17] By heating **4** up to 220°C , a stereospecific Cope rearrangement takes place.^[22] Cope rearrangement of (Z,Z)-cyclonona-1,5-diene: E. Vogel, W. Grimme, E. Dinné, *Angew. Chem.* **1963**, *75*, 1103.
- [18] a) Review of anionic oxy-Cope rearrangements: L. A. Paquette, *Tetrahedron* **1997**, *53*, 13971–14020; b) anionic oxy-Cope rearrangement of *rac*-**6**: L. A. Paquette, G. D. Crouse, A. K. Sharma, *J. Am. Chem. Soc.* **1982**, *104*, 4411–4423. The enantiomeric ratio of **13** was determined as described in reference [7].
- [19] a) S.-Y. Wei, K. Tomooka, T. Nakai, *Tetrahedron* **1993**, *49*, 1025–1042; b) L. A. Paquette, G. D. Maynard, *Angew. Chem.* **1991**, *103*, 1392–1394; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1368–1370; c) in anionic oxy-Cope rearrangements the oxido group prefers an equatorial position: L. A. Paquette, G. Maynard, *J. Am. Chem. Soc.* **1992**, *114*, 5018–5027.
- [20] K. Tomooka, N. Komine, T. Sasaki, H. Shimizu, T. Nakai, *Tetrahedron Lett.* **1998**, *39*, 9715–9718.
- [21] Review of homoenolate chemistry: D. Hoppe, *Angew. Chem.* **1984**, *96*, 930–946; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 932–948.
- [22] A. Deiters, D. Hoppe, unpublished results.
- [23] All new compounds were analytically pure (error in C,H,N elemental analyses ± 0.4).

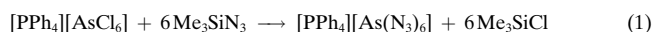
Tetraphenylphosphonium Hexaazidoarsenate(v): The First Structurally Characterized Binary As^V–Azide Species**

Thomas M. Klapötke,* Heinrich Nöth, Thomas Schütt, and Marcus Warchhold

Dedicated to Professor Heinrich Vahrenkamp on the occasion of his 60th birthday

The chemistry of covalent arsenic–azide species was opened by Revitt and Sowerby^[1] with the reported synthesis, spectroscopic properties, and thermal decomposition of some organoarsenic(III) azides. However, very little is known about their reactivity or structures. To our knowledge, only two molecular arsenic(III) azides have been structurally characterized: the perfluoroalkyl species bis(trifluoromethyl)arsenic azide, ((CF₃)₂AsN₃)₂,^[2a] and trifluoromethylarsenic diazide, CF₃As(N₃)₂,^[2b] which were investigated by gas-phase electron diffraction. Recently we reported on the reaction between AsCl₃ and [AsCl₄][AsF₆] with activated sodium azide, which resulted in the formation of the first binary arsenic azide, As(N₃)₃, and the first reported As^V–azide species, [As(N₃)₄]⁺.^[3a,b] As far as we are aware, prior to this study there has been no report on a hexacoordinated arsenic–azide compound nor has the structure of any arsenic–azide species through X-ray analysis been determined experimentally.^[4a,b] Herein, we report on the formation and the crystal structure^[5] of the extremely nitrogen rich, binary hexaazidoarsenate(v) anion [As(N₃)₆][−].

In contrast to the synthesis of As(N₃)₃ and [As(N₃)₄]⁺, which were prepared from NaN₃ and the corresponding As–Cl compound, in this study we used trimethylsilylazide, (CH₃)₃SiN₃, as the azide group-transferring agent. The compound [PPh₄][AsCl₆] was prepared as described in the literature^[6] and treated with an excess of trimethylsilylazide in a solution of CH₂Cl₂ at room temperature to yield tetraphenylphosphonium hexaazidoarsenate(v) **1** [Eq. (1)].



Compound **1** was isolated and fully characterized by elemental analysis, IR, Raman, and multinuclear NMR spectroscopies, and the molecular structure was determined by single crystal X-ray diffraction.

The IR and Raman spectra of **1** show, in addition to the expected vibrations which can be attributed to the [PPh₄]⁺ ion, all the characteristic modes of a covalently bound azide species. The covalent nature of the [As(N₃)₆][−] ion is indicated by both the simultaneous appearance of the asymmetric

(2110–2084 cm^{−1}) and the symmetric (1270–1279 cm^{−1}) azide stretch modes and by the presence of a strong As–N vibration at 416 cm^{−1} in the IR and the Raman spectra.^[7] An exact assignment of the normal modes of the [As(N₃)₆][−] ion, which has idealized S₂ symmetry, was not possible due to lowering of the symmetry in the solid state.

Compound **1** was characterized by ¹H, ¹³C, ¹⁴N, ³¹P, and ⁷⁵As NMR spectroscopy. The most suitable NMR method for the characterization of covalent azides is undoubtedly the ¹⁴N NMR spectroscopy. The ¹⁴N NMR spectrum (Figure 1) shows

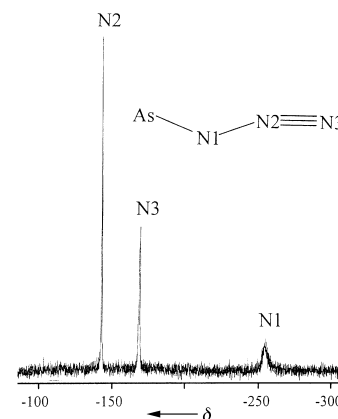


Figure 1. ¹⁴N NMR spectrum of **1**.

three well resolved resonances, which can be assigned to the chemically inequivalent nitrogen atoms of the covalently bound azide group. The assignment was made on the basis of examples given in the literature.^[8] The N2 atom shows a sharp signal at δ = −141.1, the N3 atom at δ = −165.4, and the N1 atom, as expected, gives a very broad resonance at δ = −256.2. In the ⁷⁵As NMR spectrum, **1** shows a resonance at δ = +4 (standard: KAsF₆, δ = 0) and appears at significantly lower shift compared to the starting material [PPh₄][AsCl₆] (δ = −392).^[9d] The relatively sharp signal in the ⁷⁵As NMR spectrum of [As(N₃)₆][−] indicates a very symmetrical environment around the central As atom, since the large quadrupolar moment of the ⁷⁵As nucleus means this element can be detected only in very symmetrical environments.^[9a–d]

The X-ray structure analysis of **1** (Figure 2)^[5] revealed the presence of an ionic compound with [PPh₄]⁺ cations and [As(N₃)₆][−] anions which do not show any significant cation–anion interaction. The arsenic atom, which exhibits a slightly

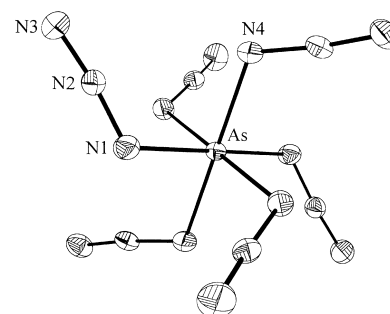


Figure 2. The molecular structure of **1** (the counterions are omitted for clarity). The thermal ellipsoids represent a 25% probability. Selected bond lengths [Å] and angles [°]: As–N1 1.931(2), N1–N2 1.229(3), N2–N3 1.123(3); As–N1–N2 115.6(2), N1–N2–N3 173.6(3), N1–As–N4 91.5(1).

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distorted octahedral environment with idealized S_2 symmetry, is bound to six nitrogen (N1) atoms. The As–N1 distances span 1.920(3)–1.938(2) Å and the N1–As–N4 angles lie between 88.2(1)–91.8(1)°. While the N1–N2 bond lengths range 1.222(3)–1.238(3) Å, the terminal N2–N3 lengths range 1.120(3)–1.123(3) Å and are both in good accord with the bond lengths of bis(trifluoromethyl)arsinic azide^[2a] and trifluoromethylarsinic diazide.^[2b] The N1–N2–N3 angle lies within 173.6(3)–176.3(3)° and in good agreement with N–N–N angles of other previously reported covalent azides.^[10] The angle between the arsenic atom and the azide group (As–N1–N2) is 116.2(2)°.

Compound **1** shows only moderate impact, friction, and electrostatic sensitivity but explodes when subject to a thermal shock test.^[11] The relatively high kinetic stability of **1** can be explained by the fact that the large and bulky $[PPh_4]^+$ ions separate the $[As(N_3)_6]^-$ ions in the solid state and therefore cause a high activation barrier for the overall exothermic decomposition. This phenomenon has also been observed for the $[I(N_3)_2]^-$ ion, which is kinetically stable as the $[PPh_4]^+$ salt but highly explosive as the $[NMe_4]^+$ salt.^[12]

Experimental Section

Caution: Covalent and ionic azides as well as arsenic compounds are very toxic and covalent azides are potentially explosive. Appropriate safety precautions should be taken.

Apparatus: NMR: Jeol EX400 Delta (^1H , ^{13}C : relative to $\delta_{TMS} = 0.00$; ^{31}P : relative to $\delta_{H_3PO_4} = 0.00$; ^{14}N : relative to $\delta_{CH_3NO_2} = 0.00$; ^{75}As : relative to $\delta_{KAsF_6} = 0.00$). IR: Nicolet 520 FTIR. Raman: Perkin Elmer Spectrum 2000R NIR FT. Elemental (C, H, N) analysis: Analysator Elementar Vario EL.

Compound **1** was synthesized under an inert gas atmosphere at 25 °C by the slow addition of neat $(CH_3)_3SiN_3$ (0.53 mL, 4 mmol) with stirring to a solution of $[PPh_4][AsCl_6]$ (0.314 g, 0.5 mmol) in CH_2Cl_2 (25 mL). After 5 hours the solvent and $(CH_3)_3SiCl$ were removed by a dynamic vacuum. Colorless crystals were obtained after recrystallization from CH_2Cl_2 at –25 °C. Yield: 0.237 g (71 %, based on $[PPh_4][AsCl_6]$); elemental analysis for $C_{24}H_{20}AsN_{18}P$: calcd: C 43.25, H 3.02, N 37.83; found: C 42.89, H 2.91, N 37.44; IR (powder between CsI plates): $\tilde{\nu}$: 2085 (vs, $\nu_{as}(N_3)$), 1585 (w), 1481 (m), 1434 (s), 1270 (s, $\nu_s(N_3)$), 1108 (s), 996 (w), 888 (m), 783 (s), 688 (s, $\delta(N_3)$), 526 (s), 416 (s, $\nu(AsN)$), 305 cm^{-1} (s); Raman (525 scans, 200 mW, 180° geometry, 25 °C): $\Delta\tilde{\nu}$: 3068 (6.5), 2110 ($\nu_{as}(N_3)$ in plane, 3.5), 2084 ($\nu_{as}(N_3)$ out of plane, 1.5), 1587 (4.5), 1279 ($\nu_s(N_3)$, 3.5), 1187 (0.5), 1163 (0.5), 1099 (1.5), 1027 (2.5), 1001 (6.5), 681 ($\delta(N_3)$, 1.5), 669 ($\delta(N_3)$, 1.5), 416 ($\nu(AsN)$, 10), 267 (1.5), 252 (1.5), 116 (8) cm^{-1} ; ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 117.5 (d, C1), 130.8 (d, C2), 134.4 (d, C3), 135.9 (d, C4); ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.8–7.5 (Ph); ^{31}P NMR (109 MHz, $CDCl_3$, 25 °C): δ = 23.9 (s); ^{14}N NMR (29 MHz, $CDCl_3$, 25 °C): δ = –141.1 (N2), –165.4 (N3), –256.2 (N1); ^{75}As NMR (46 MHz, $CDCl_3$, 25 °C): δ = 4.

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- [1] D. M. Revitt, D. B. Sowerby, *J. Chem. Soc. Dalton Trans.* **1972**, 7, 847–850.
- [2] a) H. G. Ang, W. L. Kwik, Y. W. Lee, S. Liedle, H. Oberhammer, *J. Mol. Struct.* **1992**, 268, 389–394; b) H. G. Ang, W. L. Kwik, Y. W. Lee, H. Oberhammer, *Inorg. Chem.* **1994**, 33, 4425–4426.
- [3] a) T. M. Klapötke, P. Geissler, *J. Chem. Soc. Dalton Trans.* **1995**, 3365–3366; b) P. Geissler, T. M. Klapötke, H. J. Kroth, *Spectrochim. Acta Part A* **1995**, 51, 1075–1078.
- [4] a) I. C. Tornieporth-Oetting, T. M. Klapötke, *Angew. Chem.* **1995**, 107, 509; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 511; b) T. M. Klapötke, *Chem. Ber.* **1997**, 130, 443.
- [5] X-ray structure analysis: Siemens CCD Area Detector, hemisphere scan type, MoK_{α} radiation, $\lambda = 0.71073$ Å, $T = 183$ K, range $3.72^\circ <$

$2\theta < 58.48^\circ$ in $-27 \leq h \leq 12$, $-7 \leq k \leq 8$, $-24 \leq l \leq 24$, 8213 measured reflections, 2869 independent reflections ($R_{int} = 0.0343$), 2399 observed reflections ($F > 4\sigma(F)$). Structure solution program: SHELXS-97 (G. M. Sheldrick, University of Göttingen, Germany, **1997**), solution with direct methods, data to parameter ratio 11.9:1 (10.0:1 [$F > 4\sigma(F)$]), $R1 = 0.0375$, $wR2 = 0.0922$, $R1 = 0.0501$, $wR2 = 0.0986$ (all data), $GOF(F^2) = 1.035$, max./min. residual electron density 0.574, –0.436 $e \text{ Å}^{-3}$, structure refinement program: SHELXL-97 (G. M. Sheldrick, University of Göttingen, Germany, **1997**). Crystal data for **1**: $C_{24}H_{20}AsN_{18}P$ ($M_r = 666.47$), colorless prisms, $0.20 \times 0.10 \times 0.05$ mm, monoclinic, space group $C2/c$, $a = 22.147(3)$, $b = 7.1943(8)$, $c = 18.766(2)$ Å, α , $\gamma = 90.00$, $\beta = 98.635(2)^\circ$, $V = 2956.1(6)$ Å³, $Z = 4$, $\rho_{calcd} = 1.498$ g cm^{-3} , $\mu = 1.255$ mm^{–1}, $F(000) = 1352$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140555. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [6] J. Bebenorf, U. Müller, *Z. Naturforsch. B* **1990**, 45, 927–930.
- [7] M.-J. Crawford, T. M. Klapötke, *Internet J. Vib. Spec.* **1999/2000**, 3(6), 3 (<http://www.ijvs.com>).
- [8] W. Beck, W. Becker, K. F. Chew, W. Derbyshire, N. Logan, D. M. Revitt, D. B. Sowerby, *J. Chem. Soc. Dalton Trans.* **1972**, 7, 245–247.
- [9] a) G. Baliman, P. S. Pregosin, *J. Magn. Reson.* **1977**, 26, 283; b) C. Brevard, P. Granger, *Handbook of High Resolution Multinuclear NMR*, Wiley, New York, **1981**, pp. 136–137; c) H. P. A. Mercier, J. C. P. Sanders, G. J. Schrobilgen, *J. Am. Chem. Soc.* **1994**, 116, 2921; d) M. F. A. Dove, J. C. P. Sanders, E. L. Jones, M. J. Parkin, *J. Chem. Soc. Chem. Commun.* **1984**, 1578.
- [10] W. Beck, T. M. Klapötke, J. Knizek, H. Nöth, T. Schütt, *Eur. J. Inorg. Chem.* **1999**, 523–526.
- [11] “Covalent Inorganic Nonmetal Azides”: I. C. Tornieporth-Oetting, T. M. Klapötke in *Combustion Efficiency and Air Quality* (Eds.: I. Hargittai, T. Vidoczy), Plenum, New York, **1995**, p. 51.
- [12] U. Müller, R. Dübgen, K. Dehnicke, *Z. Anorg. Allg. Chem.* **1980**, 463, 7–13.

Preparation of Bioconjugates through an Ugi Reaction**

Thomas Ziegler,* Sonja Gerling, and Martin Lang

Dedicated to Professor Franz Effenger
on the occasion of his 70th birthday

The specific preparation of bioconjugates, which is, in general, the covalent linking of a low molecular weight compound to a protein or an oligonucleotide or bond formation between two proteins, can be regarded as one of the most important methods in modern biochemistry.^[1] Conjugates which are constructed out of a protein or oligonucleotide (for example, an enzyme or a DNA fragment) and a pigment or a radioactive compound are widely used for

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distorted octahedral environment with idealized S_2 symmetry, is bound to six nitrogen (N1) atoms. The As–N1 distances span 1.920(3)–1.938(2) Å and the N1–As–N4 angles lie between 88.2(1)–91.8(1)°. While the N1–N2 bond lengths range 1.222(3)–1.238(3) Å, the terminal N2–N3 lengths range 1.120(3)–1.123(3) Å and are both in good accord with the bond lengths of bis(trifluoromethyl)arsinic azide^[2a] and trifluoromethylarsinic diazide.^[2b] The N1–N2–N3 angle lies within 173.6(3)–176.3(3)° and in good agreement with N–N–N angles of other previously reported covalent azides.^[10] The angle between the arsenic atom and the azide group (As–N1–N2) is 116.2(2)°.

Compound **1** shows only moderate impact, friction, and electrostatic sensitivity but explodes when subject to a thermal shock test.^[11] The relatively high kinetic stability of **1** can be explained by the fact that the large and bulky $[PPh_4]^+$ ions separate the $[As(N_3)_6]^-$ ions in the solid state and therefore cause a high activation barrier for the overall exothermic decomposition. This phenomenon has also been observed for the $[I(N_3)_2]^-$ ion, which is kinetically stable as the $[PPh_4]^+$ salt but highly explosive as the $[NMe_4]^+$ salt.^[12]

Experimental Section

Caution: Covalent and ionic azides as well as arsenic compounds are very toxic and covalent azides are potentially explosive. Appropriate safety precautions should be taken.

Apparatus: NMR: Jeol EX400 Delta (^1H , ^{13}C : relative to $\delta_{TMS} = 0.00$; ^{31}P : relative to $\delta_{H_3PO_4} = 0.00$; ^{14}N : relative to $\delta_{CH_3NO_2} = 0.00$; ^{75}As : relative to $\delta_{KAsF_6} = 0.00$). IR: Nicolet 520 FTIR. Raman: Perkin Elmer Spectrum 2000R NIR FT. Elemental (C, H, N) analysis: Analysator Elementar Vario EL.

Compound **1** was synthesized under an inert gas atmosphere at 25 °C by the slow addition of neat $(CH_3)_3SiN_3$ (0.53 mL, 4 mmol) with stirring to a solution of $[PPh_4][AsCl_6]$ (0.314 g, 0.5 mmol) in CH_2Cl_2 (25 mL). After 5 hours the solvent and $(CH_3)_3SiCl$ were removed by a dynamic vacuum. Colorless crystals were obtained after recrystallization from CH_2Cl_2 at –25 °C. Yield: 0.237 g (71 %, based on $[PPh_4][AsCl_6]$); elemental analysis for $C_{24}H_{20}AsN_{18}P$: calcd: C 43.25, H 3.02, N 37.83; found: C 42.89, H 2.91, N 37.44; IR (powder between CsI plates): $\tilde{\nu}$: 2085 (vs, $\nu_{as}(N_3)$), 1585 (w), 1481 (m), 1434 (s), 1270 (s, $\nu_s(N_3)$), 1108 (s), 996 (w), 888 (m), 783 (s), 688 (s, $\delta(N_3)$), 526 (s), 416 (s, $\nu(AsN)$), 305 cm^{-1} (s); Raman (525 scans, 200 mW, 180° geometry, 25 °C): $\Delta\tilde{\nu}$: 3068 (6.5), 2110 ($\nu_{as}(N_3)$ in plane, 3.5), 2084 ($\nu_{as}(N_3)$ out of plane, 1.5), 1587 (4.5), 1279 ($\nu_s(N_3)$, 3.5), 1187 (0.5), 1163 (0.5), 1099 (1.5), 1027 (2.5), 1001 (6.5), 681 ($\delta(N_3)$, 1.5), 669 ($\delta(N_3)$, 1.5), 416 ($\nu(AsN)$, 10), 267 (1.5), 252 (1.5), 116 (8) cm^{-1} ; ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C): δ = 117.5 (d, C1), 130.8 (d, C2), 134.4 (d, C3), 135.9 (d, C4); ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.8–7.5 (Ph); ^{31}P NMR (109 MHz, $CDCl_3$, 25 °C): δ = 23.9 (s); ^{14}N NMR (29 MHz, $CDCl_3$, 25 °C): δ = –141.1 (N2), –165.4 (N3), –256.2 (N1); ^{75}As NMR (46 MHz, $CDCl_3$, 25 °C): δ = 4.

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- [1] D. M. Revitt, D. B. Sowerby, *J. Chem. Soc. Dalton Trans.* **1972**, 7, 847–850.
- [2] a) H. G. Ang, W. L. Kwik, Y. W. Lee, S. Liedle, H. Oberhammer, *J. Mol. Struct.* **1992**, 268, 389–394; b) H. G. Ang, W. L. Kwik, Y. W. Lee, H. Oberhammer, *Inorg. Chem.* **1994**, 33, 4425–4426.
- [3] a) T. M. Klapötke, P. Geissler, *J. Chem. Soc. Dalton Trans.* **1995**, 3365–3366; b) P. Geissler, T. M. Klapötke, H. J. Kroth, *Spectrochim. Acta Part A* **1995**, 51, 1075–1078.
- [4] a) I. C. Tornieporth-Oetting, T. M. Klapötke, *Angew. Chem.* **1995**, 107, 509; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 511; b) T. M. Klapötke, *Chem. Ber.* **1997**, 130, 443.
- [5] X-ray structure analysis: Siemens CCD Area Detector, hemisphere scan type, MoK_{α} radiation, $\lambda = 0.71073$ Å, $T = 183$ K, range $3.72^\circ <$

$2\theta < 58.48^\circ$ in $-27 \leq h \leq 12$, $-7 \leq k \leq 8$, $-24 \leq l \leq 24$, 8213 measured reflections, 2869 independent reflections ($R_{int} = 0.0343$), 2399 observed reflections ($F > 4\sigma(F)$). Structure solution program: SHELXS-97 (G. M. Sheldrick, University of Göttingen, Germany, **1997**), solution with direct methods, data to parameter ratio 11.9:1 (10.0:1 [$F > 4\sigma(F)$]), $R1 = 0.0375$, $wR2 = 0.0922$, $R1 = 0.0501$, $wR2 = 0.0986$ (all data), $GOF(F^2) = 1.035$, max./min. residual electron density 0.574, –0.436 $e \text{ Å}^{-3}$, structure refinement program: SHELXL-97 (G. M. Sheldrick, University of Göttingen, Germany, **1997**). Crystal data for **1**: $C_{24}H_{20}AsN_{18}P$ ($M_r = 666.47$), colorless prisms, $0.20 \times 0.10 \times 0.05$ mm, monoclinic, space group $C2/c$, $a = 22.147(3)$, $b = 7.1943(8)$, $c = 18.766(2)$ Å, α , $\gamma = 90.00$, $\beta = 98.635(2)^\circ$, $V = 2956.1(6)$ Å³, $Z = 4$, $\rho_{calcd} = 1.498$ g cm^{-3} , $\mu = 1.255$ mm^{–1}, $F(000) = 1352$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-140555. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [6] J. Bebenorf, U. Müller, *Z. Naturforsch. B* **1990**, 45, 927–930.
- [7] M.-J. Crawford, T. M. Klapötke, *Internet J. Vib. Spec.* **1999/2000**, 3(6), 3 (<http://www.ijvs.com>).
- [8] W. Beck, W. Becker, K. F. Chew, W. Derbyshire, N. Logan, D. M. Revitt, D. B. Sowerby, *J. Chem. Soc. Dalton Trans.* **1972**, 7, 245–247.
- [9] a) G. Baliman, P. S. Pregosin, *J. Magn. Reson.* **1977**, 26, 283; b) C. Brevard, P. Granger, *Handbook of High Resolution Multinuclear NMR*, Wiley, New York, **1981**, pp. 136–137; c) H. P. A. Mercier, J. C. P. Sanders, G. J. Schrobilgen, *J. Am. Chem. Soc.* **1994**, 116, 2921; d) M. F. A. Dove, J. C. P. Sanders, E. L. Jones, M. J. Parkin, *J. Chem. Soc. Chem. Commun.* **1984**, 1578.
- [10] W. Beck, T. M. Klapötke, J. Knizek, H. Nöth, T. Schütt, *Eur. J. Inorg. Chem.* **1999**, 523–526.
- [11] “Covalent Inorganic Nonmetal Azides”: I. C. Tornieporth-Oetting, T. M. Klapötke in *Combustion Efficiency and Air Quality* (Eds.: I. Hargittai, T. Vidoczy), Plenum, New York, **1995**, p. 51.
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The specific preparation of bioconjugates, which is, in general, the covalent linking of a low molecular weight compound to a protein or an oligonucleotide or bond formation between two proteins, can be regarded as one of the most important methods in modern biochemistry.^[1] Conjugates which are constructed out of a protein or oligonucleotide (for example, an enzyme or a DNA fragment) and a pigment or a radioactive compound are widely used for

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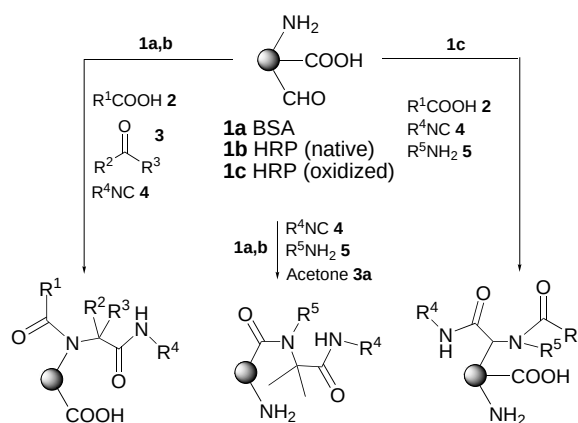
[**] This work was financially supported by the Fonds der Chemischen Industrie and Aventis Research & Technologies, Frankfurt am Main.

analytical and histological purposes or as devices for genetics. Antibody–enzyme conjugates are important and indispensable for almost all immunological tests. Similarly, bioconjugates, like neoglycoconjugates, which are prepared from a saccharide and a protein, or conjugates constructed from a pharmaceutically active substance, are applied as vaccines and therapeutic tools in medicine. The potential applications of bioconjugates in all these fields are legion. Although the repertory of established procedures for the preparation of bioconjugates is quite remarkable, it is still desirable to develop new methods. An overview of current procedures for the preparation of bioconjugates can be found in ref. [2].

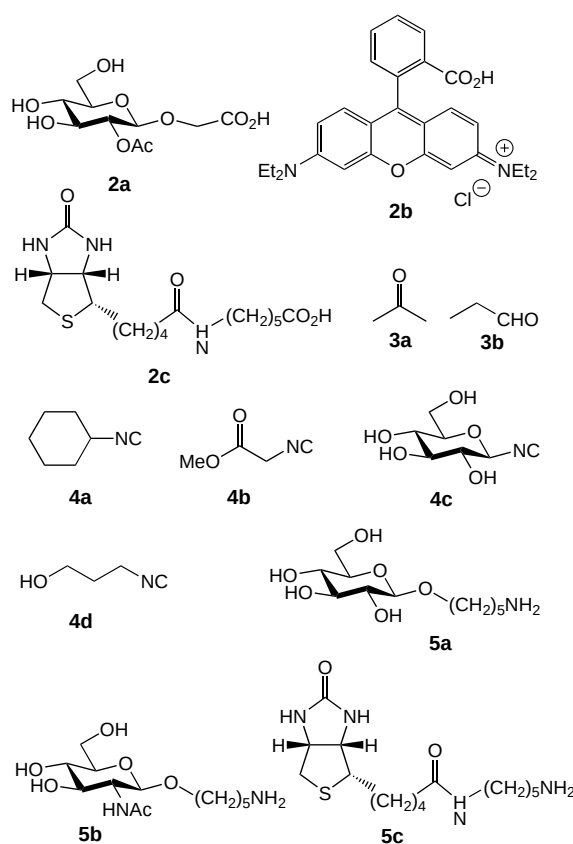
Usually, the preparation of bioconjugates from proteins is based on condensation or addition of free amino, carboxylic, thio, or aldehyde functional groups of the protein to low molecular weight compounds that also contain reactive groups. Different condensation reagents or bifunctional coupling reagents can be used for this purpose and the established standards are quite high. However, the possibilities to couple a biopolymer to several low molecular weight compounds simultaneously and in a well defined ratio is restricted. Multiple low molecular weight compounds may be conjugated to a protein by standard procedures but the exact stoichiometric ratio cannot be controlled. On the other hand, it would be highly desirable to have procedures which allow for the conjugation of multiple reporter groups to a protein in a well defined ratio.^[3] Our recent work on Ugi reactions of isocyanoglycosides^[4, 5] prompted us to investigate if such a multiple compound reaction can be used for the preparation of bioconjugates. In general, the Ugi reaction combines an isonitrile, an amine, a carbonyl compound, and a carboxylic acid in a fixed ratio to afford a peptide.^[6, 7] To the best of our knowledge, Ugi reactions have so far been solely applied to the immobilisation of proteins by condensation of a protein with an isonitrile-modified solid support.^[8]

In order to apply the Ugi reaction to the preparation of bioconjugates of the aforementioned type we reacted two proteins, bovine serum albumin (BSA) **1a** and horseradish peroxidase (HRP) **1b,c**, under various conditions with different carboxylic acids **2**, carbonyl compounds **3**, isocyanides **4**, and amines **5**.^[9] The free functional groups of both BSA and HRP were used in Ugi reactions, either as amine groups or carboxylic acids (for native proteins **1a,b**) or as aldehyde groups (for NaIO₄ oxidized HRP **1c**), to afford bioconjugates as shown in Scheme 1.

As the representative components for these Ugi reactions we chose the compounds shown in Scheme 2. In particular, glycoside **2a**, rhodamine B **2b**, and the biotin derivative **2c** were chosen as the carboxylic acids.^[11] The latter was chosen in order to demonstrate the preparation of the important class of biotinylated proteins^[12] by this method. As carbonyl compounds, we used acetone **3a** and propionic aldehyde **3b** although other carbonyl compounds can also be applied as long as no denaturation of the protein occurs. As isocyanide components^[13] for the Ugi reactions, we chose **4a** and **4b** for heterogeneous couplings and the water soluble isocyanides **4c** and **4d** for homogeneous ones. Finally, several amines **5**^[14] were used in order to demonstrate the flexibility of this method.



Scheme 1. Principle for the formation of bioconjugates from proteins **1** with low molecular weight compounds **2–5** through Ugi reactions.



Scheme 2. The low molecular weight compounds **2–5** which were used.

Table 1 shows the dependence of conjugate formation by Ugi reactions of BSA **1a** with the components **3a**, **4a**, and **5a** on the buffer, the buffer's pH value, and the concentration of the components. The obtained epitope density of the prepared conjugates (molar ratio of low molecular weight compounds and the respective protein) was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS).^[15] Optimal conditions for high epitope densities in the bioconjugates turned out to be 0.01–0.1M phosphate buffer, pH 7.5, with an expected proportionality between epitope density and the initial ratio of **1a** and **5a**. In tris/HCl buffer, as well as at lower pH values, the obtained

Table 1. Dependence of conjugate formation between **1a** and **3a–5a** on buffer, pH value, and concentration of the components.

buffer	pH value	mol. ratio 5a/1a	epitope density
0.01M phosphate buffer	6.0	100/1	1.9
0.01M phosphate buffer	7.5	100/1	4.0
0.1M phosphate buffer	7.5	100/1	1.2
0.1M phosphate buffer	7.5	1000/1	6.2
0.1M phosphate buffer	7.5	2000/1	6.5
0.1M phosphate buffer	7.5	3000/1	7.7
0.1M tris/HCl buffer	7.5	100/1	1.0
0.1M tris/HCl buffer	7.5	1000/1	1.6

epitope densities were significantly lower because of probable unselective reactions of the components with the buffer or because of simple hydrolysis of the isonitrile.

Table 2 summarizes the results for the conjugations of the proteins **1** with the components from Scheme 2. Once again, the epitope density was measured by MALDI-TOF MS. Furthermore, for conjugates with rhodamine B **2b**, the

Table 2. Preparation of bioconjugates from proteins **1** by an Ugi reaction with components **2–5** in 0.1M phosphate buffer, pH 7.5 at 25 °C.

Entry	protein 1	mol. ratio 2–5/1	components 2–5	<i>t</i> [d]	epitope density	yield [%]	activity ^[a] [%]
1	1a	1000/1	3a, 4b, 5a	2	3.4	100	–
2	1a	3000/1	3a, 4a, 5b	2	9.4	100	–
3	1a	4000/1	2a, 3a, 4a	2	9.9	100	–
4	1a	3000/1	2b, 3a, 4a	1	4.1	49	–
				2	4.8	8.3	–
5	1a	3000/1	2b, 3a, 4c	2	0.5	100	–
				4	1.0	80	–
6	1a	1000/1	2c, 3a, 4d	3	0.0	n.d. ^[b]	–
7	1b	100/1	2c, 3a, 4d	2	0.0	n.d. ^[b]	88
8	1b	100/1	3a, 4d, 5c	2	0.0	n.d. ^[b]	95
9	1b	1000/1	2c, 3b, 4a	1	3.0	75	62
10	1c	1000/1	2b, 4a, 5c	1	3.5	11	93
11	1c	2000/1	2c, 4a, 5c	1	1.0	67	157

[a] Relative to the original activity of the native enzyme. [b] Not determined.

epitope density was also determined photometrically by measuring the extinction of an aqueous solution of the conjugates at 575 nm. Similarly, the epitope densities of conjugates with biotin derivatives **2c** and **5c** were determined by a 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA)–avidine test^[16] and by a streptavidine enzyme-linked immunosorbent assay (ELISA).^[17] In some cases, a photometric determination of the yield of conjugate was performed and, in the case of HRP, determination of the remaining enzymatic activity was also measured by a 2,2'-azino(3-ethyl-benzylthiazoline-6-sulfonic acid) (ABTS) test.^[17]

For reactions of **1a** with isonitriles **4a–c** (Table 2, entries 1–5) an average epitope density of about 1–10 was obtained. This is in the range for optimal epitope densities of bioconjugates.^[18] The Ugi reactions with BSA **1a** as the carboxylic acid component (Table 2, entries 1, 2) and those with BSA **1a** as the amine component (Table 2, entries 3–5) afforded comparable results. However, in all cases a deposition of denaturated protein appeared after three to four days due to probable cross linking of the proteins through an Ugi

reaction. This was significant for the conjugation of BSA and **4a** with rhodamine B **2b** as the carbonyl component (Table 2, entry 4). After two days of reaction time, only 8.3% of the respective conjugate was obtained without any improvement of the average epitope density. In contrast, the water soluble isonitrile **4c** (Table 2, entry 5) did not show significant denaturation of the protein even after four days. No formation of conjugates by Ugi reaction could be detected for the reactive water soluble isonitrile **4d** (Table 2, entries 6–8). Neither with BSA **1a** nor with HRP **1b** could any conjugates be detected by mass spectrometry. Thus, the determination of the protein yield was not performed in these cases. The failure of the conjugation in this case can be explained by a significantly higher reactivity of **4d** relative to **4c** which leads to a rather unselective reaction of the isonitrile with the protein. This was also evident from the depletion of the enzymatic activity of **1b** (Table 2, entries 7, 8) without any formation of conjugates by an Ugi reaction. Similar experiments for Ugi reactions of bovine insulin, which showed a better resolution in MALDI-TOF MS than BSA or HRP, revealed that adducts from protein and isonitrile were initially formed in a fast reaction followed by slow hydrolysis during three to four days. Reaction of HRP as the amine or the carbonyl component with isonitrile **4a** (Table 2, entries 9–11) afforded the respective conjugates with average epitope densities of 1–3 in 11–75% yield. Although the reactivity of **4a** and **4d** can be judged as almost equal, the Ugi reaction of **4a** is heterogeneous and thus, proceeds without any unselective blocking of the functional groups of the protein. The retained enzymatic activity of the conjugates with HRP (Table 2, entries 9, 10) was found to be in the range of 62–95% of the initial activity. Thus, the Ugi reaction did not cause any dramatic loss in enzymatic activity. Furthermore, in one case (Table 2, entry 11) the conjugation even resulted in an increase of the enzymatic activity during the Ugi reaction.

In summary, the examples presented here show that Ugi reactions are well suited for the preparation of bioconjugates with up to three components in an exactly defined ratio, provided that careful optimisation of the reaction conditions is done.

Experimental Section

Reaction no. 3 from Table 2: An aqueous solution of acetone (5%, 89.6 µL) and 2 drops of **4a** were added to a solution of BSA (**1a**, *M* = 66431 Da, 1.0 mg, 15 nmol) and **2a**^[11] (19.1 mg, 60 mmol) in 0.1M phosphate buffer (3 mL, pH 7.5) at 25 °C. The mixture was set aside for 2 d and shaken occasionally. Aliquots (500 µL) were withdrawn, centrifuged, and dialysed with water. The molecular weight of the conjugate was determined by MALDI-TOF MS: *M* = 70975 Da; average epitope density = 9.9.

Reaction no. 10 from Table 2: 2 drops of **4a** were added to a solution of HRP freshly oxidized with NaIO₄^[10] **1c** (*M* = 43 183 Da, 1.8 mg, 45 nmol), **2b** (21.6 mg, 45 mmol), and **5c** (14.8 mg, 45 mmol) in 0.1M phosphate buffer (4 mL, pH 7.5) at 25 °C. The mixture was set aside for 1 d and shaken occasionally, centrifuged, and dialysed with 0.01M phosphate buffer pH 7.5. The amount of protein was determined by measuring the extinction at 280 nm: Yield 4.8 nmol (11%); remaining enzymatic activity of the conjugate (ABTS test^[17]) 93%. The molecular weight of the conjugate was determined by MALDI-TOF MS: *M* = 46 118 Da; average epitope density was 3.4. The amount of **2b** in the conjugate was determined

photometrically as 3.5 by measuring the extinction at 575 nm. The biotin density was determined to be 3.3 by a HABA–avidine test.^[16]

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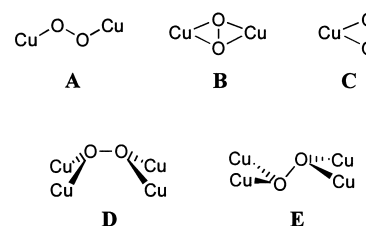
- [1] *Bioconjugate Chemistry* (Ed.: C. F. Mears), American Chemical Society, New York, **1999**.
- [2] a) G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, **1998**; b) *Neoglycoconjugates, Preparation and Applications* (Eds.: Y. C. Lee, R. T. Lee), Academic Press, San Diego, **1994**.
- [3] a) B. E. Rothenberg, B. K. Hayes, D. Toomre, A. E. Manzi, A. Varki, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11939–11943; b) D. K. Toomre, A. Varki, *Glycobiology* **1994**, *4*, 653–663.
- [4] T. Ziegler, R. Schlömer, C. Koch, *Tetrahedron Lett.* **1998**, *39*, 5957–5960.
- [5] T. Ziegler, H.-J. Kaisers, R. Schlömer, C. Koch, *Tetrahedron* **1999**, *55*, 8397–8408.
- [6] I. Ugi, *Isonitrile Chemistry*, Academic Press, New York, **1971**.
- [7] a) I. Ugi, A. Dömling, B. Gruber, M. Almstetter, *Croatia Chim. Acta* **1997**, *70*, 631–647; b) I. Ugi, A. Dömling, W. Hörl, *Endeavour* **1994**, *18*, 115–122.
- [8] a) M. Marek, J. Jary, O. Valentova, Z. Vodrazka, *Czech. Biotechnol. Lett.* **1983**, *5*, 653–658; b) M. Marek, O. Valentova, J. Kas, *Biotechnol. Bioeng.* **1984**, *26*, 1223–1226; c) R. Axen, P. Vretblad, Porath, *Acta Chem. Scand.* **1971**, *25*, 1129–1132.
- [9] T. Ziegler, S. Gerling, M. Lang, G. Kretschmar (Aventis Research & Technologies), patent registration, **1999**.
- [10] P. K. Nakane, A. Kawaoi, *J. Histochem. Cytochem.* **1974**, *22*, 1084–1091.
- [11] Compound **2a** was prepared from *n*-butyloxycarbonylmethyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (B. Helferich, K. F. Wedemeyer, *Liebigs Ann.* **1949**, 563, 139–145) by saponification with 1M aqueous KOH in MeOH (24 h, 25 °C) and was obtained in 99% yield. $[\alpha]_D^{20} = -13.6$ ($c = 1.0$ in H₂O); ¹³C NMR (D₂O): $\delta = 181.8$ (CO), 177.7 (CO₂), 102.7 (C-1), 76.3, 75.9, 73.5 (C-3, C-4, C-5), 69.9 (C-2), 68.9 (OCH₂), 61.0 (C-6), 23.6 (CH₃). Compounds **2b** and **2c** were purchased from Pierce.
- [12] M. Wilchek, E. A. Bayer, *Anal. Biochem.* **1988**, *171*, 1–32.
- [13] Compounds **4a** and **4b** were purchased from Fluka. Compound **4c** was prepared from 1-isocyano-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranose^[5] by Zemplén saponification (cat. NaOMe in MeOH) and used without further purification. Compound **4d** was prepared from *N*-(3-hydroxypropyl)formamide (L. Goldstein, A. Niv, *Appl. Biochem. Biotechnol.* **1993**, *42*, 19–35) with diphosgene according to W. P. Fehlhammer, K. Bartel, B. Weinberger, U. Plaia, *Chem. Ber.* **1985**, *118*, 2220–2234.
- [14] For the preparation of compound **5a**, see: E. Eckhardt, T. Ziegler, *Carbohydr. Res.* **1994**, *264*, 253–269. Compound **5b** was prepared by hydrogenolysis (Pd/C) from 5-benzyloxycarbonylpentyl-2-acetamido-2-deoxy- β -D-glucopyranoside (T. Ziegler, *Carbohydr. Res.* **1994**, *262*, 195–212) and used without any further purification. Compound **5c** was purchased from Pierce.
- [15] The determination of the epitope density was performed with a Biflex III (Bruker) MALDI-TOF mass spectrometer with sinapinic acid as a matrix and by calibration of the spectrometer with BSA (66431 Da) and bovine insulin (5734.6 Da). The determination of the average epitope density was done according to the formula: (mass of conjugate – mass of native protein)/(mass of linked remainder).
- [16] N. M. Green, *Biochem. J.* **1965**, *94*, 23c–24c.
- [17] The ABTS test for HRP (A. Stutowicz, *Anal. Biochem.* **1984**, *138*, 86) was performed on IN-5008c (Innova) streptavidine microtiter plates using an SLT Spectrafluor plus (Tecan) ELISA reader at 405 nm.
- [18] B. F. Erlanger, *Methods Enzymol.* **1980**, *70*, 85–103.

μ_4 -Peroxo versus Bis(μ_2 -Hydroxo) Cores in Structurally Analogous Tetracopper(II) Complexes**

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In biological systems, nature often uses the combined redox power of several adjacent metal ions for mediating multi-electron redox transformations. In particular, oligonuclear copper enzymes play a pivotal role in the reversible binding and in the activation of O₂ for oxidation and oxygenation reactions, respectively,^[1]—well known representatives include the O₂ carrier protein hemocyanin^[2] as well as the enzymes catechol oxidase and tyrosinase.^[3] In view of the interest in the use of O₂ in catalytic oxidation reactions, extensive research has been devoted to the search of stable copper–dioxygen adducts^[4, 5] to gain insight into the binding of O₂ at oligonuclear copper sites and to understand the function of such metalloenzymes.^[6] In this context, structural investigations of copper–peroxo systems are of prime importance.

To date, copper–dioxygen adducts characterized by X-ray crystallography are a set comprised of a complex with *trans*- μ -1,2-peroxo bridge (type **A**),^[7] two model complexes for the O₂-binding protein hemocyanin that feature a μ - η^2 : η^2 -peroxo group (**B**),^[5, 8] and a mononuclear η^2 -superoxo copper(II) compound (**C**).^[9] In addition, a μ_4 -peroxo coordination mode



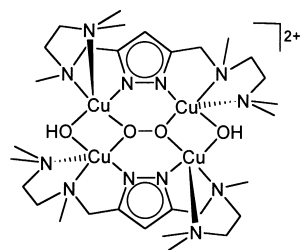
unique in copper chemistry, in which a peroxo group spans four copper(II) ions (**D**), has been described by Krebs et al.^[10] Herein, we report a novel example of such unusual μ_4 -peroxo coordination (**E**), as well as the X-ray crystallographic characterization of a structurally analogous complex in which the O–O linkage is formally cleaved and replaced by two OH units, while at the same time the overall tetranuclear framework is fully conserved.

The new copper complexes are based on a multidentate pyrazolate ligand L[–].^[11] Ligands of this type have proven suitable to hold two metal ions in close proximity and to therefore enable cooperative action of the two metal centers.^[12] The metal–metal separation can be tuned by the lengths of the chelating side arms attached to the heterocycle: In complexes of L[–] bearing “short” side arms, long metal–metal distances are enforced and small ions like OH[–] are

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prevented from occupying a secondary bridging position.^[13] For the O₂ binding by a dicopper(I) complex with L[−], an initial *cis*-μ-1,2-peroxo coordination is predicted from molecular models.



1

Diffusion of O₂ into a solution of L[−]/2[Cu(MeCN)₄]PF₆ in EtCN that has previously been layered with Et₂O at −80 °C affords dark green crystals of the peroxo complex 1·2PF₆. The molecular structure of 1 has been resolved crystallographically^[14] and is depicted in Figure 1. Figure 2 shows details of its central coordination core.

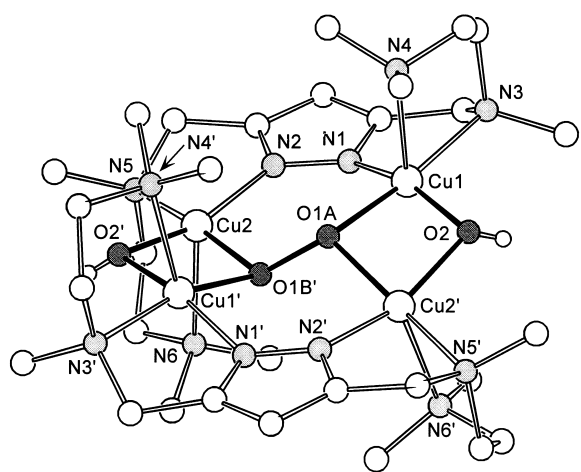


Figure 1. Molecular structure of 1 in the solid state. Only one position of the disordered peroxo group is shown. Selected distances [Å] and angles [°]: Cu1–N1 1.920(3), Cu1–N3 2.149(3), Cu1–N4 2.323(4), Cu1–O1A 2.040(4), Cu1'–O1B' 1.950(4), Cu1–O2 1.934(3), Cu2–N2 1.911(2), Cu2–N5 2.153(3), Cu2–N6 2.351(3), Cu2–O1B' 2.015(4), Cu2'–O1A' 1.968(4), Cu2–O2' 1.919(3), N1–N2 1.357(3), O1A–O1B' 1.497(5), Cu1...Cu2 3.902, Cu1...Cu2' 2.986, O2...O2' 3.013, Cu1–O2–Cu2' 101.6(2), Cu1–O1A–Cu2' 96.3(2), Cu1–O1A–O1B' 121.6(3), Cu2'–O1A–O1B' 115.4(2), Cu2–O1B'–Cu1' 97.7(2), Cu2–O1B'–O1A 123.8(3), Cu1'–O1B'–O1A 113.0(2).

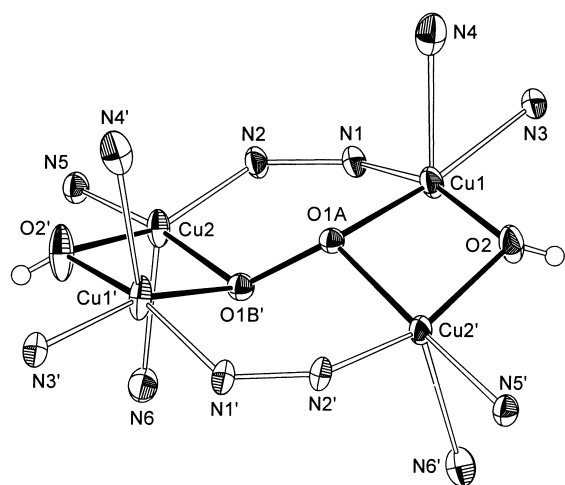


Figure 2. Central coordination core of 1.

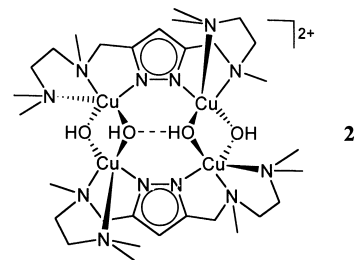
Compound 1 is a tetranuclear complex with a central O₂^{2−} peroxo ligand. As expected from the geometric constraints imposed by the ligand matrix L[−], the diatomic peroxo unit bridges the copper ions Cu1 and Cu2 of a bimetallic LCu₂ entity in the *cis*-μ-1,2-fashion. However, this peroxo binding mode is stabilized by a second dicopper(II) moiety and leads to the formation of an overall tetranuclear framework with a μ₄-coordination of the O₂^{2−} unit in 1. The two shorter edges of the nonplanar rectangle of four copper(II) ions (dihedral angle 23.6°) are each spanned by an additional hydroxide, the H atom of which could be located in a bridge to the PF₆[−] counteranion. The accompanying O–H stretching mode gives rise to a sharp band at 3641 cm^{−1} in the IR spectrum.

The copper(II) ions in 1 are nested in Jahn–Teller-distorted square–pyramidal environments, where the terminal N-donors of the L[−] ligand side arms (N4 and N6) are found in the apical positions at expected, albeit long, Cu–N distances (2.323(4) and 2.351(3) Å, respectively). The center of the tetranuclear cation is located on a two-fold crystallographic axis and the peroxo ligand is disordered over two positions. The O1A–O1B' bond length (1.497(5) Å) lies well within the range characteristic for an O₂^{2−} ligand, although it is the longest O–O distance of all copper–peroxo complexes characterized crystallographically to date. The geometry of the {Cu₄(O₂)} core in 1 differs from those of the related μ₄-peroxo tetracopper(II) complex reported by Krebs et al.^[10] In that compound, the peroxo ligand caps a nearly planar Cu₄ rectangle and thus adapts an eclipsed orientation with respect to the O–O bond (*cis*-μ₄-peroxo, type D). Such features have also been found in three other complexes (of Fe, Mo, and Sb) which show μ₄-coordination of a peroxo ligand.^[15] In addition, planar μ₄-O₂^{2−} coordination has been observed in the case of a hexanuclear iron complex.^[16] In contrast, the oxygen atoms of the O₂^{2−} group in 1 are situated on different sides of the Cu₄ framework and the peroxo group is surrounded by the metal centers in an approximately *trans*-staggered arrangement (*trans*-μ₄-peroxo, type E).

The peroxo complex 1·2PF₆ is stable at room temperature. Its composition is further confirmed by mass spectrometry: The fast atom bombardment (FAB) mass spectrum (matrix = nitrobenzylalcohol) shows a signal at *m/z* 1055 with an isotopic distribution pattern characteristic for the [L₂Cu₄(O₂)(OH)₂(PF₆)₂]⁺ ion.

When a solution of 1·2PF₆ exposed to air is stored at room temperature some, blue crystals of a second compound 2·2PF₆ form over a few days. The molecular structure of 2, as determined by X-ray crystallography, is depicted in Figure 3.^[14] Figure 4 gives a detailed view of its central coordination unit.

In complex 2, the tetranuclear core, built of two bimetallic LCu₂ fragments and two linking OH bridges (O1 and O4) as is found in 1, is fully conserved. However, two further hydroxo bridges (O2 and O3), instead of the central peroxo ligand, are present in 2. The H atoms of all four hydroxo bridges could be



2

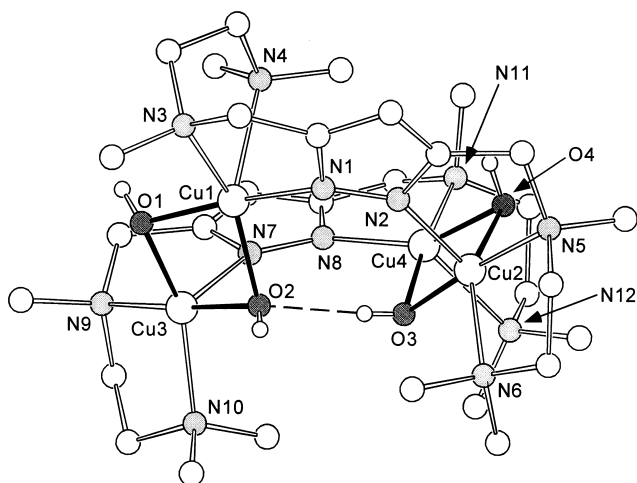


Figure 3. Molecular structure of **2** in the solid state. Selected distances [Å] and angles [°]: Cu1–N1 1.939(4), Cu1–N3 2.138(4), Cu1–N4 2.384(4), Cu1–O1 1.928(4), Cu1–O2 1.989(4), Cu2–N2 2.142(4), Cu2–N5 2.071(4), Cu2–N6 2.116(4), Cu2–O3 1.917(4), Cu2–O4 1.995(3), Cu3–N7 2.197(4), Cu3–N9 2.065(4), Cu3–N10 2.069(4), Cu3–O1 1.954(4), Cu3–O2 1.937(4), Cu4–N8 1.980(4), Cu4–N11 2.081(4), Cu4–N12 2.323(5), Cu4–O3 1.915(4), Cu4–O4 1.993(3), N1–N2 1.364(5), N7–N8 1.365(5), Cu1...Cu2 4.348, Cu3...Cu4 4.530, Cu1...Cu3 2.925, Cu2...Cu4 2.925, O2...O3 2.747; Cu1–O1–Cu3 97.8(2), Cu1–O2–Cu3 96.3(2), Cu2–O3–Cu4 99.5(2), Cu2–O4–Cu4 94.4(2).

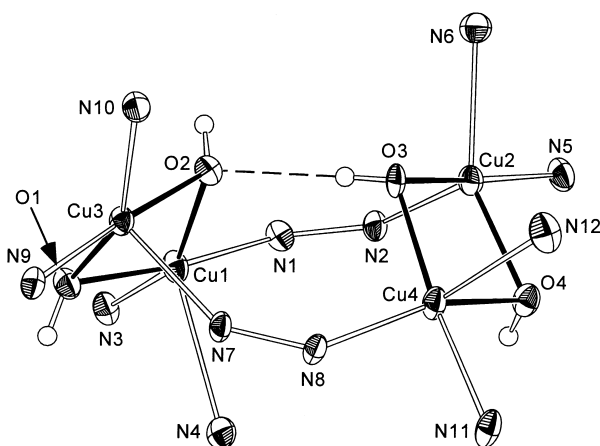


Figure 4. Central coordination core of **2**.

located: The H atom bound to O3 forms a short hydrogen bridge between the central O atoms ($d(\text{O2} \cdots \text{O3}) = 2.747 \text{ Å}$), all other oxygen-bound H atoms are found in bridges to PF_6^- counteranions or to a EtCN solvent molecule. Three of the four terminal N donor atoms of the chelating side arms of the ligands **L** are located on one side of the Cu_4 framework (N6, N10, and N12), thus shielding three quadrants of the central (H)O...HO-group surroundings. Only atom N4 is situated on the opposite side of the Cu_4 framework and leaves sufficient space for the H atom bound to O2. The IR spectrum of **2** shows two bands in the range typical for the O–H stretch ($3644, 3589 \text{ cm}^{-1}$).

The Cu_4 core, which consists of two LCu_2 fragments, is present in both **1** and **2**. The tetranuclear framework thereby allows for variability of the central O–O distance (1.497 Å for the peroxo group in **1** versus 2.747 Å for the (H)O...HO bridge in **2**) in two ways. First, different Cu...Cu separations are adopted in each LCu_2 bimetallic moiety (3.902 Å in **1**

versus $4.348/4.530 \text{ Å}$ in **2**) and, second, the planes of the two Cu_2O_2 four-membered rings are tilted severely with respect to each other in **2** (99.4°). From a purely structural point of view, a mutual transformation of the $\{\text{Cu}_4(\text{O}_2)\}$ and $\{\text{Cu}_4(\text{OH})_2\}$ units of such tetrametallic cores should obviously be feasible; the formation of the central $\{\text{Cu}_4(\text{OH})_2\}$ fragment in **2** formally reflects a two electron reduction and concomitant double protonation of the $\{\text{Cu}_4(\text{O}_2)\}$ fragment in **1**. Complex **2** thus indicates to what the reductive cleavage of dioxygen, after it is first captured as a μ_4 -peroxo ligand on a Cu_4 surface, might eventually lead. The question whether compound **2** results from reductive cleavage of the peroxo group in **1** or whether **2** forms by a different pathway is presently under investigation.

Experimental Section

1: $\text{HL}^{[1]}$ (0.18 g, 0.61 mmol) was dissolved in THF (20 mL) and was deprotonated by addition of $[\text{NBu}_4]\text{OH}$ (0.61 mL, 1.0 M in methanol) at 0°C . After evaporation of all volatile material under reduced pressure, the residue was extracted into EtCN (20 mL). A solution of $[\text{Cu}(\text{MeCN})_4]\text{PF}_6$ (0.45 g, 1.21 mmol) in EtCN (10 mL) was then added under argon at -80°C . After stirring for 10 min at -80°C , the yellow reaction mixture was layered with Et_2O (100 mL) and air was let to diffuse into the remaining space of the Schlenk tube. The closed Schlenk tube was then stored -80°C . The solution gradually turned green and, after two weeks, a precipitate containing small crystals of **1**· 2PF_6 formed. This was filtered and dried at room temperature (yield: 0.14 g, 0.12 mmol, 38%). Recrystallization was achieved by layering an EtCN solution of the product with Et_2O . UV/Vis (EtCN) $\lambda_{\text{max}}(\epsilon)$: 360 nm (3100), 631 nm ($260 \text{ M}^{-1}\text{cm}^{-1}/\text{Cu}_4$); elemental analysis: calcd. for $\text{C}_{30}\text{H}_{64}\text{Cu}_4\text{F}_{12}\text{N}_{12}\text{O}_4\text{P}_2$ (1201.0): C 30.00, H 5.37, N 13.99; found: C 30.53, H 5.40, N 13.54. When the solution containing **1**· 2PF_6 was stored at room temperature, blue crystals of **2**· 2PF_6 ·EtCN gradually formed.

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- [1] See, for example: a) W. Kaim, J. Rall, *Angew. Chem.* **1996**, *108*, 47–64; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 43–60; b) E. I. Solomon, U. M. Sundaram, T. E. Machonkin, *Chem. Rev.* **1996**, *96*, 2563–2605.
- [2] K. A. Magnus, H. Ton-That, J. E. Carpenter, *Chem. Rev.* **1994**, *94*, 727–735.
- [3] T. Klabunde, C. Eicken, J. C. Sacchettini, B. Krebs, *Nat. Struct. Biol.* **1998**, *5*, 1084–1090.
- [4] a) J. E. Bol, W. L. Driessen, R. Y. N. Ho, B. Maase, L. Que Jr., J. Reedijk, *Angew. Chem.* **1997**, *109*, 1022–1025; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 998–1000; b) H. Börzel, P. Comba, C. Katsichtis, W. Kiefer, A. Lienke, V. Nagel, H. Pritzkow, *Chem. Eur. J.* **1999**, *5*, 1716–1721.
- [5] M. Koder, K. Katayama, Y. Tachi, K. Kano, S. Hirota, S. Fujinami, M. Suzuki, *J. Am. Chem. Soc.* **1999**, *121*, 11006–11007.
- [6] a) Z. Tyeklár, K. D. Karlin, *Acc. Chem. Res.* **1989**, *22*, 241–248; b) N. Kitajima, Y. Moro-Oka, *Chem. Rev.* **1994**, *94*, 737–757; c) K. D. Karlin, S. Kaderli, A. D. Zuberbühler, *Acc. Chem. Res.* **1997**, *30*, 139–147; d) W. B. Tolman, *Acc. Chem. Res.* **1997**, *30*, 227–237; e) P. L. Holland, W. B. Tolman, *Coord. Chem. Rev.* **1999**, *190–192*, 855–869; f) A. P. Cole, D. E. Root, P. Mukherjee, E. I. Solomon, T. D. P. Stack, *Science* **1996**, *273*, 1848–1850.
- [7] R. R. Jacobsen, Z. Tyeklár, A. Farooq, K. D. Karlin, S. Liu, J. Zubieta, *J. Am. Chem. Soc.* **1988**, *110*, 3690–3692.
- [8] a) N. Kitajima, K. Fujisawa, Y. Moro-Oka, K. Toriumi, *J. Am. Chem. Soc.* **1989**, *111*, 8975–8976; b) N. Kitajima, K. Fujisawa, C. Fujimoto, Y. Moro-Oka, S. Hashimoto, T. Kitagawa, K. Toriumi, K. Tatsumi, A. Nakamura, *J. Am. Chem. Soc.* **1992**, *114*, 1277–1291.
- [9] K. Fujisawa, M. Tanaka, Y. Moro-Oka, N. Kitajima, *J. Am. Chem. Soc.* **1994**, *116*, 12079–12080.
- [10] a) J. Reim, B. Krebs, *Angew. Chem.* **1994**, *106*, 2040–2041; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1969–1971; b) J. Reim, R. Werner, W. Haase, B. Krebs, *Chem. Eur. J.* **1998**, *4*, 289–298.

- [11] F. Meyer, U. Ruschewitz, P. Schober, B. Antelmann, L. Zsolnai, *J. Chem. Soc. Dalton Trans.* **1998**, 1181–1186.
- [12] a) F. Meyer, H. Pritzkow, *Chem. Commun.* **1998**, 1555–1556; b) F. Meyer, E. Kaifer, P. Kircher, K. Heinze, H. Pritzkow, *Chem. Eur. J.* **1999**, 5, 1617–1630.
- [13] a) F. Meyer, K. Heinze, B. Nuber, L. Zsolnai, *J. Chem. Soc. Dalton Trans.* **1998**, 207–213; b) F. Meyer, P. Rutsch, *Chem. Commun.* **1998**, 1037–1038.
- [14] Crystal data of $1 \cdot 2\text{PF}_6$ ($\text{C}_{30}\text{H}_{64}\text{Cu}_4\text{F}_{12}\text{N}_{12}\text{O}_4\text{P}_2$, $M = 1201.0$): monoclinic, space group $C2/c$, $a = 21.309(1)$, $b = 11.421(1)$, $c = 19.174(1)$ Å, $\beta = 97.562(1)^\circ$, $V = 4625.8(5)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.725$ g cm⁻³, $\mu(\text{MoK}\alpha) = 1.981$ mm⁻¹, $2\theta_{\text{max}} = 52.7^\circ$, 4720 independent reflections, 3342 observed with $I > 2\sigma(I)$, 417 refined parameters; all H atoms have been localized and refined isotropically, $R1 = 0.037$ ($I > 2\sigma(I)$), $wR2 = 0.088$ (all data), GOF = 0.924 (refinement on F^2), max./min. residual electron density 0.661/–0.300 e Å⁻³. Crystal data of $2 \cdot 2\text{PF}_6$ ($\text{C}_{30}\text{H}_{66}\text{Cu}_4\text{F}_{12}\text{N}_{12}\text{O}_4\text{P}_2 \cdot \text{EtCN}$, $M = 1258.1$): monoclinic, space group $P2_1/c$, $a = 11.985(1)$, $b = 26.728(3)$, $c = 16.114(2)$ Å, $\beta = 100.505(2)^\circ$, $V = 5075.3(8)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.647$ g cm⁻³, $\mu(\text{MoK}\alpha) = 1.810$ mm⁻¹, $2\theta_{\text{max}} = 49.4^\circ$, 8656 independent reflections, 5111 observed with $I > 2\sigma(I)$, 644 refined parameters; the O-bound H atoms have been localized and refined isotropically, all other H atoms have been included in calculated positions, $R1 = 0.046$ ($I > 2\sigma(I)$), $wR2 = 0.108$ (all data), GOF = 0.878 (refinement on F^2), max./min. residual electron density 0.892/–0.434 e Å⁻³. Data have been collected on a Bruker AXS CCD diffractometer, MoK α radiation ($\lambda = 0.71073$ Å), $T = 173$ K, ω scan, structures have been solved using direct methods (SHELXS-86 and SHELXL-97). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-138151 ($1 \cdot (\text{PF}_6)_2$) and CCDC-138152 ($2 \cdot (\text{PF}_6)_2$). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [15] a) R. Stomberg, L. Trysberg, I. Larking, *Acta Chem. Scand.* **1970**, 24, 2678–2679; b) I. Shweky, L. E. Pence, G. C. Papaefthymiou, R. Sessoli, J. W. Yun, A. Bino, S. J. Lippard, *J. Am. Chem. Soc.* **1997**, 119, 1027–1042; c) H. J. Breunig, T. Krüger, E. Lork, *Angew. Chem.* **1997**, 109, 654–655; *Angew. Chem. Int. Ed. Engl.* **1997**, 36, 615–617.
- [16] W. Micklitz, S. G. Bott, J. G. Bentsen, S. J. Lippard, *J. Am. Chem. Soc.* **1989**, 111, 372–374.

The Use of Immobilized Templates—A New Approach in Molecular Imprinting

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Molecular imprinting is a technique that allows specific recognition sites for target molecules to be formed in synthetic polymers through the use of templates. Customary

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protocols for molecularly imprinted polymers (MIPs) are based on one of two distinct approaches: the “covalent approach” and the “noncovalent approach”. The covalent approach was pioneered by the group of Wulff^[1] and uses covalent bonds between the imprint molecules and functional monomers. The other approach, which is based on non-covalent interactions, was introduced by Mosbach and co-workers.^[2] More recently, a hybrid system was proposed that comprises a covalent imprinting step and subsequent rebinding of the imprint molecule by noncovalent interactions.^[3]

Molecular imprinting of small molecules has until now only been done with the template (imprint) molecules in free solution. These polymers will be referred to here as classical MIPs. Herein we present a novel imprinting method based on oriented immobilization of the template onto a solid support. After polymerization, the support is dissolved and thus sacrificed (Figure 1). Our aim is to demonstrate the feasibility

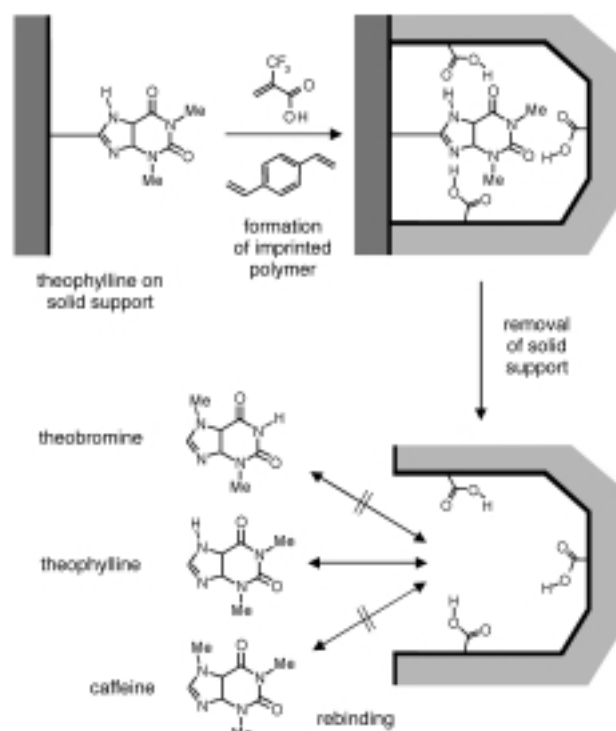


Figure 1. Schematic representation of the molecular-imprinting approach employing immobilized templates and a sacrificial solid support.

of this approach, which extends molecular imprinting technology to a new dimension. The bronchodilating drug theophylline was investigated as a model template by immobilizing its 8-carboxypropyl derivative onto a support of aminopropyl-derivatized silica gel. Immobilization of 8-carboxypropyltheophylline was achieved through the formation of amide bonds by using carbodiimide chemistry adapted from protocols used for solid-phase peptide synthesis.^[4] The amount of template coupled was determined at the end of the reaction by elemental analysis (Table 1). Approximately 75% of the free aminopropyl groups on the silica surface could be coupled with 8-carboxypropyltheophylline. Acetic anhydride was added at the end of the coupling

Table 1. Elemental analysis of derivatized silica particles and the corresponding imprinted polymers.

	Aminopropyl silica gel ^[a]	Control silica gel ^[b]	Theophylline silica gel ^[c]	Control polymer ^[d]	Imprinted polymer ^[e]
C [%]	1.13	1.8	3.3	80.6	80.1
H [%]	0.34	0.42	0.5	7.3	6.9
N [%]	0.33	0.34	1.21	b.d.	b.d.
Si [%]	n.d.	n.d.	n.d.	b.d.	b.d.

[a] Aminopropyl-derivatized silica gel as obtained from the manufacturer. [b] Control silica obtained after acetylation of aminopropyl-derivatized silica gel with acetic anhydride. [c] Prepared by coupling carboxypropyltheophylline to aminopropyl-derivatized silica gel. [d] Control polymer obtained after imprinting with control silica gel. [e] Imprinted polymer obtained after imprinting with theophylline/silica gel. b.d. = below detection limit, n.d. = not determined.

reaction to block any remaining free aminopropyl groups that were not detectable with conventional methods, such as the Kaiser^[5] or trinitrobenzenesulfonic acid (TNBS)^[6] tests. The same aminopropyl-derivatized silica gel as used for the imprinted polymer was used for the preparation of control polymers, but all the aminopropyl groups were acetylated with acetic anhydride.

In previous work we described an optimized recipe for the preparation of theophylline-imprinted polymers.^[7] It contains trifluoromethylacrylic acid (TFMAA), a negatively charged functional monomer, and divinylbenzene (DVB), an apolar cross-linker. This mixture yielded highly efficient polymers over a wide range of template:monomer ratios. This method was applied here for the imprinting of immobilized theophylline, with a molar ratio of approximately 1:4 between the immobilized theophylline and the TFMAA monomer used. The same molar ratio had yielded a good functioning MIP imprinted with free theophylline.^[7]

In contrast to classical MIPs, where a solvent capable of forming pores (porogen) is essential to yield a macroporous structure, no porogen was used for the imprinting of immobilized theophylline. In our new system uniform macropores are generated by the removal of the silica gel, the binding sites thus being situated on the surface of the pores. It was shown, however, that the addition of toluene as a porogen to the polymerization mixture did not affect analyte recognition by the polymers obtained. The silica gel was completely removed by treatment with aqueous hydrofluoric acid (HF), as determined by elemental analysis (Table 1). The nitrogen content was also determined by elemental analysis to ascertain whether theophylline molecules were still present in the polymer after removal of the silica gel. The amount of nitrogen in the polymer was found to be below the detection limit of the method. Furthermore, the elemental composition of both control and imprinted polymers was very much alike (Table 1). After removal of some incompletely polymerized material and fragments, the yield of polymer particles was approximately 80% relative to the amount of monomers initially used.

The treatment with aqueous HF is rather harsh, so its potential detrimental impact on the polymers was investigated. A polymer imprinted with free theophylline that had the same polymer composition^[7] as above was also treated with

aqueous HF. Binding and competitive assays were performed after the polymer had been washed and dried. No change of capacity or specificity of the polymer was observed, which demonstrates that imprinted DVB–TFMAA copolymers can withstand treatment with aqueous HF.

The porosity of the polymers was determined by nitrogen adsorption/desorption analysis. The results show a narrow pore size distribution with a mean pore diameter of 254 Å and 257 Å, and pore surface areas (by BET) of 35 m²g⁻¹ and 31 m²g⁻¹, for the imprinted and the control polymers, respectively. No larger pores or micropores were detected. In contrast, polymers prepared by the classical method had a much broader pore size distribution (30–1000 Å) and a larger surface area (about 500 m²g⁻¹).

Binding assays were carried out in toluene by using [³H]-theophylline as a radioligand to evaluate the capacity of the polymer imprinted with immobilized theophylline and the corresponding control polymer (Figure 2). The imprinted

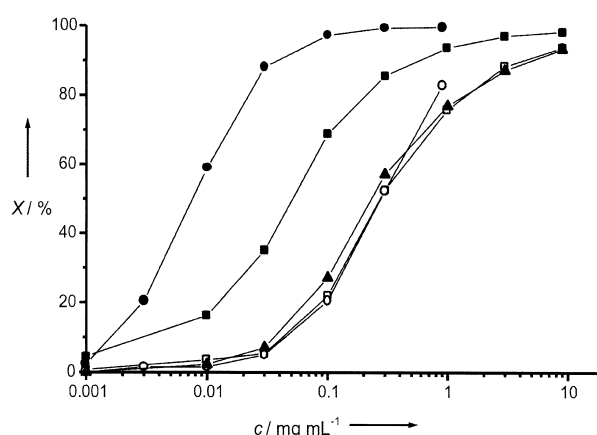


Figure 2. Adsorption of [³H]theophylline as a function of the polymer concentration. The curves represent an imprinted polymer prepared with: theophylline/silica gel (■), acetylated silica gel (control polymer; □), naphthalene/silica gel (▲), free theophylline (●), and aminopropyl-derivatized silica gel (○). The points represent mean values of three measurements.

polymer has a higher capacity for theophylline than the control polymer. At a polymer concentration where the MIP binds 50% of the radioligand, the control polymer binds only 10%. To elucidate the effects of template shape and functionality on imprinting, an additional polymer was synthesized using silica gel onto which 2-naphthylacetic acid was coupled. Naphthalene is about as bulky as theophylline, but contains no functional groups or heteroatoms. As can be seen in Figure 2 this polymer had a binding capacity that was only slightly higher than the control polymer prepared with acetylated silica gel. This result indicates that the functionalities on the theophylline molecule are responsible for the observed imprinting effect.

The binding assays of MIPs prepared in the classical way using free theophylline are shown for comparison in Figure 2. The classical MIP binds more radiolabeled theophylline than the MIP prepared with immobilized theophylline. However, a direct comparison of the capacities of the two systems is difficult because of the differences in their porosities.

Interestingly, both control polymers have very similar (low) binding capacities. Apparently, the presence of silica gel during the synthesis of the polymer does not cause increased nonspecific binding. Thus, the difference in binding between the MIP and the control polymers prepared in the presence of silica gel should be caused by the template immobilized on the silica gel surface.

The theophylline-imprinted polymer shows a high selectivity for theophylline over the related compounds theobromine and caffeine (Figure 3), which have cross-reactivities of less than 2% relative to theophylline. This result is similar to that obtained with polymers imprinted with the free template.^[7]

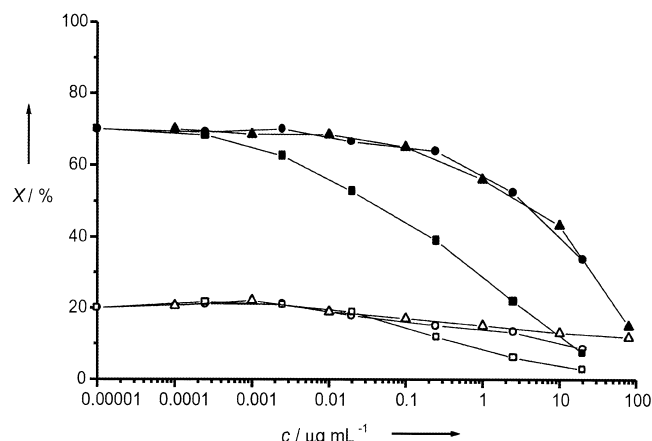


Figure 3. Competition of [³H]-theophylline binding to the imprinted polymer (filled symbols) and the control polymer (empty symbols) as a function of the concentration of nonlabeled theophylline (squares), theobromine (circles), or caffeine (triangles). Polymer concentration: 70 $\mu\text{g mL}^{-1}$.

Similar studies were conducted earlier in our group using latex and nonporous silica nanobeads as a solid support, but these only had limited success. This was found to be a consequence of the low surface area of nonporous beads, and of the incompatibility of latex particles with most of the common imprinting solvents. On the other hand, we anticipated silica gel to be a suitable material for our approach because of its stability in organic solvents and its higher density, which facilitates handling. Moreover, it can be readily removed from the imprinted polymer, for example by treatment with HF. We chose a porous silica material for its larger internal surface area, which allows for a higher template load. Recently, the possibility of using silica particles as a sacrificial material in combination with DVB-based polymers was reported by another research group.^[8] Although this study was not concerned with molecular imprinting, it was shown that these polymer–silica composites, after dissolution of the silica gel with HF, allow for the fine tuning of the polymer porosity.

There are several potential advantages in using immobilized templates for imprinting. Templates that are not soluble in the polymerization cocktail can be immobilized and then brought into contact with the monomers. The imprinting of insoluble templates would thus be made possible if they can be immobilized.

The possibility has been raised by others that aggregation of templates in pre-polymerization mixtures may occur.^[9, 10] The formation of such aggregates can be prevented by immobilizing the template on an appropriate solid support, which at the same time might provide a useful tool for further investigations of this phenomenon. Oriented immobilization of the imprint molecule should also result in a better orientation and homogeneity of the binding sites, more so as immobilization reduces the tumbling rate of the template molecule during the imprinting polymerization step.

Since the pre-polymerization mixture contained no porogen, the pore structure of the obtained polymers is formed only by uniform macropores generated by the dissolved silica gel backbone. All imprinted binding sites should therefore be located at, or close to, the surface of the pores and so greatly facilitate diffusion of the analyte to the binding sites.

The present study also opens new possibilities for the application of MIPs. For example, the template molecule can be tagged with various markers, whereby the problem of steric hindrance, which is most often encountered with classical MIPs where the binding sites are buried in the polymer structure, is avoided. This will greatly increase the usefulness of MIPs for immunoassays and related applications.

The described composite pair silica gel/acrylate, in which the silica gel acts as a dissolvable template carrier, is one of several possible composite pairs, for example, latex/acrylates, polysaccharides/acrylates, and chitosan/acrylates.

In conclusion we have demonstrated for the first time that the imprinting of immobilized templates is feasible and constitutes an alternative way of producing MIPs. Nevertheless, the phenomena involved during imprinting at liquid–solid interphases remain to be studied in more detail.

Experimental Section

Materials: Toluene, dimethylformamide (DMF), and dichloromethane (DCM; all anhydrous) were purchased from Labscan Ltd. (Dublin, Ireland). All other solvents (analytical or HPLC grade) and HF (40% aqueous solution), were obtained from Merck (Darmstadt, Germany). 8-Carboxypropyltheophylline, theophylline, caffeine, theobromine, [8-³H]-theophylline (specific activity: 0.8 mCi mol⁻¹), and diisopropylcarbodiimide (DIC) were purchased from Sigma (St. Louis, MO, USA). Aminopropyl-derivatized silica gel (7 μm , 300 Å, 0.7 mL g⁻¹) was obtained from Macherey-Nagel (Düren, Germany) and had an aminopropyl content of about 0.25 mmol per gram of silica gel. The radical polymerization initiator 2,2'-azobis(2,4-dimethylvaleronitrile) was obtained from Wako Pure Chemicals Industries (Osaka, Japan). TFMMA and technical grade DVB containing 20% ethylvinylbenzene was obtained from Aldrich (Steinheim, Germany), and was treated with basic alumina (Merck) immediately prior to use to remove polymerization inhibitors. TNBS (1% solution in DMF) and 2-naphthylacetic acid were obtained from Fluka (Buchs, Switzerland).

Preparation of the aminopropyl-derivatized silica gel surfaces for imprinting: A) Theophylline/silica gel: 8-carboxypropyltheophylline (266 mg, 1 mmol) and DIC (450 μL , 3 mmol) were dissolved in anhydrous DMF/DCM (1/1, 10 mL). Dry aminopropyl-derivatized silica gel (1 g) was then added and the suspension was shaken on a rocking-table for a minimum of 18 h at room temperature. The coupling reaction was allowed to continue until both Kaiser^[5] and TNBS^[6] tests were negative, which indicated that most aminopropyl groups on the silica surface had reacted. Subsequently, acetic anhydride (100 μL , 1 mmol) was added and the mixture incubated for another 2 h to acetylate any remaining aminopropyl groups. The silica gel was then washed on a G4-glass filter funnel with DMF, DCM, and methanol, before drying for 6 h at 45 °C and then in vacuo for a further 6 h. B) Control acetylated silica gel: aminopropyl silica (1 g) was treated with

an excess of acetic anhydride (1 mL, 10 mmol), to obtain complete acetylation of the amino groups. The control silica gel was processed as described earlier. C) Naphthalene/silica gel: The same procedure was used as for theophylline/silica gel (A), but using 2-naphthylacetic acid (186 mg, 1 mmol) instead of 8-carboxypropyltheophylline.

Preparation of the polymers: A pre-polymerization mixture consisting of DVB (2.14 mL, 12 mmol), TFMAA (336 mg, 2.4 mmol), and 2,2'-azobis(2,4-dimethylvaleronitrile) (20 mg) was prepared in a glass vial. According to the pore volume of the silica (ca. 0.65 mL g⁻¹), the amount of the mixture required to fill the pores was added to the silica gel (theophylline/silica gel, control silica gel, or naphthalene/silica gel) and gently stirred with a stainless steel spatula.^[11] The vial was flushed gently with N₂ for 2 min and the mixture was then allowed to polymerize overnight at 45 °C. After polymerization was completed (this was monitored by polymerizing a portion of the pre-polymerization mixture without silica gel) the polymer/silica gel composite was gently wet-milled in acetone with a manual mortar and pestle to disintegrate any particle aggregates. The composite was then transferred into a plastic tube with a screw cap, suspended in acetone (2 mL), and cooled in a water/ice bath. Aqueous HF (4 mL, 40%) was added portionwise whilst shaking the mixture to dissolve the silica matrix of the composite. The suspension was then allowed to react overnight on a rocking-table at room temperature. The remaining polymer was washed extensively on a G4-glass filter funnel with 20% acetone in deionized water (ca. 2 L) until the filtrate had a neutral pH value, and finally washed with methanol (0.25 L). The polymer particles were then dried in an oven at 45 °C for 6 h and in vacuo for a further 6 h.

Elemental analysis: Flash combustion elemental analysis coupled to gas chromatography was performed at MikroKemi AB (Uppsala, Sweden).

Radioligand binding assays: The polymer particles were suspended in toluene and appropriate volumes were added into 1.5-mL polypropylene test tubes, followed by the radioligand [³H]-theophylline, varying amounts of a solution of a competing ligand if appropriate, and toluene to give a total volume of 1 mL. The samples were incubated on a rocking-table for 12 h at room temperature. Particles were removed by centrifugation and supernatant (500 µL) was withdrawn and added to scintillation liquid (10 mL, Ecoscint O, National Diagnostics, Atlanta, GA, USA). The radioactivity was measured by liquid scintillation counting with a Rackbeta 1219 counter (LKB Wallac, Turku, Finland). This assay is similar to that described previously.^[12]

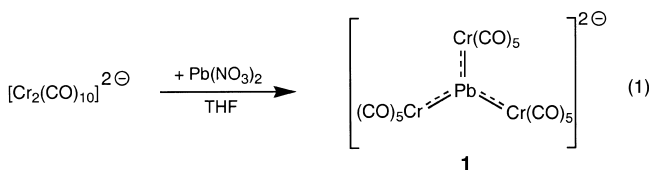
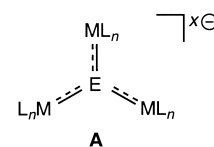
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Synthesis and Reactivity of [(CO)₅Cr]₃Pb]²⁻, an Unsaturated Compound with Trigonal-Planar Coordinated Lead

Peter Rutsch und Gottfried Huttner*

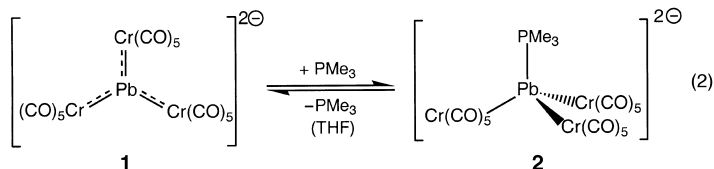
Dedicated to Professor Arndt Simon
on the occasion of his 60th birthday

Compounds **A**, which contain a main group element bound in a trigonal-planar environment to three organotransition metal 16-valence electron fragments L_nM (e.g. L_nM = (CO)₄Fe, E = In, x = 3;^[1] L_nM = (CO)₄Fe, E = Sn, Pb, x = 2;^[2] L_nM = (CO)₅Cr, E = Sn, x = 2;^[3] L_nM = (CO)₅Cr, E = Sb, x = 1;^[4] L_nM = Cp(CO)₂Mn, E = Te, x = 0^[5]), are in the broadest sense isoelectronic analogues of well known four-center, six-π-electron systems such as NO₃⁻ and CO₃²⁻. The p_π-p_π interaction in systems such as CO₃²⁻ corresponds to the metal-d_π-main group element-p_π interaction in the organometal derivatives.^[6] The unsaturated character of these organometallic π systems becomes apparent from the short M-E bonds^[1-5] as well as from their spectroscopic behavior.^[3] The NMR signals of the trigonal-planar coordinated main group elements are each shifted to low field.^[3, 7] This is also true for **1**, which is obtained from the reaction of disodium decacarbonyldichromate with lead nitrate [Eq. (1)]. The ²⁰⁷Pb NMR



signal of **1** is shifted to low field at δ = 7885 and supports, in agreement with the structural data (Figure 1),^[8] the unsaturated character of **1**.

Chemical evidence for the unsaturated character of π systems of the type **A** was hitherto unknown. We have now found that **1** in presence of PMe₃ is in equilibrium with its base adduct **2** [Eq. (2)]. From the temperature dependence of the ³¹P NMR spectra of **2**, the following thermodynamic parameters are obtained for the position of the formation equilibrium



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- [1] G. Wulff, *Angew. Chem.* **1995**, *107*, 1958–1979; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1812–1832.
- [2] K. Mosbach, O. Ramström, *Bio/Technology* **1996**, *14*, 163–170.
- [3] M. J. Whitcombe, M. E. Rodriguez, P. Villar, E. N. Vulfson, *J. Am. Chem. Soc.* **1995**, *117*, 7105–7111.
- [4] F. Albericio, L. A. Carpino, *Methods Enzymol.* **1997**, *289*, 104–126.
- [5] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595–598.
- [6] W. S. Hancock, J. E. Battersby, *Anal. Biochem.* **1976**, *71*, 260–264.
- [7] E. Yilmaz, K. Mosbach, K. Haupt, *Anal. Commun.* **1999**, *36*, 167–170.
- [8] S. A. Johnson, P. J. Ollivier, T. E. Mallouk, *Science* **1999**, *283*, 963–965.
- [9] H. S. Andersson, J. G. Karlsson, S. A. Piletsky, A.-C. Koch-Schmidt, K. Mosbach, I. A. Nicholls, *J. Chromatogr. A* **1999**, *848*, 39–49.
- [10] A. Katz, M. E. Davis, *Macromolecules* **1999**, *32*, 4113–4121.
- [11] M. Glad, P. Reinholdsson, K. Mosbach, *React. Polym.* **1995**, *25*, 47–54.
- [12] G. Vlatakis, L. I. Andersson, R. Müller, K. Mosbach, *Nature* **1993**, *361*, 645–647.

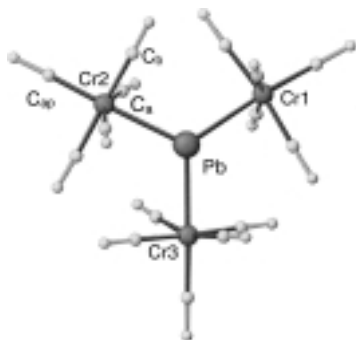


Figure 1. Structure of **1** in the crystal of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$.^[8] Selected bond lengths [pm] and angles [°]: Pb–Cr1 273.3(1), Pb–Cr2 272.6(1), Pb–Cr3 272.8(1), Cr–C_a 184.5(1)–188.5(1), Cr–C_b 186.1(1)–190.0(3), Cr–C_{ap} 183.8(2)–185.7(3); Cr1–Pb–Cr2 119.7(6), Cr1–Pb–Cr3 120.8(6), Cr2–Pb–Cr3 117.9(5), C_a–Cr–C_a 160.0(4)–169.0(6), C_b–Cr–C_b 172.1(6)–176.5(6); torsion angle τ [°]: X–Pb–Cr–C_a 18–26 (the reference atom X is located on a reference axis that runs through the lead center and is perpendicular to the plane Cr1–Cr2–Cr3).

of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{2}$: $K_{298} = 1.86$, $\Delta H = -(59 \pm 2) \text{ kJ mol}^{-1}$, $\Delta S = (-194 \pm 9) \text{ J mol}^{-1} \text{ K}^{-1}$. Whereas accordingly in equimolar mixtures of **1** and PMe_3 , **2** is only formed quantitatively at temperatures below 213 K (within the accuracy of the NMR experiment), the tetraphenylphosphonium salt of **2** can be crystallized from THF/EtOH already at room temperature. The structure analysis of **2** (Figure 2) confirms the coordination of the Lewis base PMe_3 to the coordinatively unsaturated

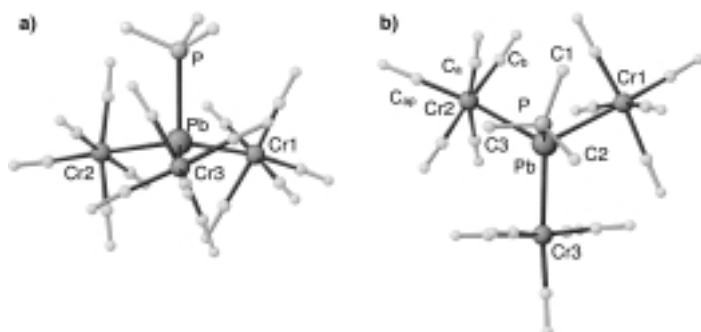


Figure 2. Two views of the structure of **2** in the crystal of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{2}$.^[8] Selected bond lengths [pm] and angles [°]: Pb–Cr1 282.2(1), Pb–Cr2 282.7(1), Pb–Cr3 281.6(1), Pb–P 284.4(2), Cr–C_a 186.5(2)–190.3(2), Cr–C_b 185.9(3)–191.4(2), Cr–C_{ap} 181.3(2)–182.5(2); Cr1–Pb–Cr2 114.8(5), Cr1–Pb–Cr3 117.5(6), Cr2–Pb–Cr3 118.2(6), P–Pb–Cr1 100.0(5), P–Pb–Cr2 101.7(6), P–Pb–Cr3 99.1(5), C_a–Cr–C_a 168.0(5)–171.9(6), C_b–Cr–C_b 172.0(6)–175.8(5); torsion angle τ [°]: P–Pb–Cr–C_a 24–27.

lead center.^[8] The Pb–P distance of 284 pm lies in the usual range.^[9] The $^2J_{\text{PbPb}}$ coupling of 1567 Hz observed in the ^{31}P NMR spectrum supports this finding.^[9] Whereas in **1** the lead center lies approximately in the plane formed by the three chromium atoms (deviation 13 pm, Figure 1), in **2** it lies 38 pm above this plane, and in fact on the side facing the ligand (Figure 2a). The angles Cr–Pb–Cr (117°) and P–Pb–Cr (101°) deviate, however, strongly from the values expected for an ideal tetrahedron; through the addition of the Lewis base PMe_3 the trigonal-planar coordination of the central element lead in **1** (Figure 1) is only slightly pyramidalized.^[10]

Consistent with their stronger saturated character (Figure 2), the Pb–Cr bonds, which are shortened through multiple-bond contributions to 273 pm in **1** (Figure 1), are extended to 282 pm in **2**. With the reaction of **1** to give **2** and the complete characterization of both compounds, the unsaturated character of four-center, six- π -electron systems of the type **A** is now also supported by reactivity studies for one example.

Experimental Section

Experimental conditions see ref. [3], ^{207}Pb NMR spectra see ref. [9c]. C, H analyses: Mikroanalytisches Laboratorium, Organisch-Chemisches Institut der Universität Heidelberg.

Synthesis of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$: $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$: $\text{Pb}(\text{NO}_3)_2$ (331 mg, 1 mmol) was added to a yellow solution of $\text{Na}_2[\text{Cr}_2(\text{CO})_{10}]$ (860 mg, 2 mmol) in THF (50 mL) under stirring. Within 4 h the solution turned intense violet, it was filtered through 5 cm Kieselgur and concentrated to 3 mL. The chromatographic purification was carried out over 15 cm silica gel. $[\text{Cr}(\text{CO})_6]$ and $\text{Na}_2[\text{Cr}_2(\text{CO})_{10}]$ (for structure see ref. [8]) were separated with THF/Et₂O (1/1). The sodium salt of **1** was eluted with EtOH as a deep purple band. After concentration, a solution of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$ (740 mg, 2 mmol) in ethanol was added, which led to the formation of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$ as a black precipitate in 47 % yield (640 mg; yield based on $\text{Pb}(\text{NO}_3)_2$). The product was dissolved in THF (3 mL) and isolated, after layering with ethanol, in the form of black crystals after six days at room temperature, yield 430 mg (32 %). ^1H NMR: $\delta = 7.92$ –7.68 (m, 40 H; H_{arom}); ^{13}C NMR: $\delta = 230.8$ (s, 3 C; C_{ax}), 223.2 (s, 12 C; C_{eq}), 136.5–118.2 (m, 48 C; C_{arom}); ^{31}P NMR: $\delta = 22.5$ (s, 2 P; Ph_4P); ^{207}Pb NMR: $\delta = 7885$ (s, 1 Pb); IR (THF): $\nu(\text{CO}) = 2008$ (w), 1977 (vs), 1911 (vs), 1857 cm^{-1} (vs); UV/Vis (THF): $\lambda_{\text{max}}(\epsilon) = 331$ (20500), 428 (5600), 534 nm ($1600 \text{ M}^{-1} \text{ cm}^{-1}$); elemental analysis (%): C 51.69 (calcd: 51.75), H 3.20 (calcd: 2.75).

Synthesis of $[(\text{PPh}_4)^+]_2 \cdot \mathbf{2}$: $[(\text{PPh}_4)^+]_2 \cdot \mathbf{2}$: $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$ (430 mg, 0.32 mmol) was dissolved in THF (4 mL) and treated with a 1.0 M PMe_3 solution in THF (1 mL, 1 mmol) under stirring. The reaction solution was layered with ethanol; after five days $[(\text{PPh}_4)^+]_2 \cdot \mathbf{2}$ (270 mg) was obtained in the form of black crystals (60 %, based on $[(\text{PPh}_4)^+]_2 \cdot \mathbf{1}$). ^1H NMR: $\delta = 7.92$ –7.68 (m, 40 H; H_{arom}), 1.07 (s, 9 H; CH_3); ^{13}C NMR: $\delta = 231.1$ (s, 3 C; C_{ax}), 223.8 (s, 12 C; C_{eq}), 136.4–118.2 (m, 48 C; C_{arom}), 15.5 (s, 3 CH_3); ^{31}P NMR (25 °C): $\delta = 22.5$ (s, 2 P; Ph_4P), –114.3 (s, 1 P; $\text{P}(\text{CH}_3)_3$); ^{31}P NMR (–90 °C): $\delta = 22.5$ (s, 2 P; Ph_4P), –180.06 (s, $^2J_{\text{PbPb}} = 1567 \text{ Hz}$, 1 P, $\text{P}(\text{CH}_3)_3$); ^{207}Pb NMR (–90 °C): $\delta = 7813$ (s, 1 Pb); IR (THF, 25 °C): $\nu(\text{CO}) = 1977$ (vs), 1907 (vs), 1857 cm^{-1} (vs); UV/Vis (THF, 25 °C): $\lambda_{\text{max}}(\epsilon) = 327$ (19800), 428 (5100), 541 nm ($1400 \text{ M}^{-1} \text{ cm}^{-1}$); elemental analysis (%): C 51.53 (calcd: 51.53), H 3.61 (calcd: 3.21).

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- [1] V. G. Albano, M. Cane, M. C. Iapaluci, G. Longoni, M. Monari, *J. Organomet. Chem.* **1991**, 407, C9–C12.
- [2] J. M. Cassidy, K. H. Whitmire, *Inorg. Chem.* **1989**, 28, 2494–2496.
- [3] P. Kircher, G. Huttner, K. Heinze, *J. Organomet. Chem.* **1998**, 562, 217–227.
- [4] G. Huttner, U. Weber, B. Sigwarth, O. Scheidsteger, H. Lang, L. Zsolnai, *J. Organomet. Chem.* **1985**, 282, 331–348.
- [5] M. Herberhold, D. Reiner, D. Neugebauer, *Angew. Chem.* **1983**, 95, 46–47; *Angew. Chem. Int. Ed. Engl.* **1983**, 22, 59–60.
- [6] R. Hofmann, *Angew. Chem.* **1982**, 94, 725–808; *Angew. Chem. Int. Ed. Engl.* **1982**, 94, 711–794.
- [7] B. Schiemenz, G. Huttner, L. Zsolnai, P. Kircher, T. Diercks, *Chem. Ber.* **1995**, 128, 187–191.
- [8] Crystal structure data: The X-ray data were collected on a Nonius-Kappa-CCD diffractometer with $\text{MoK}\alpha$ radiation ($\lambda_{\text{Mo}} = 0.71074 \text{ \AA}$). Data collection and reduction were performed with the Nonius software,^[11] the structure solution and refinement were carried out with the SHELXTL-PLUS software package; the structures were

solved by direct methods with SHELXS-86 and refined with SHELXL-93.^[12] The program XPM was used for the graphical processing of the data.^[13] The figures were produced with WINRAY-32.^[14] The refinement was carried out anisotropically against F^2 , hydrogen atoms were included in calculated positions. **1**: space group $P2_1/c$, $a = 1277.4(3)$, $b = 1956.0(4)$, $c = 2436.8(5)$ pm, $\beta = 103.63(3)^\circ$, $V = 5917 \times 10^6$ pm³, $\rho_{\text{calc}} = 1.641$ g cm⁻³, $2\theta_{\text{max}} = 52.1^\circ$, $Z = 4$, 71 438 measured reflections, 11 474 independent reflections, of which 5485 ($I > 2\sigma(I)$) observed, 758 refined parameters, $R = 0.070$, $R_w = 0.112$, max. residual electron density 1.54×10^{-6} e pm⁻³. **2**: space group $P\bar{1}$, $a = 1422.2(3)$, $b = 1902.5(4)$, $c = 2609.1(5)$ pm, $\alpha = 100.58(3)$, $\beta = 103.63(3)$, $\gamma = 108.48(3)^\circ$, $V = 6490 \times 10^6$ pm³, $\rho_{\text{calc}} = 1.543$ g cm⁻³, $2\theta_{\text{max}} = 55.0^\circ$, $Z = 4$, 58 282 measured reflections, 29 826 independent reflections, of which 19 004 ($I > 2\sigma(I)$) observed, 1463 refined parameters, $R = 0.077$, $R_w = 0.204$, max. residual electron density 2.39×10^{-6} e pm⁻³. The two crystallographically independent anions **2** are the same with regard to all the important structural features. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-137594 (**1**), -137593 (**2**), and -137592 ($\text{Na}_2[\{(\text{CO})_5\text{Cr}\}_2\text{Pb}(\text{NO}_3)_2]$). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [9] a) A. H. Cowley, D. M. Giolando, R. A. Jones, C. M. Nunn, J. M. Power, *Polyhedron* **1988**, *7*, 1909–1910; b) S. C. Goel, M. Y. Chiang, D. J. Rauscher, W. E. Buhro, *J. Am. Chem. Soc.* **1993**, *115*, 160–169; c) A. Winkler, W. Bauer, F. W. Heinemann, V. G. Montalvo, M. Moll, J. Ellermann, *Eur. J. Inorg. Chem.* **1998**, 437–444; d) A. L. Balch, D. E. Oram, *Organometallics* **1986**, *5*, 2159–2161.
- [10] The compounds $\text{H}[\text{E}(\text{Fe}(\text{CO})_4)_3]^{2-}$ ($\text{E} = \text{As}, \text{Sb}$) are in the broadest sense isoelectronic to **2**, though their relationship to the unsaturated analogues $[\text{E}(\text{Fe}(\text{CO})_4)_3]^-$ is not chemically confirmed, particularly since these are unknown to date. a) R. E. Bachmann, S. K. Miller, K. H. Whitmire, *Inorg. Chem.* **1994**, *33*, 2075–2076; b) P. Henderson, M. Rossignoli, R. C. Burns, M. L. Scudder, D. C. Craig, *J. Chem. Soc. Dalton Trans.* **1994**, 1641–1647; c) R. E. Bachmann, S. K. Miller, K. H. Whitmire, *Organometallics* **1995**, *14*, 796–803.
- [11] Collect, data collection software, Nonius, **1998** (<http://www.nonius.com>).
- [12] a) G. M. Sheldrick, SHELXS-86 Program for Crystal Structure Solution, Universität Göttingen, **1986**; b) G. M. Sheldrick, SHELXL-93 Program for Crystal Structure Refinement, Universität Göttingen, **1993** (<http://www.shelx.uni-ac.gwdg.de/shelx/index.html>); c) *International Tables for X-Ray Crystallography*, Vol. 4, Kynoch, Birmingham, **1974**.
- [13] L. Zsolnai, G. Huttner, XPM, Universität Heidelberg, **1998** (<http://www.rzuser.uni-heidelberg.de/~v54/xpm.html>).
- [14] R. Soltek, G. Huttner, WINRAY-32, Universität Heidelberg, **1998** (<http://www.rzuser.uni-heidelberg.de/~v54>).

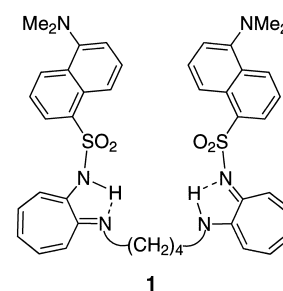
Metal-Based NO Sensing by Selective Ligand Dissociation**

Katherine J. Franz, Nisha Singh, and Stephen J. Lippard*

The large and increasing number of biological processes for which nitric oxide is implicated necessitates the development of improved methods of NO detection. Currently available techniques often rely on the identification of NO metabolites, such as nitrite and nitrate, or lack sensitivity.^[1] The application of ratiometric biosensors^[2] and ultramicro amperometric sensors^[3–5] affords one avenue to obtain selective, sensitive detection of NO in vivo. Fluorescent indicators also provide desirable properties that allow direct, real-time detection with both spatial and temporal resolution,^[6] as has been amply demonstrated for Ca^{II} sensors.^[7] Although the invention of fluorescent NO sensors is an active research area, so far these methods rely on indirect detection of more reactive NO_x species,^[8–10] display decreased fluorescence intensities upon NO binding,^[11] or require further chemistry to provide a positive fluorescence response.^[12, 13] We report herein an approach in which the formation of a transition metal nitrosyl complex triggers a positive fluorescent signal in response to NO.

The design of this NO sensor takes advantage of the fluorescence-quenching properties of transition metal ions with partly filled d shells. We prepared a ligand containing a fluorophore that is quenched by the metal center, in this case Co^{II} , in the absence of NO to give little residual signal for the “off” response. In the presence of NO, however, the formation of the metal–nitrosyl adduct selectively displaces a fluorescent ligand, thereby removing it from the quenching environment and turning the fluorescence “on”. A similar approach was reported for an Fe^{II} complex of a quinoline pendant cyclam but the fluorescence intensity decreased in the presence of NO.^[11] An analogous ligand-displacement strategy has also been applied for a pH-sensitive fluorescent probe.^[14]

In the newly designed ligand $\text{H}_2\text{DATI-4}$ (**1**), each aminotroponimate (ATI) ring is modified with a dansyl fluorophore on one of the imine nitrogen atoms and linked through the other nitrogen by a 4-methylene chain to a second such chelating unit.^[15] The yellow ligand **1** is rather in-



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soluble in organic solvents but deprotonation with KH in a THF/CH₃CN mixture affords a clear, orange solution. Addition of [Co(CH₃CN)₄](PF₆)₂ and work-up provides an 80 % yield of [Co(DATI-4)] **2**. The single-crystal X-ray diffraction study of **2** revealed its solid-state structure (Figure 1).^[16] The

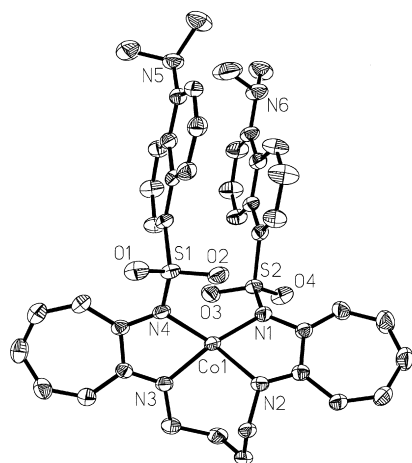
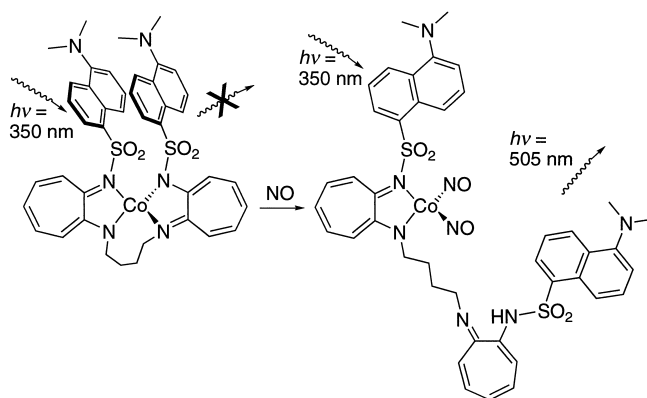


Figure 1. ORTEP diagram of [Co(DATI-4)] **2** showing 50 % thermal ellipsoids and selected atom labels. Selected bond lengths [Å] and angles [°]: Co1–N1 1.986(3), Co1–N2 1.963(2), Co1–N3 1.996(3), Co1–N4 1.977(2), N1–S2 1.607(3), N4–S1 1.618(3), S1–O1 1.440(2), S1–O2 1.442(2), S2–O3 1.439(2), S2–O4 1.437(2); N1–Co1–N2 79.78(10), N2–Co1–N3 103.94(10), N3–Co1–N4 79.93(10), N4–Co1–N1 123.78(10), N2–Co1–N4 136.82(10), N1–Co1–N3 141.36(11).

Co^{II} center has distorted tetrahedral geometry and the dansyl groups align in a parallel planar fashion. The two six-membered aromatic rings attached to the S atoms are stacked with an average aryl–aryl distance of 3.63(9) Å. Compound **2** displays only 9 % of the fluorescence emission intensity of **1**, as revealed by their quantum yields Φ of 9×10^{-4} and 0.01, respectively.

Isolated **2** is air stable in both the solid state and solution, an important property for applications as a sensor that recognizes NO but not O₂.^[17] Complex **2** reacts readily with NO, as indicated by two new IR bands at 1827 and 1751 cm⁻¹ (KBr). These bands shift to 1793 and 1719 cm⁻¹ upon isotopic labeling with ¹⁵NO, in excellent agreement with the values of 1794 and 1720 cm⁻¹ calculated by assuming a classic diatomic oscillator model. The IR bands indicate formation of a Co–dinitrosyl species and are at the low end of the range for tetrahedral {Co(NO)₂}¹⁰ complexes, which exhibit symmetric and asymmetric NO stretching bands between 1820–1876 cm⁻¹ and between 1755–1798 cm⁻¹.^[18] In some cases, formation of {Co(NO)₂}¹⁰ species proceeds by disproportionation of Co^{III}L₂ in the presence of NO to afford [Co^I(NO)₂L] and [Co^{III}L₃], where L is a bidentate ligand.^[19, 20] There is no apparent evidence for a Co^{III} species in the reaction of **2** with NO and, at present, the nature of the reductant is unknown.

The formation of a Co–dinitrosyl species with a tetrahedral coordination environment around the Co center from **2** is possible if one of the DATI arms dissociates from the metal center (Scheme 1). Similar products are obtained upon the reaction of [Co(*i*Pr₂ATI)₂] with NO, in which *Hi*Pr₂ATI is released as [Co(NO)₂(*i*Pr₂ATI)] forms.^[21] A crystal structure



Scheme 1. Formation of a cobalt–dinitrosyl complex results in ligand dissociation and fluorescence enhancement.

determination revealed the geometry of [Co(NO)₂(*i*Pr₂ATI)] in the solid state to be pseudotetrahedral with one bidentate *i*Pr₂ATI⁻ and two NO ligands. In the IR spectrum, there are two NO stretching bands at 1809 and 1730 cm⁻¹.^[21] These bands are lower in energy than those in the product of **2** with NO because of the electron-withdrawing influence of the sulfonamide groups.

The dissociation of a dansyl-containing ligand fragment suggests that fluorescence of the Co complex should be restored upon addition of NO. Figure 2a displays the fluorescence emission spectra of a solution of **2** in CH₂Cl₂ at 25 °C under a NO atmosphere. After 3 min, the earliest time point

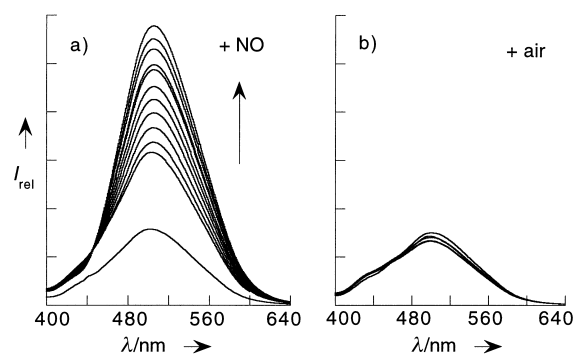


Figure 2. a) The lowest intensity spectrum is that of a 40 μM CH₂Cl₂ solution of [Co(DATI-4)] **2** under N₂ at 25 °C. Residual fluorescence of the paramagnetic complex gives an emission at 505 nm (excitation at 350 nm). Within 3 minutes after purging NO gas through the headspace, the intensity of the emission at 505 nm doubles. The intensity continues to increase over the course of 6 hours to a final fourfold increase. b) A 40 μM CH₂Cl₂ solution of **2** exposed to air over 6 hours. Residual fluorescence of the paramagnetic complex gives an emission at 505 nm (excitation at 350 nm).

recorded, the fluorescence intensity doubled over that of the starting complex. The intensity continued to rise at smaller increments over 6 h to afford a greater than four-fold increase in intensity over that of the starting material. In a separate experiment, aliquots of NO gas were added incrementally to a solution of **2** to gauge the lower limit of NO detection. Addition of 2 μL of NO caused only a slight fluorescence enhancement, whereas addition of 5 μL (2.9 equiv based on Co) of NO doubled the fluorescence intensity. The NO detection limit of **2** can therefore be estimated at 50–100 μM.

In addition to the dissociation of a DATI moiety, a metal-based mechanism may also contribute to the positive increase in fluorescence. Fluorescence quenching by transition metals can occur by energy or electron transfer between the excited state of the fluorophore and empty or partially filled d orbitals on the metal. If the d shell is full, such as in the d¹⁰ ions Zn^{II} or Cu^I, such an energy transfer is not accessible and fluorescence occurs.^[22] Formation of a cobalt–dinitrosyl species [Co(NO)₂]¹⁰ also creates a filled d¹⁰ shell and therefore this moiety may itself account for some of the fluorescence response. An additional fluorescence enhancement mechanism is conceivable if π – π stacking of the fluorophores, as observed in the solid-state structure of **2**, creates an excimer complex that is disrupted by formation of the dinitrosyl adduct. Related [Co(^RDATI)₂] where ^RDATI = alkylidansyl-aminotroponimate, complexes that exhibit similar fluorescence responses to NO do not exhibit such π – π interactions in the solid state.^[15, 21]

The ability of a sensor to report only the desired analyte while ignoring competitors is crucial. Although NO rapidly reacts with O₂ to yield NO₂, the reaction is second order with respect to NO.^[23] For regulatory functions such as vasodilation and neurotransmission, the supposed 0.1 to 10 μ M concentrations of NO translates to a possible lifetime of up to ten minutes.^[24] It is therefore essential that a sensor respond only to NO and not O₂, a situation that creates a challenge for metal-based systems. Compound **2** is stable to dioxygen indefinitely and displays no fluorescence response in the presence of dissolved O₂, as illustrated by the fluorescence spectra in Figure 2b.

In conclusion, we have demonstrated ligand dissociation from a transition metal ion to be an attractive approach to NO sensor design. The present NO sensor overcomes two major challenges: It selectively reports NO and not O₂ and it displays a *positive* fluorescence response to this important analyte. The mechanism of fluorescence enhancement involves ligand dissociation upon metal–nitrosyl binding and perhaps also involvement of {Co(NO₂)₂}¹⁰ in which energy or electron transfer quenching is not possible. We are currently using the principles learned in this study to develop NO sensors that are more sensitive, water-soluble, display greater fluorescence intensity enhancement, and can bind NO reversibly for in vivo applications.

Experimental Section

2: A slurry of **1** (164 mg, 0.22 mmol) in a THF/CH₃CN mixture (5/1, 10 mL) was added to KH (18 mg, 0.45 mmol) under a nitrogen atmosphere. A clear, orange solution was obtained after stirring for 30 min. To this solution, 3 mL of a CH₃CN solution of [Co(CH₃CN)₄](PF₆)₂ (122 mg, 0.24 mmol) was added dropwise. The resulting dark amber solution was evacuated to dryness after stirring for 3 h under a dry nitrogen atmosphere. The brown powder was extracted into CHCl₃ and filtered through Celite. A brown, microcrystalline powder (143 mg, 80% yield) was obtained by diethyl ether diffusion into a CHCl₃/MeOH (4/1) solution of the complex. X-ray quality crystals were grown from the same solvent combination. FTIR (KBr): $\tilde{\nu}$ = 2937, 1610, 1577, 1509, 1451, 1287, 1209, 1129, 1059, 864, 788, 746, 627, 571, 499, 474 cm^{–1}; UV/Vis (CH₂Cl₂): λ_{max} (ϵ) = 254 (55 000), 367 nm (26 800 M^{–1} cm^{–1}); Φ = 9×10^{-4} ; elemental analysis (%): calcd for CoC₄₂H₄₂N₆S₂O₄: C 61.68, H 5.18, N 10.28; found: C 61.54, H 5.29, N 10.01.

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- [1] M. Feelisch, J. S. Stamler in *Methods in Nitric Oxide Research* (Eds.: M. Feelisch, J. S. Stamler), Wiley, New York, **1996**, pp. 303–307.
- [2] S. L. R. Barker, H. A. Clark, S. F. Swallen, R. Kopelman, A. W. Tsang, J. A. Swanson, *Anal. Chem.* **1999**, *71*, 1767–1772.
- [3] J. Y. Jin, T. Miwa, L. Q. Mao, H. P. Tu, L. T. Jin, *Talanta* **1999**, *48*, 1005–1011.
- [4] S. Trevin, F. Bedioui, J. Devynck, *Talanta* **1996**, *43*, 303–311.
- [5] T. Malinski, Z. Taha, S. Grunfeld, S. Patton, M. Kapturczak, P. Tomboulis, *Biochem. Biophys. Res. Commun.* **1993**, *193*, 1076–1082.
- [6] A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566.
- [7] A. Minta, J. P. Y. Kao, R. Y. Tsien, *J. Biol. Chem.* **1989**, *264*, 8171–8178.
- [8] H. Kojima, K. Sakurai, K. Kikuchi, S. Kawahara, Y. Kirino, H. Nagoshi, Y. Hirata, T. Nagano, *Chem. Pharm. Bull.* **1998**, *46*, 373–375.
- [9] H. Kojima, N. Nakatsubo, K. Kikuchi, S. Kawahara, Y. Kirino, H. Nagoshi, Y. Hirata, T. Nagano, *Anal. Chem.* **1998**, *70*, 2446–2453.
- [10] H. Kojima, Y. Urano, K. Kikuchi, T. Higuchi, Y. Hirata, T. Nagano, *Angew. Chem.* **1999**, *111*, 3419–3422; *Angew. Chem. Int. Ed.* **1999**, *38*, 3209–3212.
- [11] Y. Katayama, S. Takahashi, M. Maeda, *Anal. Chim. Acta* **1998**, *365*, 159–167.
- [12] M. Bätz, H.-G. Korth, R. Sustmann, *Angew. Chem.* **1997**, *109*, 1555–1557; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1501–1503.
- [13] P. Meineke, U. Rauen, H. de Groot, H.-G. Korth, R. Sustmann, *Chem. Eur. J.* **1999**, *5*, 1738–1747.
- [14] L. Fabbri, M. Licchelli, P. Pallavicini, L. Parodi, *Angew. Chem.* **1998**, *110*, 838–841; *Angew. Chem. Int. Ed.* **1998**, *37*, 800–802.
- [15] K. J. Franz, N. Singh, B. Spingler, S. J. Lippard, unpublished results.
- [16] Crystal data for **2**: Dark brown, diamond-shaped plates, 0.36 × 0.24 × 0.08 mm. Monoclinic, space group P2₁/c, *a* = 13.239(3), *b* = 20.614(8), *c* = 14.055(5) Å, β = 92.54(2)°, *V* = 3832(2) Å³, *Z* = 4, ρ_{calcd} = 1.418 Mg m^{–3}. The data were collected at –85°C on a Siemens SMART diffractometer equipped with a CCD detector ($2\theta_{\text{max}}$ = 56.54°) using MoK α radiation (λ = 0.71073 Å). A total of 23 805 reflections were measured, of which 8811 were unique. The structure was solved by the direct methods program XS and refined by full-matrix least-squares minimization on *F*² and Fourier techniques using SHELXTL-PLUS. Absorption corrections were applied with the program SADABS. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to idealized positions and given a thermal parameter 1.2 times that of the thermal parameter of the carbon atom to which each was attached. Final residuals were *R* = 0.047 and *wR*₂ = 0.119 for 5353 observed reflections with *I* > 2 σ (*I*) and 496 variable parameters. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-139962. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [17] S. L. R. Barker, Y. Zhao, M. A. Marletta, R. Kopelman, *Anal. Chem.* **1999**, *71*, 2071–2075.
- [18] B. L. Haymore, J. C. Huffman, N. E. Butler, *Inorg. Chem.* **1983**, *22*, 168–170.
- [19] A. Del Zotto, A. Mezzetti, P. Rigo, *Inorg. Chim. Acta* **1990**, *171*, 61–69.
- [20] A. R. Hendrickson, R. K. Y. Ho, R. L. Martin, *Inorg. Chem.* **1974**, *13*, 1279–1281.
- [21] K. J. Franz, N. Singh, S. J. Lippard, unpublished results.
- [22] L. Fabbri, M. Licchelli, P. Pallavicini, *Acc. Chem. Res.* **1999**, *32*, 846–853.
- [23] D. A. Wink, J. S. Beckmann, P. C. Ford in ref. [1], pp. 29–37.
- [24] F. T. Bonner, G. Stedman in ref. [1], pp. 3–18.

Guest Penetration Deep within the Cavity of Calix[4]arene Hosts: The Tight Binding of Nitric Oxide to Distal (Cofacial) Aromatic Groups**

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Interest in the design and synthesis of modified calixarenes derives from their importance as molecular platforms or building blocks in supramolecular chemistry.^[1–3] The most relevant structural feature of calixarene hosts is the presence of a macrocyclic cavity that holds potential for binding various (guest) substrates. However, calix[4]arenes complexes such as those with various alkali as well as transition metal cations generally utilize the “hard” oxygen atoms on the rim rather than the “soft” π -basic cavity formed by the four-electron-rich aryl groups.^[4, 5] Even cationic π -acceptors such as silver(I) ions do not penetrate into the interior of the cavities of the calix[4]arenes, but position themselves on the outside of the wider rim.^[6]

Calix[4]arenes and their tetramethyl ether derivatives are conformationally mobile molecules with four rapidly interconverting conformers that contain cavities of variable size and shape.^[7, 8] Importantly, each conformer contains at least one pair of juxtaposed aromatic rings (Scheme 1). We find a cofacial arrangement of aromatic groups results in a cleft in the stilbenoid hydrocarbon ligand “Venus flytrap” that efficiently traps nitric oxide (NO)—a molecule of significant

biological interest owing to its importance as an intercellular messenger^[9] in a variety of physiological processes.^[10] Accordingly, we conjectured whether the void created by cofacial aromatic groups in various calixarene conformers would allow the actual penetration of nitric oxide into the interior of the calixarene cavities. Moreover, such an association of a guest (namely NO) with calixarene may also modulate its conformational mobility as well as the distribution of the conformers.

We now report that the conformationally mobile calix[4]-arene methyl ether derivative^[7] (**1-OMe**; Scheme 1, R = Me) readily captures nitric oxide (by complete encapsulation) deep inside its cavity, which results in all four conformations becoming immobile in solution (on the NMR time scale). Crystallization yields purple crystals of NO-bound calixarene as a single (1,3-alternate) conformer, as confirmed by X-ray crystallography and NMR spectroscopy. For comparison, we also examined the rigid conformers of the calix[4]arene propyl ether derivative^[11] (**2-OPr**; Scheme 1, R = propyl) to probe the binding properties and dynamics of differently structured calixarene cavities toward this guest.

The activation of calixarene **1-OMe** by oxidation in dichloromethane produces a bright yellow solution,^[12] which upon exposure to gaseous nitric oxide takes on a dark purple color [Eq. (1)].^[13] Infrared spectral analysis of the purple solution indicates the quantitative entrapment of NO, as gauged by the characteristic IR absorption at 1958 cm^{–1} corresponding to a single N–O stretching frequency.^[14]

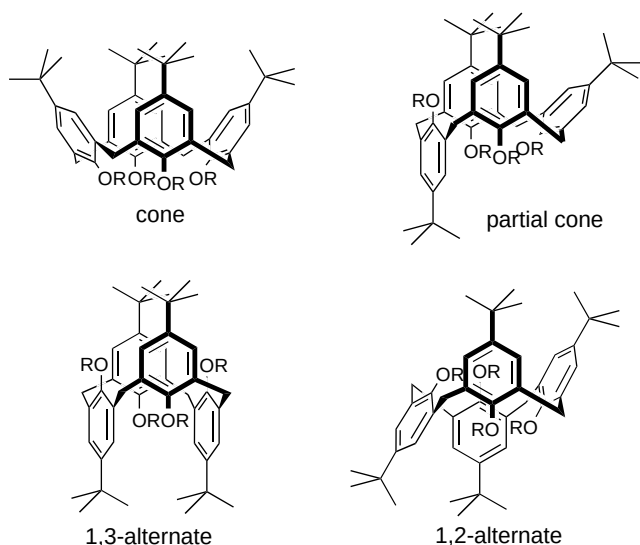


Importantly, the same purple solution is also obtained by exposure of a solution of calixarene **1-OMe** in dichloromethane to oxidized nitric oxide (NO⁺)^[15] [Eq. (2)].



Dark purple solutions of NO-bound calixarene are unusually stable and show no sign of decomposition even after standing at room temperature for several months. Such a remarkable stability is further confirmed by an exceptionally high binding constant of $K_{\text{ass}} > 5 \times 10^8 \text{ M}^{-1}$ for the association of nitric oxide with **1-OMe** in Equations (1) and (2).^[16]

To determine the structure of the NO-bound calixarene methyl ether, we crystallized [**1-OMe, NO**]⁺ from a mixture of dichloromethane and hexane at about 0 °C. Interestingly, the well-formed crop of highly colored single crystals (in 94% yield) show similar morphology despite the fact that calixarene **1-OMe** contains four conformers in solution. Indeed, the X-ray crystallographic analysis of several single crystals from different batches uniformly exhibit identical cell parameters, which are all identified as those of the 1,3-alternate conformer of [**1-OMe, NO**]⁺.^[17] Figure 1 shows that a single molecule of NO is completely entombed deep inside the calixarene cavity, and is only visible if one of the methoxy groups is omitted in the space-filling representation. The cylindrical cavity of the 1,3-alternate conformer is defined by two distal (cofacial) pairs of aromatic groups oriented orthogonally



Scheme 1. The four characteristic conformers of lower-rim-functionalized *p*-tert-butylcalix[4]arene.

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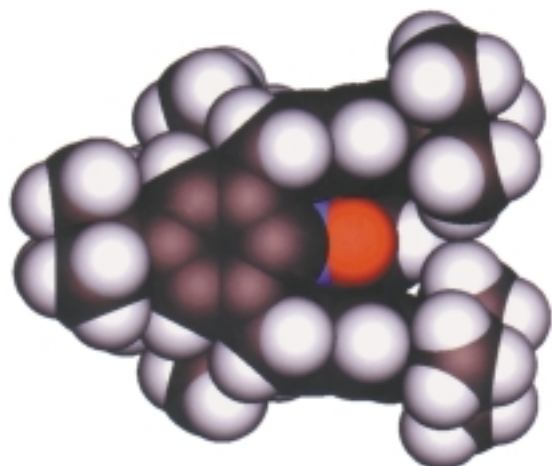


Figure 1. Space-filling representation of $[1\text{-OMe, NO}]^+$ with one of the methoxy groups omitted to reveal the deeply nestled NO.

along the cavity axis. The NO molecule is distributed equally between these chemically equivalent aromatic pairs as shown in Figure 2.^[18] Moreover, we believe that the disordered guest

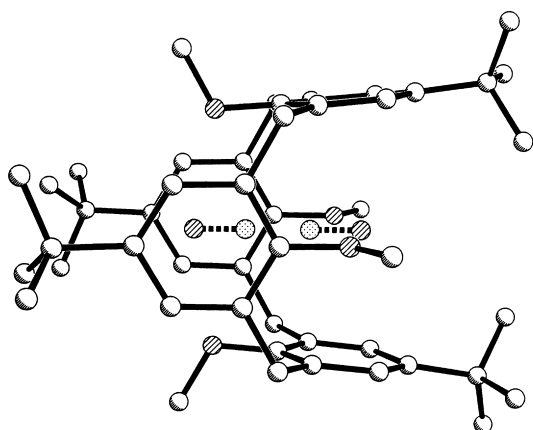


Figure 2. Molecular structure of $[1\text{-OMe, NO}]\text{SbCl}_6$ showing (disordered) nitric oxide distributed equally between the two orthogonally oriented cofacial pairs of aromatic rings that form the cylindrical cavity characteristic of the 1,3-alternate conformer. (Hydrogen atoms and the hexachloroantimonate anion are omitted for clarity.)

(NO) in the 1,3-alternate conformer interchanges its position rapidly between two symmetrically equivalent sites within the calixarene cavity—as evidenced by the simple ^1H NMR spectrum (see below), which suggests that all the aryl groups are chemically equivalent in solution even at very low temperatures. It is noteworthy that this novel molecular structure of a NO-bound calix[4]arene constitutes a unique example of a guest (namely NO) that penetrates deep inside the cavity.^[19] The strong noncovalent (charge-transfer) interactions of nitric oxide with the “soft” π -core of calixarene position it in between the cofacial aromatic rings at a distance (2.4 Å) that is substantially shorter than the van der Waals contact (3.2 Å).

It is noteworthy that the dark purple crystals of the 1,3-alternate conformer of $[1\text{-OMe, NO}]^+$ are stable indefinitely in the solid state at room temperature. We monitored the

^1H NMR spectra of the purple crystals dissolved in CD_2Cl_2 over time to determine its stability in solution. The solution of the 1,3-alternate $[1\text{-OMe, NO}]^+$ ion is stable at least for 30 min and shows four characteristic singlets in the ^1H NMR spectrum (Figure 3a) consistent with its structure.^[20] However, during the course of 24 h it slowly converts into a

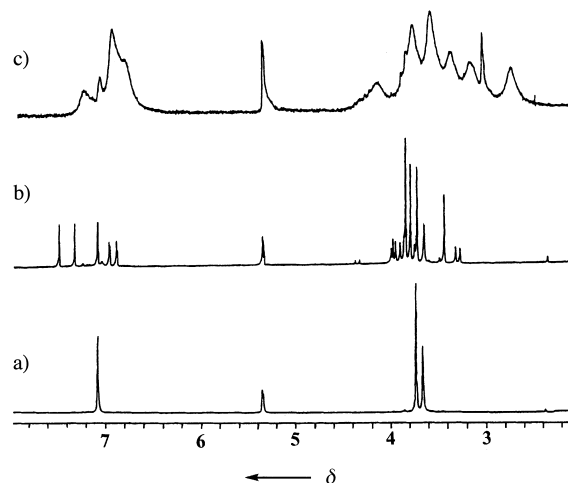
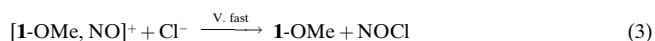


Figure 3. Partial ^1H NMR spectra of isolated 1,3-alternate conformer of $[1\text{-OMe, NO}]^+$ (a), conformational mixture of $[1\text{-OMe, NO}]^+$ at equilibrium (b), and uncomplexed **1-OMe** (c) in CD_2Cl_2 at 25 °C. (Note that the signals for the *tert*-butyl groups are omitted from the various spectra for clarity.)

mixture of conformers (Figure 3b), which is identical to the mixture of conformers obtained by merely dissolving **1-OMe** and NOSbCl_6 in CD_2Cl_2 . The observation of sharp peaks in the NMR spectrum corresponding to four conformers^[21] indicates that the association of NO with **1-OMe** inhibits any conformational mobility on the NMR time scale. In contrast, uncomplexed calixarene **1-OMe** shows broadened NMR signals (Figure 3c) owing to the rapid interconversion of its four conformations (at room temperature), and the resolved signals for the various conformations are only observed at a lower temperature (−30 °C).^[8] The striking preference in the crystallization of the 1,3-alternate (least-stable)^[7, 8] conformer of $[1\text{-OMe, NO}]^+$ points to a crystallization-induced drainage of the slow equilibrium amongst the four conformations in solution.

In an attempt to obtain pure uncomplexed 1,3-alternate conformer of **1-OMe** in solution, crystalline $[1\text{-OMe, NO}]^+$ was dissolved in CD_2Cl_2 and mixed with a prechilled solution of chloride anions (as bis(triphenylphosphoranylidene)ammonium chloride) at −78 °C. As a result, the solution bleached instantaneously as a result of the formation of uncomplexed **1-OMe** and NOCl [Eq. (3)].^[22]



The ^1H NMR spectrum of the resulting solution, recorded immediately at −78 °C, shows the presence of a characteristic mixture of conformers of uncomplexed **1-OMe**. This rapid conversion of one conformer of **1-OMe** to the mixture of conformations even at low temperatures is to be contrasted

with the slow isomerization of $[1\text{-OMe,NO}]^+$ at room temperature (see above).^[23]

To gauge the relative binding abilities of various conformers of calix[4]arene separately towards NO, we examine the rigid conformers of the propyl ether derivative (**2-OPr**).^[12] First, we note that all the conformers of **2-OPr** show blue to purple coloration upon binding to NO,^[24] and the solutions are stable for months if protected from moisture. The high stability of $[2\text{-OPr,NO}]^+$ allows the ready isolation of single crystals of NO-bound 1,3-alternate, cone, and partial cone conformers by slow evaporation of the highly colored solutions in dichloromethane at room temperature. The X-ray structure analysis shown in Figure 4 confirms that in each conformer one pair of cofacial aromatic groups is sufficient for complete entrapment of NO deep inside the calixarene cavity. The NO-bound 1,3-alternate conformers from **1-OMe** and **2-OPr** are essentially isostructural. As yet we are unable to obtain single crystals from the 1,2-alternate conformer that are suitable for X-ray crystallography.^[25]

The similarities in the structures of the various NO-bound calixarene ethers in Figures 1 and 4 are further reflected in their binding abilities towards NO.^[25] Thus, we attempted to estimate the association constants (K_{ass}) for the binding of NO with various conformers of **2-OPr**, using the ratios of integral intensities of ^1H NMR signals of the complexes and the free calixarenes which appear separately.^[26] However, in each case the integral intensity of the complex matches the amount of added NO^+ salt, which implies that K_{ass} is too large to be measured by NMR spectroscopy. However, estimates of $K_{\text{ass}} > 5 \times 10^8 \text{ M}^{-1}$ are obtained for all conformers by a competition method using the Venus flytrap.^[27] Such large binding constants found with all calixarene ethers are unprecedented, and they are readily explained by a complete incarceration of NO into the interior of the calixarene cavities by utilizing a pair of cofacial (parallel) aromatic groups.^[28] We are presently exploring ways to exploit the remarkable binding abilities of calixarenes for the construction of novel molecular sensors for NO as well as other small molecules.

Experimental Section

The calix[4]arene ethers were prepared according to literature procedures^[7,11] and crystallized from a mixture of methanol and dichloromethane. The calixarene ethers were dried in vacuo overnight prior to all complexation studies.

X-ray crystallography. The intensity data were collected at -150°C on a Siemens SMART diffractometer equipped with a CCD detector using MoK_α radiation ($\lambda = 0.71073 \text{ \AA}$). The structures were solved by direct methods^[29] and refined by full matrix least-squares procedures. Crystal Data for $[1\text{-OMe,NO}]\text{SbCl}_6 \cdot [\text{C}_{48}\text{H}_{64}\text{O}_4, \text{NO}] \cdot \text{SbCl}_6 \cdot \text{CH}_2\text{Cl}_2$, $M_r = 1154.38$, tetragonal $P4/n$, $a = 14.654(2)$, $b = 14.654(2)$, $c = 12.583(3) \text{ \AA}$, $\rho_{\text{calcd}} = 1.419 \text{ Mg m}^{-3}$, $V = 2702.0(8) \text{ \AA}^3$, $Z = 2$. The total number of reflections measured was 33028, of which 5983 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0333$ and $wR2 = 0.0656$ for 5983 reflections with $I > 2\sigma(I)$. Crystal data for cone conformer of $[2\text{-OPr,NO}]\text{SbCl}_6 \cdot [\text{C}_{36}\text{H}_{80}\text{O}_4, \text{NO}] \cdot \text{SbCl}_6 \cdot \text{CH}_2\text{Cl}_2 \cdot 0.5 \text{ C}_7\text{H}_8$, $M_r = 1312.65$, monoclinic, $P2_1$, $a = 12.6304(5)$, $b = 24.151(1)$, $c = 20.4071(9) \text{ \AA}$, $\beta = 94.408(1)^\circ$, $\rho_{\text{calcd}} = 1.339 \text{ Mg m}^{-3}$, $V = 6510.6(5) \text{ \AA}^3$, $Z = 4$. The total number of reflections measured was 62475, of which 38612 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0886$ and $wR2 = 0.2370$ for 38612 reflections with $I > 2\sigma(I)$. Crystal data for partial cone conformer of $[2\text{-OPr,NO}]\text{SbCl}_6 \cdot [\text{C}_{36}\text{H}_{80}\text{O}_4, \text{NO}] \cdot \text{SbCl}_6 \cdot \text{C}_7\text{H}_8$, $M_r = 1273.79$, triclinic, $P\bar{1}$, $a = 12.8177(5)$, $b = 14.4156(5)$, $c = 19.1344(7) \text{ \AA}$, $\alpha =$

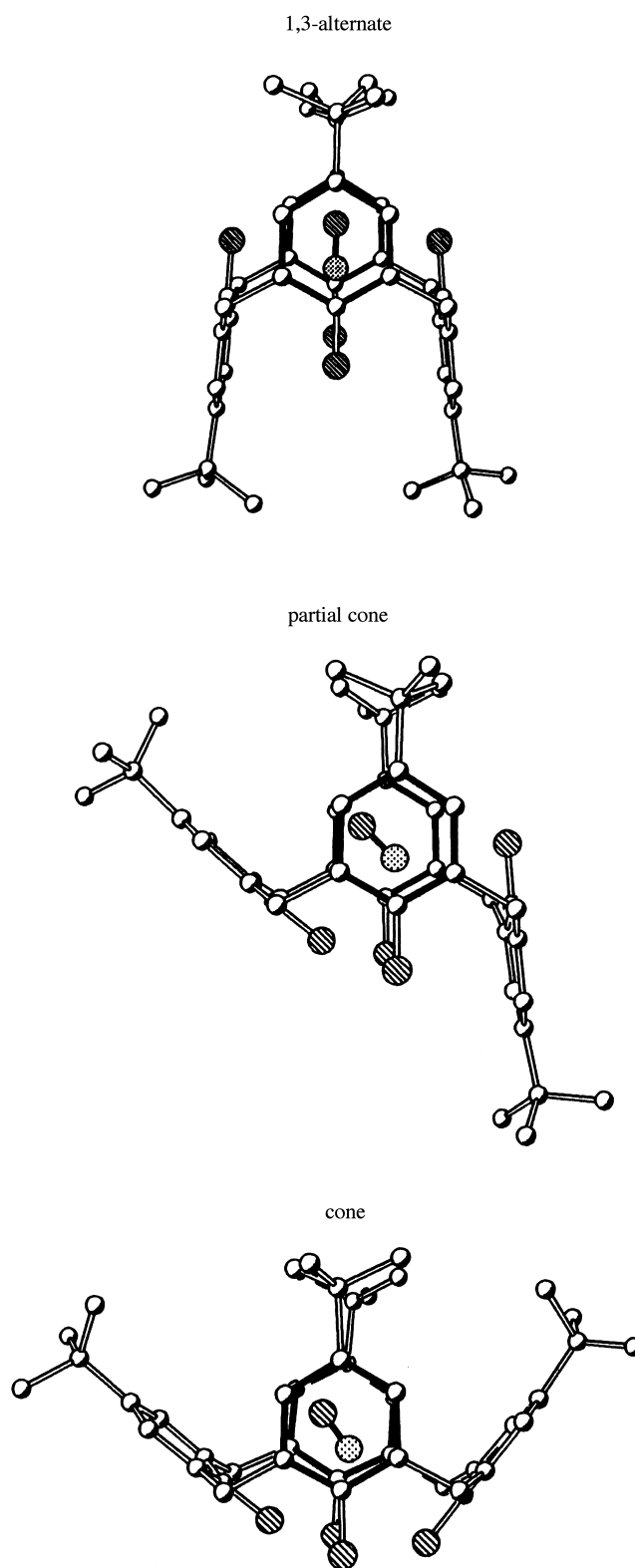


Figure 4. X-ray structures of cone, partial cone, and 1,3-alternate conformers of $[2\text{-OPr,NO}]^+$ showing nitric oxide perfectly sandwiched between a pair of cofacial aromatic groups in each structure. (Hydrogen atoms and propyl groups are removed for clarity.)

$75.745(1)^\circ$, $\beta = 70.585(1)^\circ$, $\gamma = 75.773(1)^\circ$, $\rho_{\text{calcd}} = 1.331 \text{ Mg m}^{-3}$, $V = 3179.5(2) \text{ \AA}^3$, $Z = 2$. The total number of reflections measured was 45348, of which 26946 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0508$ and $wR2 = 0.1322$ for 26946 reflections with

$I > 2\sigma(I)$. Crystal Data for 1,3-alternate conformer of [2-OPr, NO]SbCl₆: [C₅₆H₈₀O₄, NO]·SbCl₆, $M_r = 1181.66$, monoclinic, $P2_1/n$, $a = 12.573(2)$, $b = 33.922(5)$, $c = 14.484(2)$ Å, $\beta = 100.201(4)^\circ$, $\rho_{\text{calcd}} = 1.291 \text{ Mg m}^{-3}$, $V = 6079.9(15) \text{ Å}^3$, $Z = 4$. The total number of reflections measured was 45959, of which 12461 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0566$ and $wR2 = 0.1581$ for 12461 reflections with $I > 2\sigma(I)$. (Note that this structure was solved at room temperature and at -30°C .) Crystal data for [2,6-dimethyl-4-tert-butylanisole, NO]SbCl₆: [C₁₃H₂₀O, NO]·SbCl₆, $M_r = 556.75$, orthorhombic, $Pca2_1$, $a = 15.6673(9)$, $b = 15.3129(9)$, $c = 17.3455(10)$ Å, $\rho = 1.777 \text{ Mg m}^{-3}$, $V = 4161.4(4) \text{ Å}^3$, $Z = 8$. The total number of reflections measured was 58295, of which 18065 reflections were symmetrically nonequivalent. Final residuals were $R1 = 0.0277$ and $wR2 = 0.0628$ for 18065 reflections with $I > 2\sigma(I)$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-139590–139595. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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- [1] a) "Calixarenes": C. D. Gutsche *Monographs in Supramolecular Chemistry, Vol. 1* (Ed.: J. F. Stoddart), Royal Society of Chemistry, Cambridge, **1989**; b) "Calixarenes Revisited": C. D. Gutsche *Monographs in Supramolecular Chemistry* (Ed.: J. F. Stoddart), Royal Society of Chemistry, Cambridge, **1998**, and references therein; c) A. Pochini, R. Ungaro, *Comprehensive Supramolecular Chemistry, Vol. 2* (Ed.: F. Vögtle), Pergamon, NY, **1996**.
- [2] a) H. S. Park, Q. Lin, A. D. Hamilton, *J. Am. Chem. Soc.* **1999**, *121*, 8; b) A. G. Ferguson, J. F. Gallagher, A. J. Lough, A. Notti, S. Pappalardo, M. F. Parisi, *J. Org. Chem.* **1999**, *64*, 5876; c) P. Schmitt, P. D. Beer, M. G. B. Drew, P. D. Sheen, *Tetrahedron Lett.* **1998**, *39*, 6383.
- [3] a) A. Harriman, M. Hissler, P. Jost, G. Wipff, R. Ziessel, *J. Am. Chem. Soc.* **1999**, *121*, 14; b) D. M. Rudkevich, J. Rebek, Jr., *Eur. J. Org. Chem.* **1999**, 1991; c) F. Diederich, M. Gomez-Lopez, *Chem. Soc. Rev.* **1999**, *28*, 263; d) Y.-B. He, H. Huang, L.-Z. Meng, C.-T. Wu, T.-X. Yu, *Chem. Lett.* **1999**, 1329; e) T. Jin, *Chem. Commun.* **1999**, 2491.
- [4] a) S. Shinkai, *Tetrahedron* **1993**, *49*, 8933; b) A. Ikeda, T. Tsudera, S. Shinkai, *J. Org. Chem.* **1997**, *62*, 3568; c) A. Ikeda, S. Shinkai, *J. Am. Chem. Soc.* **1994**, *116*, 3102; d) A. Ikeda, S. Shinkai, *Chem. Rev.* **1997**, *97*, 1713.
- [5] A. Casnati, A. Pochini, R. Ungaro, F. Ugozzoli, F. Arnaud, S. Fanni, M.-J. Schwing, R. J. M. Egberink, F. de Jong, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1995**, *117*, 2767, and references therein.
- [6] a) A. Ikeda, H. Tsuzuki, S. Shinkai, *J. Chem. Soc. Perkin Trans. 2* **1994**, 2073, and references therein; b) Note that Ag⁺ requires a highly specific ligand arrangement within its coordination sphere, which cannot be achieved by penetrating inside the calixarene or into the cavities of other elaborately designed cyclophanes (a study describing the details of Ag⁺ binding with aromatic donors will be reported elsewhere).
- [7] a) C. D. Gutsche, B. Dhawan, J. A. Levine, K. H. No, L. J. Bauer, *Tetrahedron* **1983**, *39*, 409; b) J.-D. van Loon, L. C. Groenen, S. S. Wijmenga, W. Verboom, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1991**, *113*, 2378; c) L. C. Groenen, J.-D. van Loon, W. Verboom, S. Harkema, A. Casnati, R. Ungaro, A. Pochini, F. Ugozzoli, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1991**, *113*, 2385.
- [8] a) S. Fischer, P. D. J. Grootenhuys, L. C. Groenen, W. P. van Hoorn, F. C. J. M. van Veggel, D. N. Reinhoudt, M. Karplus, *J. Am. Chem. Soc.* **1995**, *117*, 1611; b) T. Harada, J. M. Rudzinski, S. Shinkai, *J. Chem. Soc. Perkin Trans. 2* **1992**, 2109, and references therein.
- [9] a) P. L. Feldman, O. W. Griffith, D. J. Stuehr, *Chem. Eng. News* **1993**, Dec. 20, 26; b) E. Culotta, D. E. Koshland, Jr., *Science* **1992**, *258*, 1862, and references therein; c) L. K. Keefer, *CHEMTECH* **1998**, *28*, 30.
- [10] R. Rathore, S. V. Lindeman, J. K. Kochi, *Angew. Chem.* **1998**, *110*, 1665; *Angew. Chem. Int. Ed.* **1998**, *37*, 1585; also see R. Rathore, J. K. Kochi, *J. Org. Chem.* **1998**, *63*, 8630.
- [11] K. Iwamoto, K. Araki, S. Shinkai, *J. Org. Chem.* **1991**, *56*, 4955.
- [12] The oxidation of calix[4]arene methyl ether (1-OMe) yields the yellow-green cation radical ($\lambda_{\text{max}} = 440 \text{ nm}$ and a very broad band extending beyond 1600 nm). The one-electron oxidation can also be carried out electrochemically at $E_{\text{ox}}^0 = 1.4 \text{ V}$ versus the saturated calomel electrode (SCE) in anhydrous dichloromethane (containing tetra-*n*-butylammonium hexafluorophosphate as the supporting electrolyte) or by electron transfer with either the stable cation radical of 1,1,4,4,7,7,10,10-octamethyloctahydronaphthacene (R. Rathore, P. Le Maguerès, S. V. Lindeman, J. K. Kochi, *Angew. Chem.* **2000**, *112*, 818; *Angew. Chem. Int. Ed.* **2000**, *39*, 809) or a mixture of trifluoroacetic acid and dichlorodicyanobenzoquinone in dichloromethane (R. Rathore, J. K. Kochi, *Acta Chem. Scand.* **1998**, *52*, 114).
- [13] UV/Vis spectral analysis confirms that the color arises from a broad UV/Vis absorption band at $\lambda_{\text{max}} = 546 \text{ nm}$, $\epsilon_{\text{max}} = 7600 \text{ M}^{-1} \text{ cm}^{-1}$.
- [14] Free NO absorbs at $\tilde{\nu}_{\text{NO}} = 1875 \text{ cm}^{-1}$ (W. G. Fateley, H. A. Bent, B. Crawford, Jr., *J. Chem. Phys.* **1959**, *31*, 204). For the IR data for other NO complexes, see E. K. Kim, J. K. Kochi in ref. [28].
- [15] The nitrosonium (NO⁺) cation was used as the hexachloroantimonate (SbCl₆⁻) salt.
- [16] Such a high association constant for NO is unprecedented and exceeds the value previously reported for Venus flytrap by at least two orders of magnitudes.^[10]
- [17] Note that no fewer than 10 crops from three crystallizations at different temperatures invariably yielded crystals with identical cell parameters (see Experimental Section).
- [18] The uncomplexed 1,3-alternate conformer of 1-OMe (belonging to a point symmetry S_4) occupies the special position of symmetry -4 (that is, an inversion fourfold axis) in the crystals of [1-OMe, NO]⁺. Such symmetry requires the NO to be disordered and equally distributed over two positions along the twofold axis (a subelement of -4 symmetry). Unfortunately, X-ray crystallographic studies do not distinguish between dynamic and statistical disorder.
- [19] See: a) J. L. Atwood, F. Hamada, K. D. Robinson, G. W. Orr, R. L. Vincent, *Nature* **1991**, *349*, 683; b) J. W. Steed, R. K. Juneja, J. A. Atwood, *Angew. Chem.* **1994**, *106*, 2571; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2456; c) C. Wieser-Jeunesse, D. Matt, A. De Cian, *Angew. Chem.* **1998**, *110*, 3027; *Angew. Chem. Int. Ed.* **1998**, *37*, 2861, and references therein.
- [20] The four singlets in the ¹H NMR spectrum of 1,3-alternate [1-OMe, NO]⁺ in Figure 3a appear at $\delta = 1.33$ (36H), 3.64 (8H), 3.72 (12H), and 7.08 (8H), and are deshielded relative to the uncomplexed 1-OMe.^[7]
- [21] The conformational distribution of [1-OMe, NO]⁺ in solution at equilibrium is (roughly) estimated to be 70:15:10:5 for partial cone, cone, 1,3-alternate, and 1,2-alternate, respectively. This conformer distribution is slightly different from that of the uncomplexed 1-OMe in CD₂Cl₂.^[7, 8]
- [22] NOCl was identified by comparison of the UV/Vis spectra with an authentic sample, see D. W. A. Scharp, J. Thornley, *J. Chem. Soc.* **1963**, 3557.
- [23] a) The slow equilibration of 1,3-alternate conformer of [1-OMe, NO]⁺ may be accounted by the fact that it cannot undergo isomerization while it is complexed to NO. We believe that the rapid shuttling of NO within the calixarene cavity may lead to occasional NO loss (despite the high binding constants); and the resulting uncomplexed 1-OMe undergoes rapid isomerization to the four characteristic conformers (see above) followed by complexation with NO. b) Unfortunately, we are as yet unable to cool the solution to temperatures low enough to observe a stabilized (uncomplexed) 1,3-alternate conformer of 1-OMe.
- [24] The UV/Vis spectral data for the various conformations of [2-OPr, NO]⁺ in dichloromethane are as follows: cone: $\lambda_{\text{max}} = 572 \text{ nm}$, $\epsilon_{\text{max}} = 8800 \text{ M}^{-1} \text{ cm}^{-1}$; partial cone: $\lambda_{\text{max}} = 556 \text{ nm}$, $\epsilon_{\text{max}} = 7700 \text{ M}^{-1} \text{ cm}^{-1}$; 1,3-alternate: $\lambda_{\text{max}} = 569 \text{ nm}$, $\epsilon_{\text{max}} = 5600 \text{ M}^{-1} \text{ cm}^{-1}$; 1,2-alternate: $\lambda_{\text{max}} = 540 \text{ nm}$, $\epsilon_{\text{max}} = 1900 \text{ M}^{-1} \text{ cm}^{-1}$ with a shoulder at 420 nm.^[25]
- [25] The UV/Vis absorption band of the 1,2-alternate conformer shows a prominent shoulder at 420 nm as well as a considerably reduced molar absorptivity,^[24] which suggests an incomplete overlap of nitric oxide with the aromatic groups as a result of the unique structure of this conformer. see: R. Rathore, S. V. Lindeman, J. K. Kochi in ref. [28].
- [26] ¹H NMR data for various conformers of [2-OPr, NO]⁺ in CDCl₃ at 25 °C are as follows: 1,3-alternate: $\delta = 1.03$ (t, 12H), 1.30 (s, 36H), 1.88 (m, 8H), 3.75 (t, 8H), 3.63 (s, 8H), 7.03 (s, 8H); cone: $\delta = 1.08$ (t, 12H), 1.13 (s, 36H), 1.99 (m, 8H), 3.49 (d, $J = 12.9 \text{ Hz}$, 4H), 4.02 (t, 8H), 4.42 (d, $J = 12.9 \text{ Hz}$, 4H), 7.00 (s, 8H); partial cone: $\delta = 0.7 - 1.95$

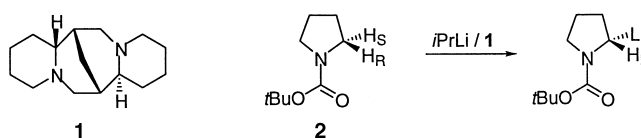
(m, 20H), 1.11 (s, 18H), 1.40 (s, 9H), 1.49 (s, 9H), 3.41 (d, $J = 12.9$ Hz, 4H), 3.75 (m, 6H), 3.94 (m, 2H), 4.06 (d, $J = 13.0$ Hz, 4H), 6.80 (d, 2H), 6.92 (d, 2H), 7.35 (s, 2H), 7.61 (s, 2H); 1,2-alternate: $\delta = 0.77$ (t, 12H), 0.90 (m, 4H), 1.44 (m, 4H), 1.48 (s, 36H), 2.91 (d, 2H), 3.55 (m, 4H), 3.62 (m, 4H), 3.63 (s, 4H), 3.97 (d, 2H), 7.12 (d, 4H), 7.22 (d, 2H).

- [27] The binding constants are estimated using the Venus flytrap ($K_{\text{ass}} = 3 \times 10^6 \text{ M}^{-1}$ for NO incarceration) according to a competition method described earlier, see E. Bosch, J. K. Kochi, *J. Org. Chem.* **1995**, *60*, 3172.
- [28] In contrast, the model 2,6-dimethyl-4-*tert*-butylanisole (DMBA) results in a pale red-brown solution upon mixing with NO^+SbCl_6 ($K_{\text{ass}} \sim 700 \text{ M}^{-1}$) in dichloromethane at 25°C . X-ray structure analysis of a single crystal of DMBA complexed with NO^+ grown from a mixture of dichloromethane and hexane at -30°C shows an approximately perpendicular orientation of NO poised centrally over the aromatic ring (see Experimental Section). This structure is similar to those observed previously with other single aromatic donors such as hexamethylbenzene (R. Rathore, S. V. Lindeman, J. K. Kochi, *J. Am. Chem. Soc.* **1997**, *119*, 9393) and mesitylene (E. K. Kim, J. K. Kochi, *J. Am. Chem. Soc.* **1991**, *113*, 4962).
- [29] G. M. Sheldrick, SHELXS-86. Program for Structure Solution, University of Göttingen; Germany (1986).

A Transition State for the Enantioselective Deprotonation of *N*-Boc-Pyrrolidine with Isopropylolithium/(–)-Sparteine**

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Over the course of the past decade, pioneering studies by the groups of Hoppe^[1] and Beak^[2] have demonstrated that the 1:1 complex of a *sec*-alkyllithium (such as *sec*-BuLi or *i*PrLi) and (–)-sparteine (**1**) is a remarkably efficient reagent for the highly enantioselective deprotonation that produce configurationally stable dipole-stabilized organolithium species.^[3] A particularly simple example of such an asymmetric deprotonation is provided by the lithiation of *N*-Boc-pyrrolidine (**2**; Boc = *tert*-butoxycarbonyl) with *i*PrLi/(–)-sparteine (Scheme 1). Beak et al. have shown that this kinetically controlled process proceeds with a remarkably high selectivity ($95 \pm 5\%$)^[2c] for abstraction of the *pro-S* hydrogen atom at C(2).^[2, 3] Moreover, **1** appears to be the ligand of choice for such a deprotonation: A variety of structurally diverse chiral



Scheme 1. Kinetically controlled deprotonation of *N*-Boc-pyrrolidine.

diamines have been screened as potential ligands for the asymmetric deprotonation of **2** but none was found to be nearly as effective as is **1**.^[2d]

In light of the current interest in development of a transition state model that accounts for the ability of *sec*-alkyllithium/(–)-sparteine to effect highly enantioselective deprotonation,^[3–5] it was of interest to investigate whether modern ab initio molecular orbital calculations could adequately describe the outcome of the asymmetric deprotonation of **2**. In this connection, we have recently reported a computational study of the conformational isomers of **1** and of the transition states for their interconversion.^[6] We also have examined the complexes of **1** with both LiH and with *n*PrLi; in each of these cases, **1** behaves as a bidentate ligand and adopts a conformation that lies just above the lowest energy conformer for the uncomplexed ligand.^[6] The structure of the LiH/(–)-sparteine complex is shown in Figure 1.

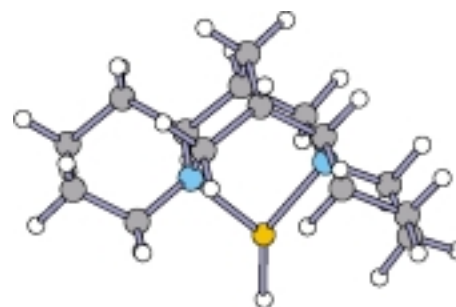


Figure 1. The LiH complex of (–)-sparteine; nitrogen atoms are blue, lithium is orange.

On the reasonable assumption that the deprotonation reaction depicted in Scheme 1 involves precomplexation of the base, ligand, and substrate,^[3, 7] the reactive species would have an isopropyl group in place of the hydrogen atom shown in Figure 1 and the carbonyl group of **2** would presumably serve as a fourth ligand, which gives a near-tetrahedral arrangement around the lithium atom. Thus, with respect to the view portrayed in Figure 1, the *i*Pr group may be either forward or behind the plane and **2** takes the other position. Similarly, the pyrrolidine ring of **2** may be either to the right or the left of the lithium atom and the *tert*-butoxy group occupies the other position. Given these preliminaries, there are four potential structures to be examined.

Because of the very large size of the complexes ($\text{C}_{27}\text{H}_{50}\text{N}_3\text{O}_2\text{Li} = 83$ atoms), the initial geometry optimizations were initially carried out at the STO-3G level and refined at the HF/3-21G level.^[8] While this theoretical level sometimes gives unsatisfactory relative energies, it is known that it usually gives quite good geometries.^[9] Of the four structures examined, the lowest energy complex **3**, depicted in Figure 2, had the shortest distance ($3.10 \text{ \AA}$) between the

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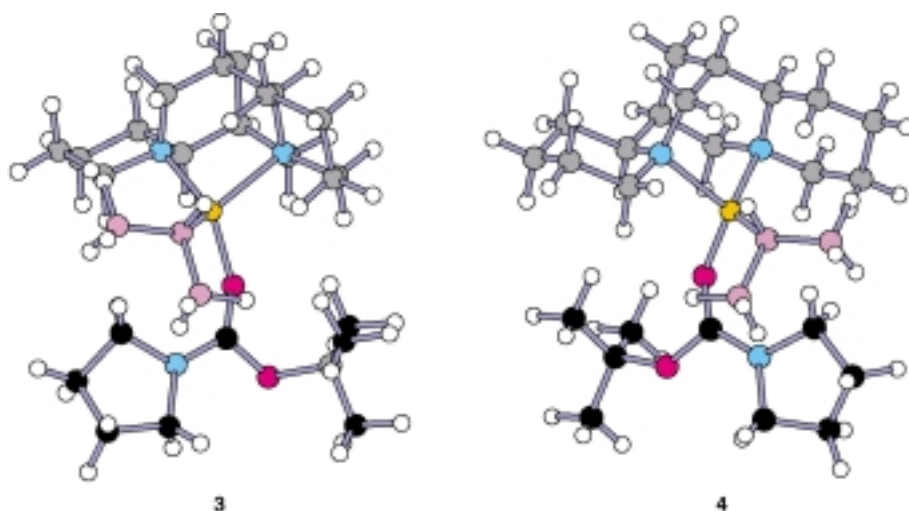


Figure 2. The lowest energy complexes, **3** and **4**, of (–)-sparteine/*i*PrLi/*N*-Boc-pyrrolidine; the oxygen atoms are red and the isopropyl carbon atoms are purple, other colors are as Figure 1.

carbanionic isopropyl center and an α -proton on the pyrrolidine ring; details are summarized in Table 1.^[10] A transition state for proton transfer from the bound pyrrolidine in **3** to the

energy (Table 1) than the transition state for transfer of the *pro-R* hydrogen within complex **3**. In order to obtain more satisfactory relative energies, B3P86/6-31G* geometry optimizations^[13] were carried out for **3** and **4** (Figure 2) as well as for their transition states (**3-TS** and **4-TS**, Figure 3 and 4). Vibrational frequencies were calculated at the HF/3-21G level and scaled by 0.917.^[9b] These results are summarized in Table 1. The calculated energies, corrected for the differences in zero-point energies, lead to an activation enthalpy for the transfer of the *pro-S* hydrogen atom of the pyrrolidine moiety within the more stable complex (**4**) of 11.0 kcal mol^{–1}. Conversely, transfer of the *pro-R* hydrogen from the pyrrolidine ring of complex **3**, which is some 3.1 kcal mol^{–1} less stable than **4**, is calculated to require an activation enthalpy of 11.8 kcal mol^{–1}. These are quite rea-

Table 1. Diastereoisomeric (–)-sparteine/*i*PrLi/*N*-Boc-pyrrolidine complexes and transition states for the proton transfer.

	Species ^[a]			
	3	3-TS	4	4-TS
HF/3-21G ^[b]	–1364.56160	–1364.52160	–1364.56792	–1364.53232
proton transfer distance [Å]	3.10		2.66	
ZPE ^[c] [kcal mol ^{–1}]	464.8	461.1	465.0	461.8
B3P86/6-31G* ^[b]	–1385.87094	–1385.84627	–1385.87620	–1385.85355
<i>E</i> _{rel} [kcal mol ^{–1}]	3.1		0.0	
$\Delta H^\ddagger$ ^[d] [kcal mol ^{–1}]		11.8		11.0

[a] See Figures 2–4. [b] Energies are given in Hartrees (1 H = 627.51 kcal mol^{–1}). [c] Zero-point energies. [d] At 0 K; the correction to 195 K did not change the results.

bound *i*PrLi was located using the synchronous transit-guided quasi-Newton method of Schlegel et al.^[11] to give the structures in Figure 3. It is apparent from a cursory examination that proton transfer within **3** leads to abstraction of a *pro-R* hydrogen atom from bound **2**; this is clearly contrary to the experimental result.^[2, 3]

Consideration of the structure of complex **3** (Figure 2) led to the realization that it is possible to rotate the pyrrolidine unit so that the *pro-S* hydrogen would lie closer to the carbanionic center of *i*PrLi. Not surprisingly, this led to a severe steric interaction between a hydrogen atom on (–)-sparteine and the *tert*-butoxy group of **2** but this could be relieved through a rotation of the latter.^[12] Geometry optimization, performed as described above, gave a slightly lower energy complex **4** (Figure 2) with a shorter distance (2.66 Å) for the proton transfer (Table 1). More significantly, the transition state for transfer of the *pro-S* hydrogen from the bound pyrrolidine within **4**, located as described above and depicted in Figure 4, was found to have a substantially lower

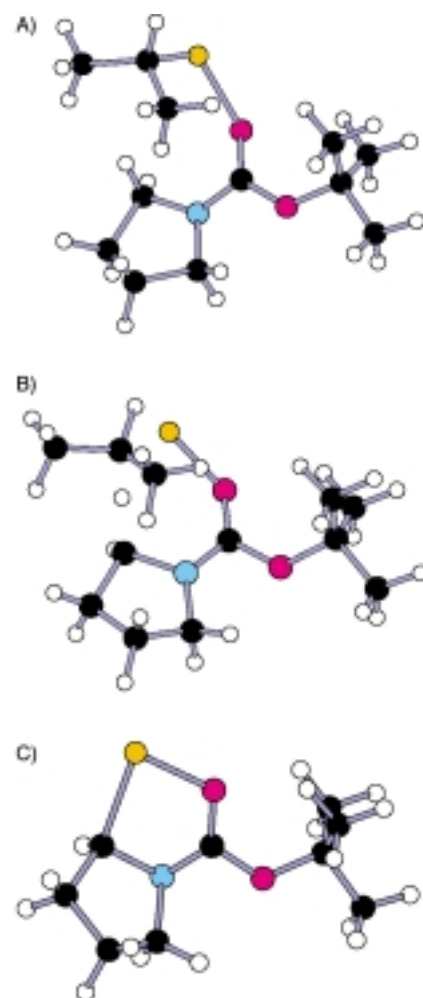


Figure 3. Proton transfer within complex **3**: A) complex **3** with (–)-sparteine removed; B) transition state (**3-TS**) for the transfer of the *pro-R* hydrogen; C) product of the reaction; atomic colors are as Figure 2.

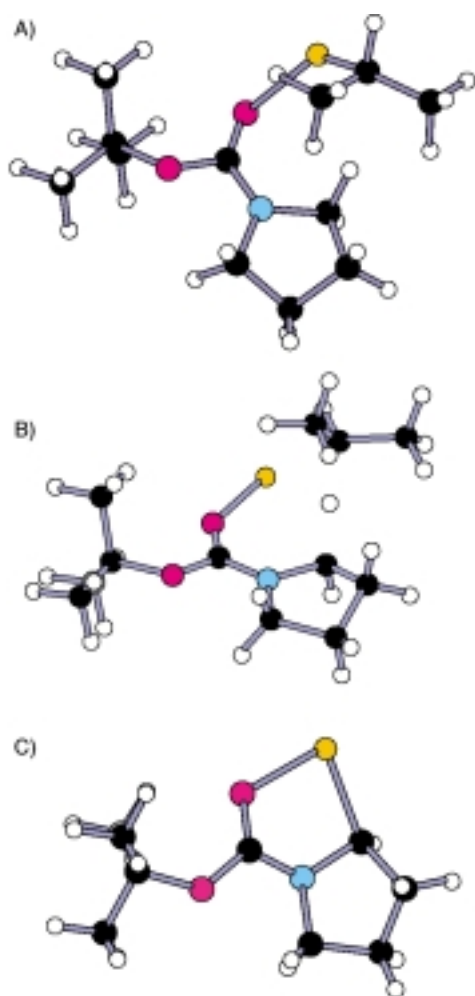


Figure 4. Proton transfer within complex **4**: A) complex **4** with (–)-sparteine removed; B) transition state (**4-TS**) for the transfer of the *pro-S* hydrogen; C) product of the reaction; atomic colors are as Figure 2.

sonable activation energies for a reaction that occurs at a relatively low temperature. An important feature of both transition states is the development of a bond between the lithium and the α -pyrrolidine carbon atoms as the proton is transferred (Figures 3 and 4). This is precisely what is expected for a kinetically controlled asymmetric deprotonation.^[2, 3]

What is the origin of the difference in energy between the transition states derived from **3** and **4**? This question was explored by examination of all calculated nonbonding distances within the range of 2 to 3 Å between the (–)-sparteine unit and the bound *i*PrLi and *N*-Boc-pyrrolidine units. For both complexes, there are several short distances (namely, 2.1–2.2 Å) between hydrogen atoms on the sparteine ligand and those on the *i*PrLi, as well as those of the *tert*-butoxy group of bound **2**. The distances are, however, shorter in **3** than **4**, leading to the difference in energy. This difference persists in the transition states derived from **3** and **4**.

Since all of the important interactions appear to be steric in origin, it should be possible to model the complexes by molecular mechanics using suitable parameters for a four-coordinate lithium center that can be derived from the present

calculations. This is being investigated and will be reported subsequently along with calculations for related systems.

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- [1] a) D. Hoppe, F. Hintze, P. Tebben, *Angew. Chem.* **1990**, *102*, 1457–1458; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1422–1423; b) J. Schwerdtfeger, D. Hoppe, *Angew. Chem.* **1992**, *104*, 1547; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1505; c) M. Oestreich, R. Fröhlich, D. Hoppe, *J. Org. Chem.* **1999**, *64*, 8616–8626, and references therein.
- [2] a) S. T. Kerrick, P. Beak, *J. Am. Chem. Soc.* **1991**, *113*, 9708–9710; b) D. J. Gallagher, S. T. Kerrick, P. Beak, *J. Am. Chem. Soc.* **1992**, *114*, 5872–5873; c) P. Beak, S. T. Kerrick, S. Wu, J. Chu, *J. Am. Chem. Soc.* **1994**, *116*, 3231–3239; d) D. J. Gallagher, S. Wu, N. A. Nikolic, P. Beak, *J. Org. Chem.* **1995**, *60*, 8148–8154.
- [3] For reviews, see: a) D. Hoppe, T. Hense, *Angew. Chem.* **1997**, *109*, 2376–2410; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2283–2316; b) P. Beak, A. Basu, D. J. Gallagher, Y. S. Park, S. Thayumanavan, *Acc. Chem. Res.* **1996**, *29*, 552–560.
- [4] Semiempirical PM3 calculations probing diastereoselectivity in the deprotonation of chiral alkyl carbamates have been reported: see, J. Haller, T. Hense, D. Hoppe, *Liebigs Ann.* **1996**, 489–499.
- [5] a) Recently, a semiempirical PM3 study supplemented with ab initio calculations has been reported for transition state models of the asymmetric deprotonation of achiral alkyl carbamates by *i*PrLi in the presence of the C_2 symmetric ligand (*R,R*)-1,2-bis(*N,N*-dimethylamino)cyclohexane: see, E. U. Würthwein, K. Behrens, D. Hoppe, *Chem. Eur. J.* **1999**, *5*, 3459–3463; b) For a discussion of amine–lithium interactions, see: A. M. Sapsee, P. von R. Schleyer, *Lithium Chemistry: a Theoretical and Experimental Overview*, Wiley, New York, **1995**.
- [6] K. B. Wiberg, W. F. Bailey, *J. Mol. Struct.* in press.
- [7] Beak et al.^[2b] has elegantly demonstrated that *i*PrLi/(–)-sparteine exists in an ethereal solution as an unsymmetrical, ether-solvated dimer [(*i*PrLi)₂·(–)-sparteine] but this structure clearly need not be maintained in the deprotonation of **2**.
- [8] Calculations were performed using Gaussian 99 (Development Version (Revision B.04), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, A. G. Baboul, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian Inc., Pittsburgh PA, **1998**).
- [9] a) W. J. Hehre, P. Radom, P. von R. Schleyer, J. A. Pople, *Ab Initio Molecular Orbital Theory*, Wiley, NY, **1985**; b) J. W. Ochterski, G. A. Petersson, J. A. Montgomery Jr., *J. Chem. Phys.* **1996**, *104*, 2598–2619.
- [10] Detailed structural data may be found in the Supporting Information.
- [11] a) C. Peng, P. Y. Ayale, H. B. Schlegel, M. J. Frisch, *J. Comput. Chem.* **1996**, *17*, 49–56; b) C. Peng, H. B. Schlegel, *Isr. J. Chem.* **1994**, *33*, 449–454.
- [12] Structures were constructed, examined, and adjusted prior to geometry optimization using Gaussview. The structures of the other three initial complexes were also modified by rotation about single bonds in order to move the bound *i*PrLi closer to the α -carbon of the pyrrolidine ring; geometry optimization for these structures did not lead to species with energies lower than those found for **3** or **4**.
- [13] a) A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652; b) J. P. Perdew, *Phys. Rev. B: Condens. Matter* **1986**, *33*, 8822–8824.

Chiral Guests and Their Ghosts in Reversibly Assembled Hosts**

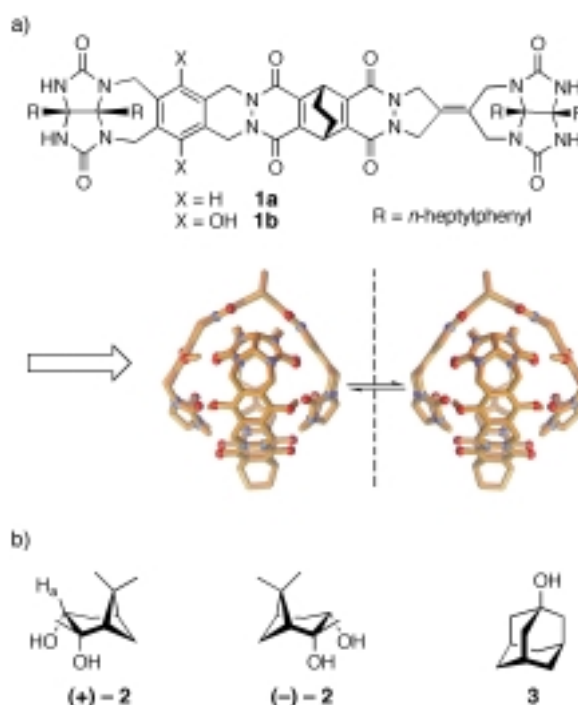
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Encapsulation complexes are assemblies in which small molecular guests are completely by large molecular hosts.^[1–4] The hosts are made up of subunits held together by intermolecular forces: hydrogen bonds, van der Waal's forces, and/or metal–ligand interactions. The assemblies are formed reversibly and are dynamic; they come together and dissipate on time scales ranging from milliseconds to days, long enough for their study by NMR methods. When multiple hosts can assemble from a given set of subunits, template effects can be expected and these have recently been reported.^[5–9]

Here we report an encapsulation complex with the additional characteristic that hydrogen bonds maintain an imprint—the ghost—of a long-departed guest. Specifically, an asymmetric microenvironment is imprinted in a reversibly formed capsular host by a chiral guest template. Removal of the template leaves a chiral, nonracemic (g)host capsule that can be characterized by NMR spectroscopy. Recognition of the guest rather than its mirror image persists for hours in organic solvents.

We recently reported a capsule known as the chiral “softball” **1a**·**1a** (Scheme 1a).^[5] It is formed when two self-complementary subunits **1a** dimerize in organic solvents through a seam of eight hydrogen bonds. The subunits feature a plane of symmetry and are achiral, but the dimer has only *C*₂ axes and exists as a pair of enantiomers. The cavity of the capsule is a distorted sphere and asymmetric guests generally prefer one enantiomer of the capsule to its mirror image. The enantiomeric capsules can interconvert (racemize) only by complete dissociation and recombination of their subunits (monomer exchange). Evidence reported elsewhere^[10–12] indicates that guests get in and out of these capsules through flaps that are opened by the breaking of hydrogen bonds as conformational changes occur.

A related structure **1b** was prepared for the imprinting studies.^[13] It dimerizes into capsules that are more robust and assemble in a number of solvents. The phenolic groups introduce four additional hydrogen bonds in the corresponding capsule **1b**·**1b** and slow its rate of racemization. Figure 1 shows how the NMR spectrum of **1b**·**1b** with added chiral



Scheme 1. a) Structure of the monomer **1b** and model of its dimeric assemblies.^[22] Some protons and *n*-heptylphenyl groups in the dimers are omitted for clarity. Atoms are colored as follows: carbon and hydrogen: orange, oxygen: red, nitrogen: blue. b) Guests used in the study.

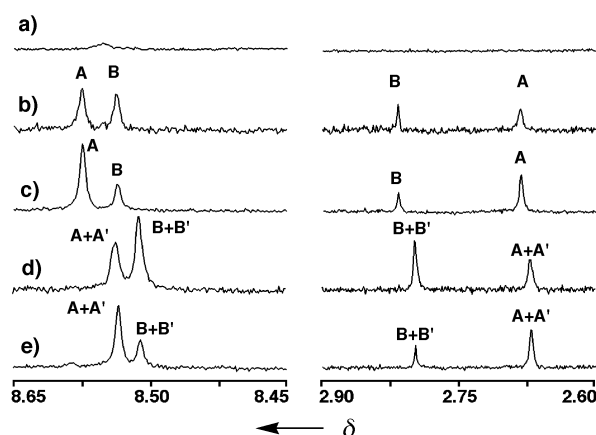


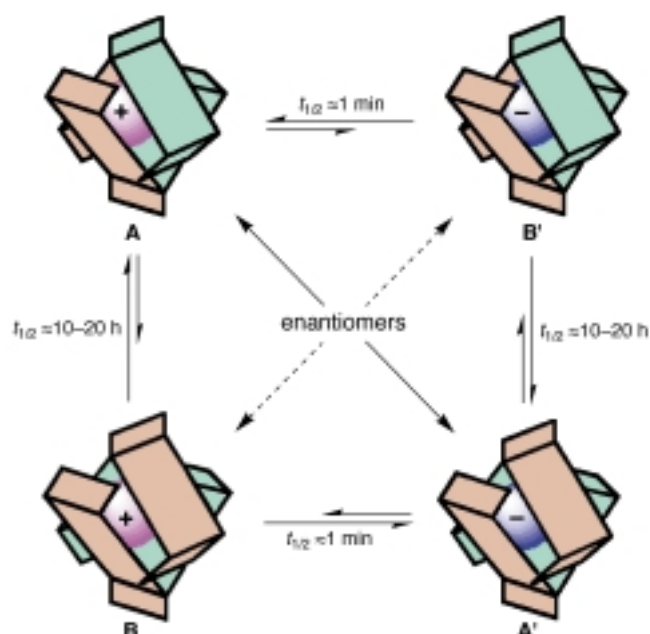
Figure 1. Portions of the ¹H NMR spectra showing the selected NH peaks of the dimer (8.65–8.45 ppm) and H_a peaks for the guest inside the capsules (2.90–2.60 ppm). The labels A and A' denote the thermodynamically more-stable complexes of (+)-**2** and (–)-**2**, respectively, while B and B' denote the respective less-stable complexes. a) **1b**·**1b** in [D₁₀]p-xylene (8.25 × 10^{–4} M); b) 4 min and c) 120 h after addition of 3 equiv of (+)-**2**; d) 17 min and e) 191 h after addition of 30 equiv of (–)-**2**.

guest, (+)-pinanediol ((+)-**2**; Scheme 1b), varies with time. The broad NMR spectrum of a solution of 0.825 mM **1b**·**1b** in [D₁₀]p-xylene is characteristic of unspecific aggregates, but the addition of three equivalents of (+)-**2** yields, within seconds, a sharp spectrum in which the two diastereomeric complexes are initially observed in nearly equal amounts (Figure 1b). Over the course of a few days the system reaches its stereochemical equilibrium—a 50% diastereomeric excess of the favored isomer (Figure 1c). The 19 h half-life for equilibration is also the lifetime of the assembly.

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Thirty equivalents of the enantiomer (–)-**2** were then added to the equilibrated mixture. Within minutes the ratio of the two diastereomers partially inverts, giving a temporary excess of the less stable diastereomer (Figure 1 d). This effect can only result from a guest exchange process that is much faster than dissociation of the assembly, so that the guest (–)-**2** is now present in excess in the capsule that formed preferentially around (+)-**2** (Scheme 2). The rate of guest exchange ($t_{1/2} \approx 1$ min) is in line with that observed in similar systems.^[12]



Scheme 2. Schematic representation of the exchange equilibria. Horizontal equilibria represent guest exchanges and vertical equilibria represent monomer exchanges. The pictures are illustrative only and do not imply the exact stereochemistry of the assemblies.

The diastereomers then re-equilibrate, with a half-life of 9.5 h, until the original 50 % *d.e.* is achieved (Figure 1 e). The rate is twice as fast as that measured for the initial equilibration, and may reflect the greater concentration of the polar, hydrogen-bonding compound **2** in the latter experiment.^[14]

The preference of the ghost for its template was established through competition experiments. When the (+)-**2**-templated capsule is exposed to a mixture of (+)-**2** and (–)-**2**, the ratio of the diastereomers ($[A + A']:[B + B']$, Scheme 2) after guest exchange reflects the relative affinity of (+)-**2** and (–)-**2** for the capsule. We observe that $[A + A']:[B + B'] = 1:1.3$ for a guest ratio (–)-**2**:(+)-**2** = 10:1. This is the expected value based on the 50 % *d.e.* observed in the original templation, and confirms that the ghost prefers the template to its enantiomer.^[15]

The memory persists through multiple guest exchanges and physical manipulations. A solution of **1b**·**1b** was templated with (+)-**2**, and the solvent was removed by evaporation. The excess guest was extracted with hexane, leaving only **1b**·(+)-**2**·**1b**. The complex was dissolved in benzene and an excess of 1-adamantanol (**3**, Scheme 1) was added to displace the remaining (+)-**2**. The NMR spectrum shows that the adamantanol rinses out the chiral guest. The solvent

was removed and extraction gives a solution free of (+)-**2**. To this solution was added an excess of (–)-**2**, which displaced adamantanol from the capsule interior. The corresponding NMR spectrum shows a 2:1 excess of the less-stable diastereomeric complex, which establishes that the chiral memory is maintained even in the absence of the chiral guests. The reaction mixture slowly re-equilibrates, and overnight shows a 2:1 excess of the more stable complex.

The memory effect reflects the relative rates of guest and monomer exchange. While monomer exchange involves the complete dissociation of the capsule (Figure 2 a), guest

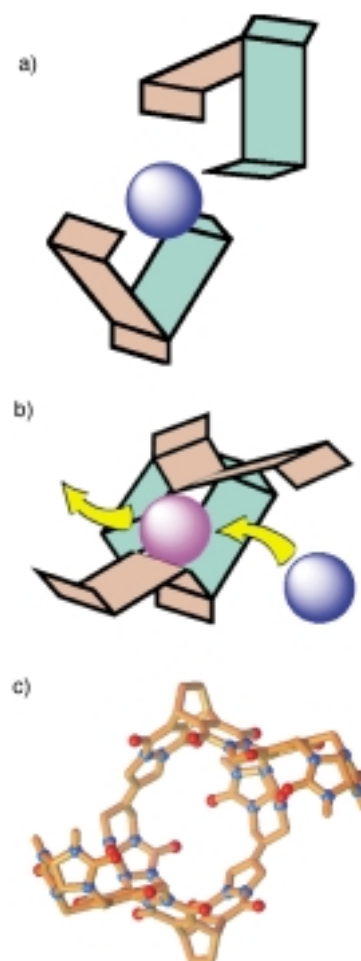


Figure 2. Putative intermediates a) for monomer exchange and b) guest exchange. c) Structural depiction of intermediate (b) showing the “doorways” for guest exchange.

exchange must proceed through doorways that form without disrupting the entire hydrogen-bonded seam of the capsule (Figure 2 b). The pyridazinyl ring of **1b** has a very low barrier to inversion, a process that creates openings in the dimer that are large enough to accommodate incoming and outgoing guests (Figure 2 c).^[11] On average, hundreds to many thousands of molecules enter and depart the capsule during its lifetime, and each guest in turn will experience the imprinted asymmetric microenvironment of the capsule.

Encapsulation influences the conformation, dynamics, and reactivity of the guest,^[1] but examples of nonracemic chiral

encapsulation complexes are rare.^[16–18] Imprinting^[19, 20] in hydrogen-bonded assemblies provides another route to such systems, and recent work promises that behaviors similar to those observed here will also be found in metal–ligand assemblies.^[8] The result augurs well for the application of these capsules in dynamic combinatorial libraries and other enantioselective processes, such as the catalysis of reactions with rates comparable to that of guest exchange.^[21]

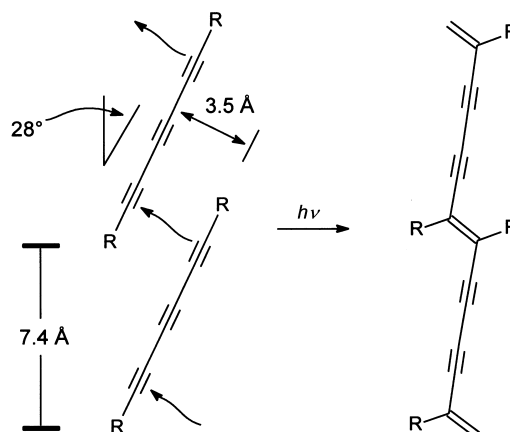
Received: February 7, 2000 [Z14661]

- [1] M. M. Conn, J. Rebek, Jr., *Chem. Rev.* **1997**, 97, 1647–1668.
- [2] T. Kusukawa, M. Fujita, *J. Am. Chem. Soc.* **1999**, 121, 1397–1398.
- [3] T. N. Parac, D. L. Caulder, K. N. Raymond, *J. Am. Chem. Soc.* **1998**, 120, 8003–8004.
- [4] R. G. Chapman, J. C. Sherman, *J. Am. Chem. Soc.* **1998**, 120, 9818–9826.
- [5] J. M. Rivera, T. Martín, J. Rebek, Jr., *Science* **1998**, 279, 1021–1023.
- [6] M. Scherer, D. L. Caulder, D. W. Johnson, K. N. Raymond, *Angew. Chem.* **1999**, 111, 1690–1694; *Angew. Chem. Int. Ed.* **1999**, 38, 1588–1592.
- [7] F. Ibukuro, T. Kusukawa, M. Fujita, *J. Am. Chem. Soc.* **1998**, 120, 8561–8562.
- [8] S. Hiraoka, M. Fujita, *J. Am. Chem. Soc.* **1999**, 121, 10239–10240.
- [9] F. Hof, C. Nuckolls, J. Rebek, Jr., unpublished results.
- [10] T. Szabo, G. Hilmersson, J. Rebek, Jr., *J. Am. Chem. Soc.* **1998**, 120, 6193–6194.
- [11] X. Wang, K. N. Houk, *Org. Lett.* **1999**, 1, 591–594.
- [12] J. Santamaría, T. Martín, G. Hilmersson, S. L. Craig, J. Rebek, Jr., *Proc. Natl. Acad. Sci. USA* **1999**, 96, 8344–8347.
- [13] J. M. Rivera, T. Martín, J. Rebek, Jr., unpublished results.
- [14] Even low concentrations of hydrogen-bonding molecules can have dramatic effects on the rates of assembly processes. R. K. Castellano, S. L. Craig, C. Nuckolls, J. Rebek, Jr., *J. Am. Chem. Soc.*, submitted.
- [15] After templation, A:B=3:1. By assuming that the capsule of A prefers (+)-2 and the capsule of B prefers (–)-2, each by the same 3:1 ratio, then, if (–)-2:(+)-2=10:1, $Aa(3) \times (3) \times (1) = 9$, $A'a(1) \times (3) \times (10) = 30$, $Ba(1) \times (1) \times (1) = 1$, and $B'a(3) \times (1) \times (10) = 30$. We expect, then, that $(A + A'):(B + B') = (31):(39) = 1:1.26$. If, on the other hand, there were no preference for the template, the ratio would be 1:2.4.
- [16] R. K. Castellano, B. H. Kim, J. Rebek, Jr., *J. Am. Chem. Soc.* **1997**, 119, 12671–12672.
- [17] R. K. Castellano, C. Nuckolls, J. Rebek, Jr., *J. Am. Chem. Soc.* **1999**, 121, 11156–11163.
- [18] C. Nuckolls, F. Hof, T. Martín, J. Rebek, Jr., *J. Am. Chem. Soc.* **1999**, 121, 10281–10285.
- [19] For examples of imprinting in covalent materials, see a) K. J. Shea, *Trends Polym. Sci.* **1994**, 2, 166–173; b) G. Wulff, *Angew. Chem.* **1995**, 107, 1958–1979; *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 1812–1832; c) K. Mosbach, O. Ramstroem, *Biotechnology* **1996**, 14, 163–170; d) H. Shi, W.-B. Tsai, M. D. Garrison, S. Ferrari, B. D. Ratner, *Nature* **1999**, 398, 593–597; e) K. Polborn, K. Severin, *Chem. Commun.* **1999**, 24, 2481–2482; f) K. Dabulis, A. M. Klibanov, *Biotechnol. Bioeng.* **1992**, 39, 176–185.
- [20] For examples of memory effects in noncovalent materials, see a) Y. Furusho, T. Kimura, Y. Mizuno, T. Aida, *J. Am. Chem. Soc.* **1997**, 119, 5267–5268; b) E. Yashima, K. Maeda, Y. Okamoto, *Nature* **1999**, 399, 449–451.
- [21] J. Kang, J. Santamaría, G. Hilmersson, J. Rebek, Jr., *J. Am. Chem. Soc.* **1998**, 120, 7389–7390.
- [22] F. Mohamadi, N. G. Richard, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, 11, 440.

A Supramolecular Solution to a Long-Standing Problem: The 1,6-Polymerization of a Triacetylene**

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The 1,6-polymerization of a triacetylene is an unknown transformation.^[1, 2] Unsuccessful attempts to accomplish this polymerization were reported as early as 1972, soon after the remarkable discovery of the topochemically controlled 1,4-polymerization of diacetylenes.^[3] It was recognized that a successful 1,6-polymerization would require preorganization of the reactants. In 1994 Enkelmann gave a more up-to-date report of other unsuccessful attempts of the 1,6-polymerization of triacetylenes and provided a more complete analysis of the criteria necessary for a successful polymerization.^[4] The structural parameters needed for a topochemical triacetylene polymerization can be derived if one assumes that the monomeric units must be preorganized with a defined simple translational distance of 7.4 Å, matching the geometric parameters of the expected polymer (Scheme 1). If the



Scheme 1. A triacetylene should polymerize if it is preorganized with a defined geometry that matches the geometrical parameters of the expected polymer product.

monomers are spaced at this distance and tilted at an angle just under 30° then the neighboring triacetylene functionalities will be in 3.5 Å van der Waals contact, a condition that should maximize the chances of polymerization. These exacting structural requirements are unlikely to be met by any randomly chosen triacetylene.

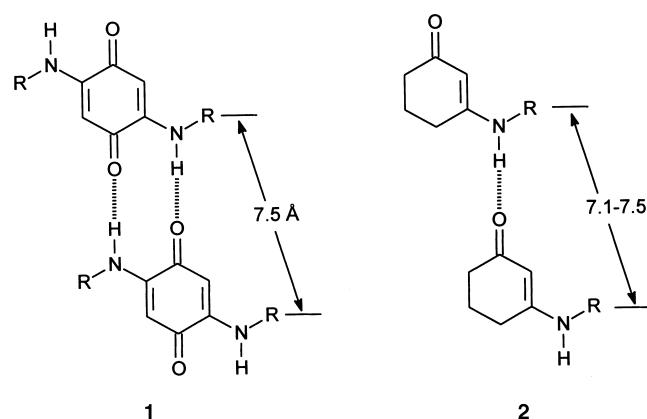
The 1,6-polymerization of a triacetylene is thus a curious chemical problem. It is a long-standing synthetic problem of

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considerable interest and the requirements are very well defined, yet no one has been able to devise a successful synthesis. We believed that the solution to the problem could be found in the fledgling field of supramolecular chemistry. The successful synthesis of a polytriacetylene through a designed 1,6-polymerization would serve as a significant test for supramolecular synthesis in the same manner that complex natural products have often served as a test for conventional molecular syntheses.

The most critical structural parameter to control is the required simple translation distance of about 7.4 Å. If a chemical functionality could be discovered that would self-assemble at such a repeat distance, then one could use this functionality to achieve the necessary preorganization of the triacetylene chains. Inspection of models^[5] suggested the 2,5-diaminoquinone functionality (**1**) would use complementary hydrogen bonds to self-assemble by simple translation with a molecular repeat distance (7.46 Å) close to that believed to be required for polymerization (7.4 Å). Molecular modeling predictions were confirmed by experiment. The glycine ethyl ester derivative of benzoquinone (**1**, R = CH₂CO₂C₂H₅; Scheme 2) was prepared and a single-crystal X-ray structure confirmed that it self-assembled with a molecular repeat of 7.54 Å. Unfortunately, as a class, the diaminoquinones proved to be extremely insoluble, limiting their application to the organization of polytriacetylenes for a topochemical polymerization.



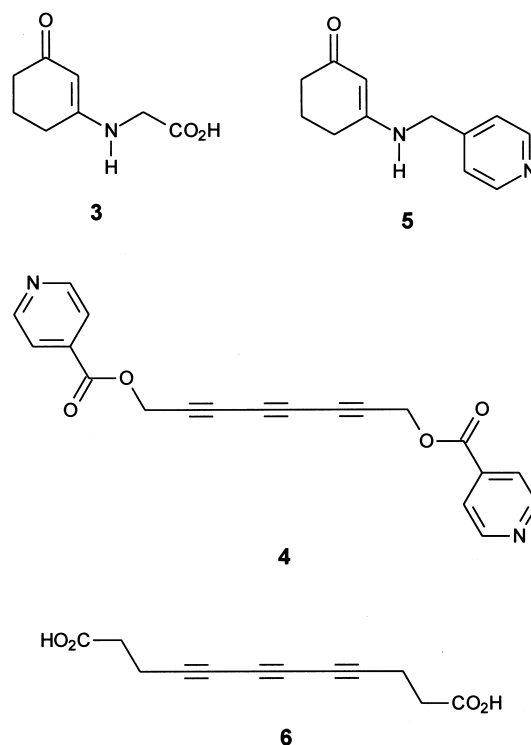
Scheme 2. The diaminoquinones (**1**) and the aminoquinones (**2**) are functional groups capable of self-assembling with a translational distance of approximately 7.4 Å.

Molecular modeling studies also suggested that derivatives of vinylogous amide **2**^[6] could self assemble to give a molecular repeat close to that required for polymerization. They should also have better solubility properties. However, the presence of only one complementary hydrogen bond suggested that the self-assembly of vinylogous amides by simple translation (Scheme 2) might not be dependable. Inspection of the Cambridge Structural Database (CSD) confirms this analysis. Among the six simple derivatives of **2** reported in the CSD only one assembled by simple translation (7.31 Å).^[7] The other five reported vinylogous amides all assembled using the anticipated hydrogen-bonding motif, but neighboring molecules were related by a glide plane or screw

axis. These types of symmetry double the crystallographic repeat distance to an undesirable 14–15 Å. However, we were encouraged by this data and believed the desired spacing could be accomplished by good design.

The next step in the organization of the triacetylene for a topochemical polymerization was to address the problem of associating the triacetylene monomer with the vinylogous amide. The most straightforward approach would be to create a covalent bond between the vinylogous amide and the triacetylene functional groups. Although this approach would probably succeed, one of its disadvantages is the complexity of the organic synthesis and of the ultimate polymer.

An alternative approach is to use host–guest chemistry, that is, to prepare two molecules, the vinylogous amide (the host) and the triacetylene (the guest). The host provides the required supramolecular structural elements and the guest provides the function. The association of these two molecules can be accomplished using a noncovalent link, such as a hydrogen bond. Suitable hydrogen-bonding functionalities that we have previously used^[8] are carboxylic acids and pyridines.^[9] Possible compounds to explore this strategy are the pyridines and carboxylic acids shown in Scheme 3. The



Scheme 3. Vinylogous amide hosts and triacetylene hosts suitable for the topochemical preparation of a polytriacetylene.

major advantages of this approach are the simplification of the molecular synthesis and flexibility. One good host can be used to organize a number of different guests or a number of different hosts can be applied to a specific guest of interest. The disadvantage of this approach is that it requires predictable assemblies using weak noncovalent bonds.

Because of solubility differences between the carboxylic acid host **3** and the dipyridine **4**, preliminary attempts to grow

co-crystals were not promising. Therefore, we turned our attention to the pyridine host **5**. We were pleased to observe that **5** readily co-crystallizes (2:1) with simple dicarboxylic acids such as succinic and adipic acid. Single-crystal X-ray structure determinations demonstrate that the host molecules assemble as illustrated in Scheme 2 with simple translational distances of 7.20 and 7.18 Å, respectively. Curiously, glutaric acid, with an odd number of methylene groups, forms a 1:1 co-crystal with **5**, but with a completely different pattern of hydrogen bonds.

Encouraged by the above results, the triacetylene diacid **6** was prepared. Evaporation of a 2:1 solution of pyridine host **5** and the triacetylene diacid **6** in methanol produced a host–guest compound as pale red crystals. A single-crystal X-ray structure of the host–guest complex (Figure 1a) clearly

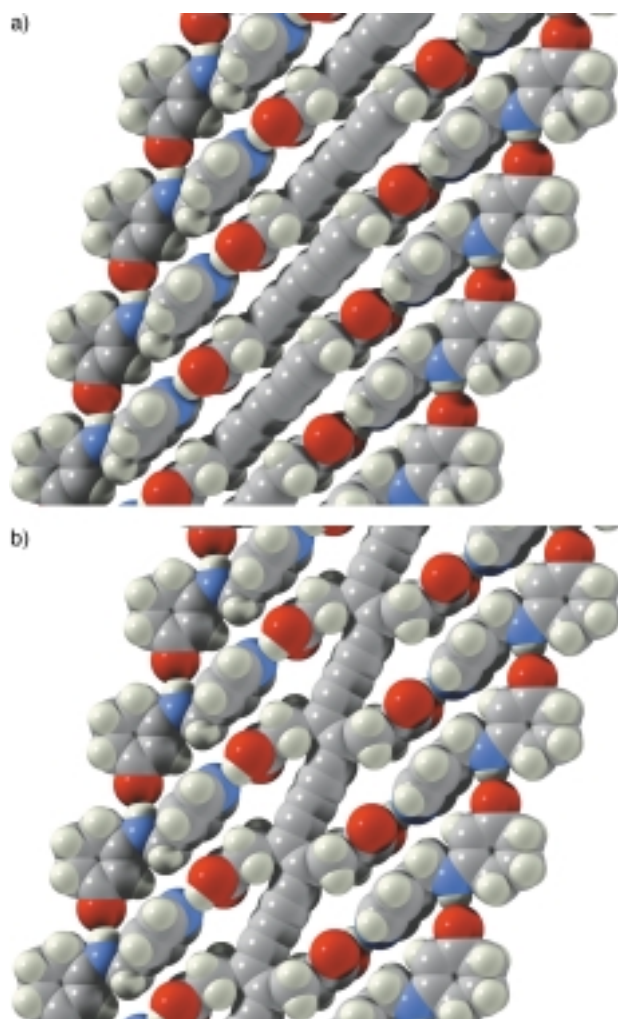


Figure 1. X-ray crystallographic structures of a) the host–guest triacetylene monomer structure and b) the resulting triacetylene polymer.

shows that the molecules have self-assembled in accordance with our design. Equivalent atoms are spaced at 7.143(2) Å, with the triacetylene core at an angle of 29.2° with respect to the translational direction. The triacetylene chains are in van der Waals contact with the crucial nonbonded C1 to C6 distance of adjacent triacetylene molecules of 3.487(2) Å.

Although the repeat distance of 7.143(2) Å is slightly shorter than the targeted value of 7.4 Å, overall these parameters are in good agreement with the numbers illustrated in Scheme 1.

Preliminary experiments to effect polymerization with both heat and light were disappointing: No significant polymerization was observed when the crystals were heated to 109 °C for 72 h or irradiated for 72 h with a Hanovia 550 W medium-pressure mercury lamp. However, exposure of the host–guest crystals to ^{60}Co γ -radiation^[10] resulted in the crystals becoming dark red, a color transformation consistent with polymerization. The topochemical polymerization was followed by X-ray crystallography. Individual single crystals were mounted and the structure of each individual crystal was determined repeatedly after radiation doses of about 8–10 Mrads of γ radiation. The analysis of the diffraction data showed that the irradiated crystals consist of a solid solution of the monomer and its polymer. The polymerization was about 70 % complete after 40 Mrads of radiation (Figure 1b). Further irradiation and polymerization brings about a sudden phase change in the crystals to an amorphous “glassy” state that no longer gives useful diffraction data.

The polytriacetylene was isolated by first extracting the irradiated crystals with concentrated HCl to remove the pyridine host and then by extracting the residue with methanol to remove any unreacted triacetylene. A red, amorphous solid was isolated in about 70 % yield that does not melt up to 300 °C. It is insoluble in common organic solvents such as methanol, acetone, ethyl acetate, and dichloromethane. The carboxylic acid polymer slowly dissolved in sodium hydroxide to form a red solution in concentrated 1M NaOH or alternatively a yellow solution in dilute 0.1M NaOH.^[11] The UV/Vis spectrum (0.011 g L⁻¹ in 0.1M NaOH) of the polymer shows a $\lambda_{\text{max}} = 446$ nm.

Raman spectroscopy has proven to be an excellent method for characterizing conjugated polymers.^[12] The nonpolar multiple bonds usually display intense Raman bands. The polytriacetylene described here is no exception. The Raman spectrum of the polytriacetylene (0.011 g L⁻¹) shows only two intense bands in the 2500–500 cm⁻¹ region at 2148 and 1552 cm⁻¹. These can be assigned to the C≡C and C=C bonds, respectively.

The poly(triacetylenes) are conjugated polymers with a delocalized π system along the polymer backbone. This structural feature imparts special properties to these compounds making them attractive candidates for the development into advanced materials.^[13] The host–guest strategy described above allows for the preparation of other derivatives of this new conjugated polymer and provides a dramatic illustration of the natural evolution of synthetic chemistry from the molecular to the supramolecular level.

Experimental Section

X-ray structure analysis: The host–guest complex (Figure 1) crystallizes in space group $P\bar{1}$ with $a = 7.143(2)$, $b = 10.695(3)$; $c = 11.2825(3)$ Å, $\alpha = 77.28(2)$, $\beta = 90.421(2)$, $\gamma = 80.15(2)^\circ$. $R_w = 4.8$, $R = 6.5$ for 221 variables and 1064 unique observations with $I > 3\sigma$. Freshly prepared crystals consisting of the host and the triacetylene monomer are of high quality. Upon γ irradiation (^{60}Co , 0.8 Mrad h⁻¹), the polymerization begins and the cell constants of the single crystals begin to change. After irradiation the

single crystals consist of solid solutions of the monomer and the polymer (Figure 1b). A typical crystal after 30 Mrads of γ radiation maintained its $P\bar{1}$ space group symmetry with $a = 7.201(2)$, $b = 10.751(3)$, $c = 11.1421(3)$ Å, $\alpha = 76.94(2)$, $\beta = 87.67(2)$, $\gamma = 80.18(2)^\circ$. $R_w = 5.7$, $R = 6.3$ for 208 variables and 1211 unique observations with $I > 3\sigma$. The occupancy of the three independent central atoms of the triacetylene polymer refined to 0.58 for this particular crystal. The experiment was repeated for many different crystals all of which gave similar, but not identical parameters. The a axis of the triclinic unit cell corresponds to the critical polymerization direction. In a fresh crystal this axis has length of 7.143(2) Å. As the polymerization proceeds, the a axis lengthens in proportion, but only slightly. The highest observed value of 7.210(6) Å was for a crystal polymerized to an extent of 70%. Since this value is still considerably shorter than the 7.4 Å repeat distance predicted for the polymer, it is likely that strain may build up in the crystals as the polymerization proceeds. As the degree of polymerization exceeds 70%, this accumulated strain in the crystals may be the driving force of the change to an amorphous state. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-138504–135509. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

The host compounds **3** and **5** were prepared by refluxing 1,3-cyclohexanedione with one equivalent of the appropriate amine in benzene, and the water produced during the reaction was removed with a Dean–Stark trap. Glycine and 4-aminomethylpyridine gave 71% and 91% yields of the vinylous amides **3** (m.p. 215 °C) and **5** (m.p. 138 °C), respectively.

The triacetylenes were prepared by two successive Cadiot–Chodkiewicz reactions (CuCl (0.1 equiv), $\text{NH}_2\text{OH}\cdot\text{HCl}$ (0.3 equiv), and propylamine (3 equiv) in methanol). Triacetylene **6** was prepared by treating 4-pentynoic acid with 1-bromo-2-triethylsilylacetylene under the above Cadiot–Chodkiewicz reaction conditions to give the triethylsilyl-protected diacetylene (59%). The protecting group was removed (95%) using 1 M tetrabutylammonium fluoride (TBAF) in THF and the diacetylene was coupled with 5-bromo-4-pentynoic acid under the above Cadiot–Chodkiewicz reaction conditions^[14] to give the triacetylene **6** (95%).

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- [1] U. H. F. Bunz, *Angew. Chem.* **1994**, *106*, 1127–1130; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1073–1076.
- [2] Although the poly(triacetylenes) have not been prepared by the direct polymerization of a triacetylene, they have been prepared by clever indirect means: R. E. Martin, T. Mäder, F. Diederich, *Angew. Chem.* **1999**, *111*, 834–838; *Angew. Chem. Int. Ed.* **1999**, *38*, 817–821.
- [3] J. Kiji, J. Kaiser, G. Wegner, R. C. Schulz, *Polymer* **1973**, *14*, 433–439.
- [4] V. Enkelmann, *Chem. Mater.* **1994**, *6*, 1337–1340.
- [5] Macromodel version 6.2 using the MM2 force field was used in modeling calculations: F. Mohamadi, N. B. G. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
- [6] P. Lue and J. V. Greenhill, *Adv. Heterocycl. Chem.* **1997**, *67*, 207–343.
- [7] This derivative ($R = m$ -nitrophenyl) displayed a simple translational distance of 7.313 Å.
- [8] J. J. Kane, R.-F. Liao, J. W. Lauher, F. W. Fowler, *J. Am. Chem. Soc.* **1995**, *117*, 12003–12004.
- [9] C. M. Paleos, D. Tsiourvas, *Angew. Chem.* **1995**, *107*, 1839–1855; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1696–1711.
- [10] The γ -ray source used had an output of 0.8 Mrad h⁻¹ and was located at Brookhaven National Laboratories, Upton, NY.
- [11] H. R. Bhattacharjee, A. F. Preziosi, G. N. Patel, *J. Polym. Sci. Polym. Symp.* **1984**, *71*, 259–270.
- [12] *Conjugated Polymers and Related Materials: The Interconnection of Chemical and Electronic Structure* (Eds. W. R. Salaneck, I. Lundström, Rånby), Oxford University Press, Oxford, **1993**.
- [13] R. E. Martin, F. Diederich, *Angew. Chem.* **1999**, *111*, 1440–1469; *Angew. Chem. Int. Ed.* **1999**, *38*, 1351–1377.
- [14] G. A. Krafft, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **1981**, *103*, 5459–5466.

Mixed Ferrocene–Cobaltocenium Dendrimers: The Most Stable Organometallic Redox Systems Combined in a Dendritic Molecule**

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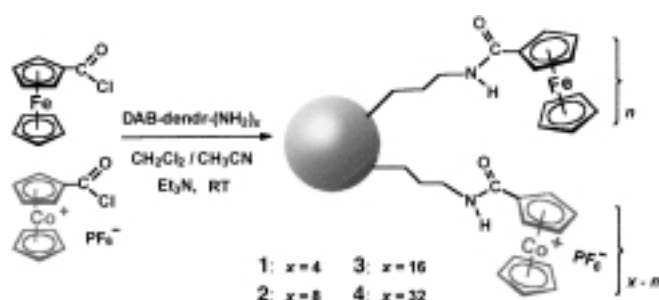
Exciting advances continue to be made in dendrimer chemistry.^[1] The incorporation of metals in dendritic structures has attracted growing interest as it generates new metallodendrimers^[2] with interesting redox, magnetic, catalytic, and photo-optical properties. At the same time, new synthetic methods have been used for the introduction of functionally diverse terminal groups in the same molecule,^[3] which give access to dendritic macromolecules having special properties and a variety of functions.

In the last few years, we reported the synthesis of several families of silicon- and nitrogen-based dendritic molecules containing organometallic moieties as surface functional groups.^[4] In particular, poly(propylene imine) dendrimers functionalized with ferrocene or cobaltocenium moieties were shown to act as multisite redox-active guests for inclusion complexation by β -cyclodextrins, which yielded soluble supramolecular assemblies of high molecular weight.^[5] Here we report on the synthesis and redox properties of novel metallodendrimers containing both ferrocene and cobaltocenium units. To our knowledge, this is the first time that multiple neutral and cationic redox-active organometallic moieties are present in a dendritic structure.^[6, 7] These peripherally heterogeneous dendrimers combine the advantages of both metallocenes, in particular, their stability and redox reversibility on oxidation and reduction, respectively. Electrode surfaces modified with these metallodendrimers were successfully used as a novel type of glucose sensor.

The new mixed-metal dendrimers **1–4** were obtained by treatment of the first four generations of poly(propylene imine) dendrimers DAB-dendr-(NH₂)_x ($x = 4, 8, 16, 32$) with an equimolar mixture of freshly prepared chlorocarbonylferrocene and the PF₆⁻ salt of chlorocarbonylcobaltocenium in CH₂Cl₂/CH₃CN (1/1) (Scheme 1). They were isolated as air-stable orange-brown shiny solids. Ideally, each metallodendritic molecule should have the same number of ferrocene and cobaltocenium units on the surface (see the schematic representation of **4**). However, the experimental results

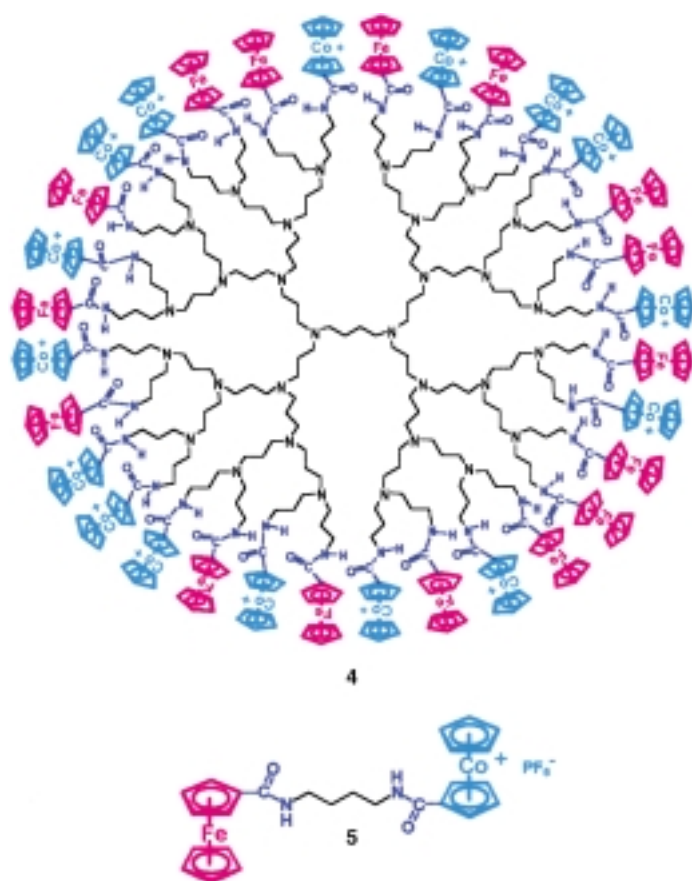
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Scheme 1. Synthesis of heteromultimetallic dendrimers **1–4**.

showed that, as expected, the formation of a single defined product is unlikely. In fact, after treatment of the reaction products with CH_2Cl_2 and CH_3CN , several fractions with different ratios of the organometallic end groups on the dendritic surface were isolated. Reaction of 1,4-diaminobutane with a mixture of $[(\text{C}_5\text{H}_4\text{COCl})\text{Fe}(\text{C}_5\text{H}_5)]$ and $[(\text{C}_5\text{H}_4\text{COCl})\text{Co}(\text{C}_5\text{H}_5)]\text{PF}_6$ (one equivalent each) afforded the heterodinuclear compound **5**, which is a valuable model for the characterization of the mixed-metal dendrimers of higher nuclearity.



The presence of neutral ferrocene and cationic cobaltocenium units imparts an interesting solubility behavior to the heteromultimetallic dendrimers, which can be modified by varying the ratio of the organometallic moieties at the periphery. Dendrimers with a higher loading of cobaltocenium moieties are soluble in H_2O and insoluble in CH_2Cl_2 ,

and they become increasingly soluble in CH_2Cl_2 as the loading of ferrocene units increases.

The new heterometallic dendrimers were characterized by ^1H and ^{13}C NMR spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and total X-ray fluorescence (TXRF).^[8] The NMR spectra show the same signals as the corresponding homogeneous ferrocene and cobaltocenium dendrimers,^[5a,c] as well as those of **5**. The signals of the two peripheral organometallic moieties are observed in the range $\delta = 4.3\text{--}5.9$ for the ferrocene units and $\delta = 5.8\text{--}6.4$ for the cobaltocenium fragments. On the basis of these signals, the ratio of the two peripheral organometallic units can be estimated. For dendrimers that are insoluble in CH_2Cl_2 , the loading of the NH_2 end groups with cobaltocenium is significantly higher than with ferrocene. This was further confirmed by TXRF analyses, which showed a ratio of cobaltocenium to ferrocene units of 3:1 for **1**, 6.5:1.5 for **2**, 13:3 for **3**, and 19:13 for **4**. The ESI mass spectra of the heterometallic dendrimers show different peaks corresponding to species with different states of charge, which result from successive loss of counteranions.

The electrochemical behavior of the novel heterometallic dendrimers **1–4** and the model compound **5** was investigated by cyclic voltammetry (CV) in CH_3CN and aqueous solutions. All four dendrimers show only one reversible oxidation process at about $+0.60\text{ V}$, which was clearly assigned to the simultaneous one-electron oxidation of the multiple peripheral noninteracting ferrocene units (see Figure 1a). Likewise,

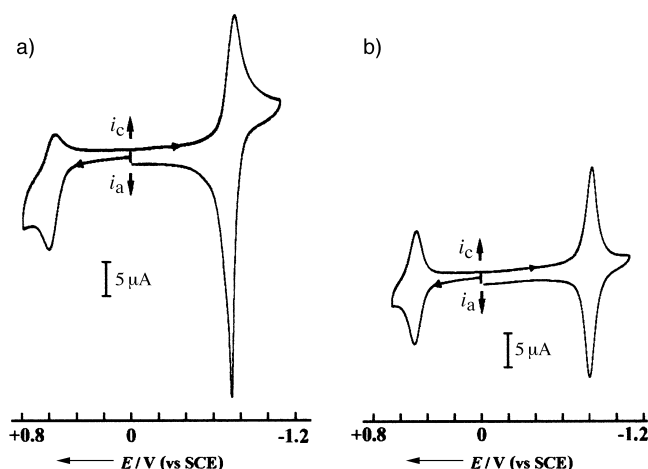


Figure 1. a) Cyclic voltammogram of **3** measured in $\text{CH}_3\text{CN}/0.1\text{M Bu}_4\text{NPF}_6$. b) Cyclic voltammetric response of a glassy carbon disk electrode modified with a film of **3** in $\text{H}_2\text{O}/0.1\text{M LiClO}_4$. Scan rate 100 mV s^{-1} .

the appearance of a single reduction wave near -0.70 V confirms the presence of multiple noninteracting terminal cobaltocenium moieties. In this case, a change in solubility of the dendrimers accompanied the change in oxidation state. Reduction of the dendrimers appeared to result in the precipitation of the reduced neutral dendrimers onto the electrode surfaces, and on the reverse scan the dendrimer redissolved as it was oxidized. The ferrocene/cobaltocenium ratio calculated from the CV is in close agreement with that estimated from the NMR spectrum.

One remarkable feature of these heterogeneous organo-metallic dendrimers is their ability to modify electrode surfaces.^[8] Figure 1 b shows the voltammetric response of an electrodeposited film of the heteromultinuclear dendrimer **3** in aqueous solution as a representative example. Two well-defined, reversible systems are observed, with formal potential values of +0.47 and –0.83 V, which correspond to the ferrocene/ferrocenium and cobaltocenium/cobaltocene systems, respectively, whose voltammetric features unequivocally indicate the surface-confined nature of the electroactive dendrimer film. These modified electrodes are extremely durable and reproducible. Cyclic voltammetric scans can be carried out hundreds of times, with no loss of electroactivity. To our knowledge, this is the first example of electrode surfaces modified with films of electroactive molecules that incorporate both ferrocene and cobaltocenium sites.

The surface-confined heterometallic dendrimers were shown to exhibit a double function: While the ferrocene units act as mediators in enzymatic processes under anaerobic conditions, the cobaltocenium moieties take part in an electrocatalytic process in reactions conducted in the presence of oxygen. We immobilized glucose oxidase (GOx) by depositing the enzyme into conducting films of **3**.^[9] Initial experiments to test these modified electrodes as glucose sensors were successful. A major advantage of these electrodes is the large amount of enzyme due to electrostatic interactions between the positive ferrocenium and cobaltocenium moieties and the negatively charged enzyme. Furthermore, the presence of cobaltocenium units prevents loss of GOx due to the reduction of ferrocenium groups, and this increases the long-term stability of the sensor. Glucose was detected by measuring the amperometric response due to the mediation of the enzymatic reaction by the electrochemical oxidation of ferrocene (Figure 2a)^[10] and, alternatively, by

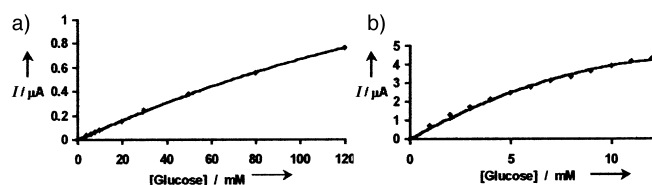


Figure 2. Variations of the steady-state current with glucose concentration, measured with a **3**/enzyme-modified electrode in 0.1 M phosphate buffer, pH = 7.3, containing 0.1 M NaClO₄, compared to a solution without glucose. a) Increases observed at +0.5 V in a N₂-saturated solution. b) Decreases observed at –0.65 V in an O₂-saturated solution.

monitoring the reduction of dissolved oxygen in the presence of the substrate (Figure 2b).^[10] At potentials where the ferrocene moieties act as mediators, the enzyme electrodes respond rapidly to glucose, and steady-state currents are achieved in less than 10 s after each addition. Likewise, significant sensitivity and linearity to clinically relevant glucose concentrations were obtained. On the other hand, the catalytic activity of the cobaltocenium centers was proved by CV.^[11] The application of these modified dendrimer/GOx electrodes allows the reduction of oxygen to occur at a less negative potential (thus diminishing interference) and with higher intensity than with bare glassy carbon electrodes. By

holding the potential at –0.65 V a decrease in the current due to oxygen reduction is detected as the concentration of glucose increases. Such intense currents are not obtained for a glucose-oxidase-coated bare electrode held at the same potential.

Experimental Section

All dendrimers were prepared by the same procedure.

3: To a solution of 1-chlorocarbonylferrocene (0.60 g, 2.42 mmol) and 1-chlorocarbonylcobaltocenium hexafluorophosphate (0.96 g, 2.42 mmol) in CH₂Cl₂/CH₃CN (1/1) (100 mL) was added dropwise a solution of DAB-dend-(NH₂)₁₆ (commercially available from DSM; 0.51 g, 0.30 mmol) and 3 mL of Et₃N in CH₂Cl₂/CH₃CN (1/1) (40 mL). The reaction mixture was stirred for about 12 h under argon at room temperature. After removal of the solvent in vacuum, the resulting brown residue was washed several times with CH₂Cl₂, then dissolved in acetonitrile and precipitated into dichloromethane. The precipitate was dried in vacuum and an orange-brown shiny solid was obtained (0.55 g). Removal of the solvent from the dichloromethane solution afforded a reddish-orange solid (0.31 g).

Selected data for **3** as a representative example: ¹H NMR (300 MHz, [D₆]DMSO, 25 °C): δ = 9.25 (CONH_[Cb]), 8.06 (CONH_[Fc]), 6.39, 5.91 (C₅H₄[Cb]), 5.85 (C₅H₅[Cb]), 4.82, 4.31 (C₅H₄[Fc]), 4.13 (C₅H₅[Fc]), 2.68, 2.95, 1.83 (CH₂); ¹³C NMR (500 MHz, [D₆]DMSO, 25 °C): δ = 170.3 (CO_[Fc]), 162.3 (CO_[Cb]), 94.6 (C₅H₄[Cb]), 86.8 (C₅H₅[Cb]), 86.5, 85.0 (C₅H₄[Fc]), 77.4, 70.9 (C₅H₅[Fc]), 70.3 (C₅H₅[Fc]), 69.2 (C₅H₄[Fc]), 51.4, 38.2, 37.5, 25.2, 21.9 (CH₂); ESI-MS (*m/z*) of the CH₂Cl₂-insoluble fraction: 856.0 [*M*_(3Fc, 13Cb) – 7PF₆]⁷⁺, 730.7 [*M*_(3Fc, 13Cb) – 8PF₆]⁸⁺, 633.4 [*M*_(3Fc, 13Cb) – 9PF₆]⁹⁺, 555.5 [*M*_(3Fc, 13Cb) – 10PF₆]¹⁰⁺, 492.4 [*M*_(3Fc, 13Cb) – 11PF₆]¹¹⁺, 834.2 [*M*_(4Fc, 12Cb) – 7PF₆]⁷⁺, 712.2 [*M*_(4Fc, 12Cb) – 8PF₆]⁸⁺, 616.7 [*M*_(4Fc, 12Cb) – 9PF₆]⁹⁺, 540.5 [*M*_(4Fc, 12Cb) – 10PF₆]¹⁰⁺, 478.1 [*M*_(4Fc, 12Cb) – 11PF₆]¹¹⁺, 813.3 [*M*_(5Fc, 11Cb) – 7PF₆]⁷⁺, 693.2 [*M*_(5Fc, 11Cb) – 8PF₆]⁸⁺, 600.2 [*M*_(5Fc, 11Cb) – 9PF₆]⁹⁺, 525.7 [*M*_(5Fc, 11Cb) – 10PF₆]¹⁰⁺, 465.0 [*M*_(5Fc, 11Cb) – 11PF₆]¹¹⁺. Fc = C₅H₄FeC₅H₅, Cb = C₅H₄CoC₅H₅.

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- a) G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules: Concepts, Synthesis, Perspectives*, VCH, Weinheim, **1996**; b) M. Fischer, F. Vögtle, *Angew. Chem.* **1999**, *111*, 934; *Angew. Chem. Int. Ed.* **1999**, *38*, 884; c) P. R. Dvornic, D. A. Tomalia, *Curr. Opin. Colloid Interface Sci.* **1996**, *1*, 221; d) N. Ardooin, D. Astruc, *Bull. Soc. Chim. Fr.* **1995**, *132*, 875; e) A. W. Bosman, H. M. Janssen, E. W. Meijer, *Chem. Rev.* **1999**, *99*, 1665; f) J.-P. Majoral, A.-M. Caminade, *Chem. Rev.* **1999**, *99*, 845.
- Recent reviews on metallo-dendrimers: a) G. R. Newkome, E. He, C. N. Moorefield, *Chem. Rev.* **1999**, 1689; b) M. Hearshaw, J. R. Moss, *Chem. Commun.* **1999**, 1; c) I. Cuadrado, M. Morán, C. M. Casado, B. Alonso, J. Losada, *Coord. Chem. Rev.* **1999**, *193–195*, 395; d) C. Gorman, *Adv. Mater.* **1998**, *10*, 295.
- a) A. Archut, S. Gestermann, R. Hesse, C. Kauffmann, F. Vögtle, *Synlett* **1998**, 546; b) C. J. Hawker, K. L. Wooley, J. M. Fréchet, *J. Chem. Soc. Perkin Trans. 1* **1993**, 1287; c) C. Galliot, C. Larré, A.-M. Caminade, J.-P. Majoral, *Science* **1997**, *277*, 1981; d) G. R. Newkome, C. D. Weis, C. N. Moorefield, G. R. Baker, B. J. Childs, J. Epperson, *Angew. Chem.* **1998**, *110*, 318; *Angew. Chem. Int. Ed.* **1998**, *37*, 307.
- a) I. Cuadrado, C. M. Casado, B. Alonso, M. Morán, J. Losada, V. Belsky, *J. Am. Chem. Soc.* **1997**, *119*, 7613; b) B. Alonso, I. Cuadrado, M. Morán, J. Losada, *J. Chem. Soc. Chem. Commun.* **1994**, 2575; c) I. Cuadrado, M. Morán, J. Losada, C. M. Casado, C. Pascual, B. Alonso, F. Lobete in *Advances in Dendritic Macromolecules*, Vol. 3 (Ed.: G. R. Newkome), JAI Press, Greenwich, CT, **1996**, p. 151.
- a) I. Cuadrado, M. Morán, C. M. Casado, B. Alonso, F. Lobete, B. García, M. Ibisate, J. Losada, *Organometallics* **1996**, *15*, 5278; b) R. Castro, I. Cuadrado, B. Alonso, C. M. Casado, M. Morán, A. Kaifer, *J. Am. Chem. Soc.* **1997**, *119*, 5760; c) B. González, C. M. Casado, B. Alonso, I. Cuadrado, M. Morán, Y. Wang, A. E. Kaifer, *Chem. Commun.* **1998**, 2569.

- [6] For heterometallic dendrimers see, for example: a) S. Serroni, A. Juris, M. Venturi, S. Campagna, I. Resino, G. Denti, A. Credi, V. Balzani, *J. Mater. Chem.* **1997**, 7, 1227; b) S. Achar, C. E. Immoos, M. G. Hill, V. J. Catalano, *Inorg. Chem.* **1997**, 36, 2314; c) G.-X. Liu, R. J. Puddephatt, *Organometallics* **1996**, 15, 5257.
- [7] For other families of ferrocene and cobaltocenium dendrimers, see a) C. Valerio, E. Alonso, J. Ruiz, J.-C. Blais, D. Astruc, *Angew. Chem.* **1999**, 111, 1855; *Angew. Chem. Int. Ed.* **1999**, 38, 1747; b) C. Valerio, J.-L. Fillaut, J. Ruiz, J. Guittard, J.-C. Blais, D. Astruc, *J. Am. Chem. Soc.* **1997**, 119, 2588; c) E. Alonso, C. Valerio, J. Ruiz, D. Astruc, *New J. Chem.* **1997**, 21, 1139.
- [8] The heterometallic dendrimers were electrodeposited onto Pt or carbon glassy electrodes by continuous scanning between 0 and -1.10 V versus SCE in degassed CH_3CN dendrimer-containing solutions. The electrodes thus coated were rinsed with CH_3CN to remove any adhering solution and dried in air. The surface coverage Γ of the dendrimer on the electrode was determined from the integration of the redox waves, and for the example shown in Figure 1B was found to be $9.7 \times 10^{-10} \text{ mol Fcm}^{-2}$ and $1.8 \times 10^{-9} \text{ mol Cbcm}^{-2}$.
- [9] The dendrimer-modified electrodes were immersed in an electrochemical cell containing an enzyme solution (0.1 % of GOx in acetate buffer, pH = 6.3, and NaClO_4 0.1 M). A potential of +0.6 V versus SCE was applied to the dendrimer-coated electrodes for 30 min with stirring, and the electrodes were subsequently rinsed in deionized water and air-dried before use.
- [10] The electrode enzymatic reactions are $\text{S} + \text{enzyme}_{\text{ox}} \rightarrow \text{P} + \text{enzyme}_{\text{red}}$ and $\text{enzyme}_{\text{red}} + \text{M}_{\text{ox}} \rightarrow \text{enzyme}_{\text{ox}} + \text{M}_{\text{red}}$, where M = mediator, $\text{M}_{\text{ox}} = \text{O}_2$ or ferrocenium, S = substrate (glucose), P = product (gluconolactone).
- [11] E_p of the O_2 reduction -0.83 V versus SCE at a bare carbon glassy electrode, and -0.70 V at the dendrimer modified electrode.

Structure of Homoleptic $\text{Cu}^I(\text{CO})_3$ Cations in Cu^I -Exchanged ZSM-5 Zeolite: An X-ray Absorption Study**

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Copper is a transition metal that plays a remarkable role in redox catalysis by several selective, three-dimensionally organized, inorganic, organic, and biological systems.^[1–3] In

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all these catalysts the structure of the active sites is rather complex, and often the interplay between two adjacent copper centers is required for their operation.

Copper(I)-exchanged zeolites can be considered the best known type of inorganic three-dimensionally organized catalysts, because of their crystalline structure and the controlled dispersion of the metal centers, which impart a good structural definition to the adsorbing sites.^[4] Copper-exchanged zeolites are usually prepared by standard exchange procedures with aqueous solutions of Cu^{II} salts. Depending on the preparation and pretreatment conditions, they contain variable mixtures of copper species in different oxidation and aggregation states.^[1, 5] Products containing exclusively isolated Cu^{I} ions can be prepared by reaction of H-zeolites with CuCl in the gas phase.^[6] Structural and spectroscopic studies have shown that the Cu-ZSM-5 thus obtained contains solely isolated Cu^{I} ions,^[7] located in structurally and energetically well defined positions.^[8] Hence, these materials have model character and are suitable for theoretical investigations.^[9, 10]

In addition, Cu complexes of well-defined structure can be synthesized in zeolitic materials, and the zeolitic framework can often stabilize complexes that would otherwise be unstable.^[11, 12] Although binary compounds of the type $\text{Cu}^I(\text{CO})_n$ were observed in strongly acidic media such as H_2SO_4 , $\text{CF}_3\text{SO}_3\text{H}$, FSO_3H , $\text{BF}_3 \cdot \text{H}_2\text{O}$, and HF ,^[3] none of these complexes could be isolated as solids. Only recently did Rack et al.^[13] report the formation and the structural and spectroscopic characterization of $[\text{Cu}^I(\text{CO})_n]^+ \text{AsF}_6^-$ ($n = 1-3$).^[14]

Infrared spectroscopy (Figure 1) has been very informative on the structure of intrazeolitic $[\text{Cu}^I(\text{CO})_n]^+$ species ($n = 1-3$), and the main results can be summarized as follows:^[6b, 8] 1) at low CO equilibrium pressures p_{CO} linear $\text{Cu}^I(\text{CO})$ complexes are formed;^[15] 2) on increasing p_{CO} the formation of C_{2v} -symmetric $\text{Cu}^I(\text{CO})_2$ adducts is observed;^[16] 3) on decreasing the temperature, $\text{Cu}^I(\text{CO})_3$ complexes of C_{3v}

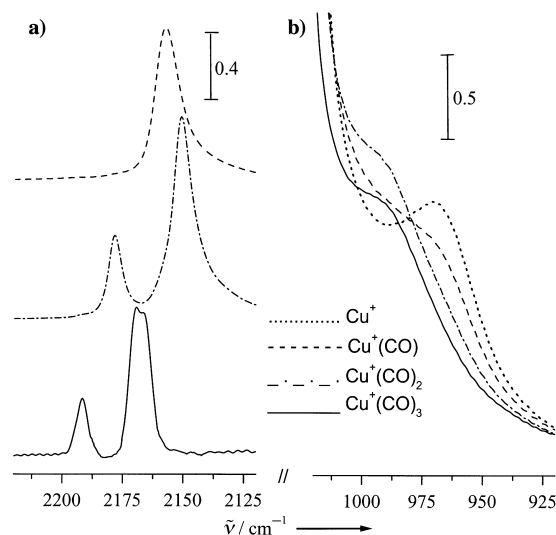


Figure 1. IR spectra of $[\text{Cu}^I(\text{CO})_n]^+$ species ($n = 1-3$) in ZSM-5 zeolite in the C=O (a) and framework stretching (b) regions. The spectrum obtained before CO addition was used as background and was subtracted from the spectra in (a). The spectrum of the $[\text{Cu}^I(\text{CO})_3]^+$ adduct is shown after additional subtraction of the band of the physisorbed component at 2138 cm^{-1} .^[17]

symmetry are obtained at about 80 K (see Figure 1a), characterized by the appearance of a new IR doublet at $\tilde{\nu}(\text{CO}) = 2167, 2192 \text{ cm}^{-1}$.^[17] Note that the homogeneous counterparts form planar adducts of D_{3h} symmetry.^[13] The distortion from ideal linear and planar symmetry observed for the di- and tricarbonyl species is associated with interactions with the zeolite walls, which act as polydentate ligands. According to ref.[3e], this distortion can be considered as an external parameter that reflects the interaction with the negatively charged framework, which is the heterogeneous counterpart of the AsF_6^- ion in the homogeneous complexes synthesized by Rack et al.^[13]

Further and more direct information about the internal and external parameters can be obtained by X-ray absorption spectroscopy (XAS). In particular, previous extended X-ray absorption fine structure (EXAFS) studies^[8, 11] showed that the formation of $\text{Cu}^{\text{I}}(\text{CO})_2$ complexes at room temperature causes a consistent elongation of the distance between the Cu^{I} ions and the framework oxygen atoms (from 2.00 to 2.16 Å). This experimental evidence clearly indicates both the high complexation capability of CO molecules with respect to Cu^{I} ions hosted in the Cu^{I} -ZSM-5 matrix and the mobility of the Cu^{I} ions under the influence of the interaction with CO adsorbates.

To more deeply investigate the analogies between the chemistry of Cu^{I} in Cu^{I} -ZSM-5 and that of Cu^{I} in superacidic media, we performed an EXAFS and X-ray absorption near-edge structure (XANES)^[18] study aimed at determining the three-dimensional structure of the $\text{Cu}^{\text{I}}(\text{CO})_3$ complexes formed in the channels of ZSM-5 at 80 K. The combined use of EXAFS and XANES techniques (the results interpreted by using a multiple-scattering approach) is the only method that can determine the local structure of such carbonylcopper ions. In fact, powder XRD can not be used in this case because the low Cu content in ZSM-5 (due to the high Si/Al ratio) makes the contribution of Cu nearly insignificant with respect to that of the framework and the solvation effect induced on Cu^{I} cations by interaction with CO implies a consistent loss of long-range ordering of the resulting $\text{Cu}^{\text{I}}(\text{CO})_3$ complexes.

The results are illustrated in Figure 2 and Table 1. In Figure 2a the calculated and experimental EXAFS signals together with the partial contributions of the different two- and three-body configurations are compared; Figure 2b compares the Fourier transforms (FT) of the experimental and calculated spectra. Table 1 lists the results of the quantitative analysis. The Cu–C distance obtained for the $\text{Cu}^{\text{I}}(\text{CO})_3$ complex (1.93 ± 0.02 Å) is very close to that previously measured for the $\text{Cu}^{\text{I}}(\text{CO})_2$ complex at room temperature (1.95 Å)^[11] and is in good agreement with the values recently obtained by advanced quantum mechanical calculations: Lupinetti et al.^[10a] (1.891 Å), Ramprasad et al.^[10b] (1.900 Å), and Sodupe et al.^[10c] (1.969 Å). The

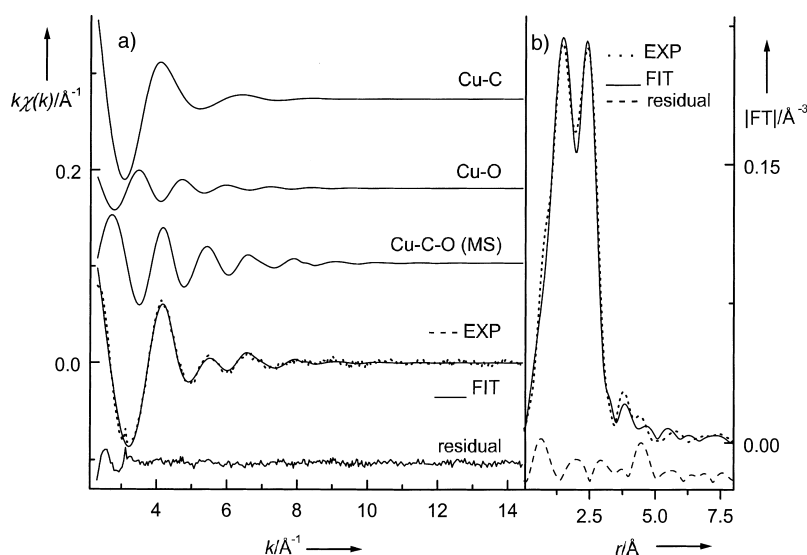


Figure 2. a) k -weighted k functions of (from top to bottom): Cu-C single-scattering (SS) contribution, Cu-O SS contribution, Cu-C-O MS contribution, the sum of the three previous theoretical contributions (FIT), the experimental curve (EXP), superimposed for comparison, and the corresponding residual function. b) Phase-uncorrected FTs of the last three functions, giving a view in r space.

Table 1. Quantitative results of the EXAFS data analysis with the coordination number N fixed at three; errors were calculated by a χ^2 statistical analysis with a confidence level of 95 %.

$r_{\text{Cu-C}}$ [Å]	$\sigma_{\text{Cu-C}}^2$ [Å ²]	$r_{\text{C-O}}$ [Å]	$\sigma_{\text{C-O}}^2$ [Å ²]	$\theta_{\text{Cu-C-O}}$ [°]	$\sigma_{\text{Cu-C-O}}^2$ [° ²]	C_{RR}
1.93 ± 0.02	0.022 ± 0.006	1.12 ± 0.03	0.005 ± 0.02	180 ± 10	100 ± 50	-0.65 ± 0.30

Cu–O_{CO} distance extrapolated from this determination is 3.03 Å with a related Debye–Waller factor of $\sigma_{\text{Cu-O}}^2 = 0.013$ Å². Note that the C–O distance (1.12 ± 0.03 Å) is in good agreement with the gas-phase value (1.128 Å) and that the Cu–C–O bond angle is linear within the error bars, in agreement with indirect IR evidence.^[6b, 8] The negative correlation parameter C_{RR} of the Cu–C and C–O vibrations indicates that while the former stretches the latter compresses, and vice versa. This behavior is exactly what is expected for the vibrational motion of the carbonylcopper adduct on the basis of the orbital approach.^[19]

It is noteworthy that we were able to simulate the experimental signal using a bare $\text{Cu}^{\text{I}}(\text{CO})_3$ cluster, and this implies that the complexation effect, already evident at room temperature in the $\text{Cu}^{\text{I}}(\text{CO})_2$ complex,^[11] is now stronger and that $\text{Cu}^{\text{I}}(\text{CO})_3$ moieties have only a very weak interaction with the framework oxygen atoms and exhibit mobility. This result confirms the conclusion based on the observation of the influence of CO polyadsorption on the ν_{asym} stretching of a Si–O–Al moiety that is perturbed by the presence of Cu^{I} (see Figure 1b). In fact, the stepwise formation of intrazeolitic $\text{Cu}^{\text{I}}(\text{CO})_n$ complexes is accompanied by the simultaneous stepwise perturbation of the IR band at 980 cm^{-1} , already attributed to $\nu_{\text{asym}}(\text{Si-O-Al})$ stretching that is perturbed by Cu^{I} , which progressively shifts in frequency and nearly disappears upon formation of $\text{Cu}^{\text{I}}(\text{CO})_2$ and $\text{Cu}^{\text{I}}(\text{CO})_3$ complexes.^[11] In other words, the $\nu_{\text{asym}}(\text{Si-O-Al})$ frequency (usually in the range 1100 – 1250 cm^{-1} for zeolites) moves from a value (980 cm^{-1}) reflecting the perturbation induced by the

bonded Cu^I ion at $r_{\text{Cu-O}} = 2.00 \text{ \AA}$ to a higher value (more similar to that of an unperturbed Si-O-Al moiety) which reflects the lower perturbation induced by a Cu^I ion at a larger $r_{\text{Cu-O}}$.^[11] The IR skeletal mode and the $r_{\text{Cu-O}}$ values measured by EXAFS are very sensitive to “external parameters” and thus give information on the bonding between Cu^I(CO)_{*n*} and the zeolite. Unfortunately, due to the weakness of the C-Cu-C three-body signal, determination of the global geometry (planar or pyramidal) of the Cu^I(CO)₃ cluster from EXAFS data was not possible.

Further information on the geometry of the Cu^I(CO)₃ cluster was obtained by analyzing the absorption coefficient in the vicinity of the edge (XANES). In this region the photoelectron has a wavelength longer than the interatomic distances and a long mean free path, and this results in a marked sensitivity of the shape and position of the edge structures to the local geometry of the complex. On the basis of the IR evidence (Figure 1), we considered a series of Cu^I(CO)₃ clusters with C_{3v} “umbrella-shaped” structures by starting from the planar configuration (*D*_{3h}; $\alpha = 90^\circ$) and reducing the polar angle α of the CO ligands with respect to the *z* axis down to $\alpha = 30^\circ$. From these trial geometries, the XANES spectra were calculated in the region between 0 and 4 Ry (54.4 eV) above the interstitial potential level and compared with the experimental results. The results of the simulations for α values between 30 and 90° (planar cluster) are shown in Figure 3. The position and relative intensities of peaks A and B are reasonably reproduced for $70 < \alpha < 90^\circ$. At

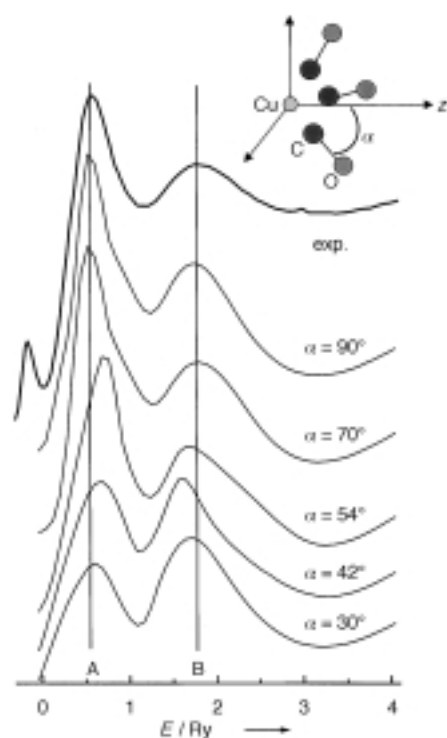


Figure 3. Comparison between experimental (top curve) and simulated XANES curves as a function of the angle α of the Cu(CO)₃ adduct in C_{3v} symmetry (1 Ry $\approx$ 13.6 eV). Simulated spectra are calculated above the interstitial potential level only ($E > 0$). Vertical lines indicate the position of bands A and B of the experimental spectrum. The schematic diagram represents the Cu^I(CO)₃ adduct in C_{3v} symmetry and defines the angle α .

$\alpha = 54^\circ$ the two peaks are too close, and for smaller angles peak B becomes more intense than peak A. The XANES results are thus compatible with a planar or slightly bent molecule with a minimum acceptable bending angle of $\alpha = 60^\circ$. A value of $\alpha = 90^\circ$ or close to 90° must be rejected on the basis of the IR spectroscopic data^[6b, 8] (only one IR-active band is expected for a Cu^I(CO)₃ complex with *D*_{3h} symmetry). In addition, the steric hindrance of the zeolitic walls makes values of α larger than 80° unreasonable. Hence, we concluded that, due to the interaction with the zeolitic walls, the structure of the Cu^I(CO)₃ complexes deviates from the planar form typical of the homogeneous counterparts, with an α value in the range of $60\text{--}80^\circ$. The interaction must however be sufficiently weak to preclude the determination of the Cu^I–O distance by EXAFS. From the above XAS data, the high structural definition of the Cu^I complexes in ZSM-5 is emerging.^[20] This result allows them to be classified among the best heterogeneous counterparts of the homoleptic Cu^I(CO)_{*n*} cations ($n = 1\text{--}3$) synthesized under homogeneous conditions.

Experimental Section

Cu^I-ZSM-5 (Si/Al = 14/1) was prepared by reaction of the corresponding protic form with gaseous CuCl at 673 K.^[6] For IR measurements, we have used an IR cell designed to allow in situ high-temperature treatment, gas dosage, and low-temperature measurements. The IR spectra were recorded with a resolution of 2 cm^{-1} on a BRUKER FTIR-66 spectrometer equipped with a MCT detector. X-ray absorption measurements were performed at the Cu K edge at GILDA, the general purpose Italian beamline at the ESRF. The monochromator was equipped with a pair of Si(311) flat crystals; efficient harmonic rejection was achieved by using a pair of Pd-coated mirrors with an energy cutoff of 21 keV. Measurements were performed at 80 K in transmission mode with N₂- and Ar-filled ion chambers for measuring the incident (*I*₀) and transmitted (*I*₁) beams, respectively. A reliable energy calibration was achieved by measuring the fluorescence of Cu metal foil placed after *I*₁. XAS and IR spectra were obtained by using the same cell equipped with Mylar and KBr windows, respectively. The Cu^I(CO)₃ complex was measured at 80 K (*p*_{CO} = 10 Torr), and the Cu^I(CO)₂ and Cu^I(CO) complexes at room temperature (*p*_{CO} = 1 and 10 Torr, respectively).

EXAFS data were analyzed in the framework of the multiple-scattering theory by using GNXAS.^[21] Theoretical calculations of XANES spectra were made on the basis of the multiple-scattering formalism and the Muffin Tin approximation by using the CONTINUUM package developed at the Laboratori Nazionali di Frascati of INFN.^[22] Muffin Tin radii were chosen according to the Norman criterion,^[23] and a 10% overlap was allowed between contiguous spheres. A detailed description of both EXAFS and XANES computations is available as supporting information.

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- [1] a) Y. Li, W. K. Hall, *J. Phys. Chem.* **1990**, *94*, 6145–6148; b) M. Iwamoto, H. Hamada, *Catal. Today* **1991**, *10*, 57–71; M. Shelef, *Chem. Rev.* **1995**, *95*, 209–225; G. Centi, S. Perathoner, *Appl. Catal. A* **1995**, *132*, 179–259.
- [2] G. Wilkinson, *Comprehensive Coordination Chemistry*, Vol. 5, Pergamon, Oxford, **1987**, p. 576.
- [3] a) E. I. Solomon, U. M. Sundaram, T. E. Machonkin, *Chem. Rev.* **1996**, *96*, 2563–2605; b) R. H. Holm, P. Kennepohl, E. I. Solomon, *Chem. Rev.* **1996**, *96*, 2239–2314; c) S. Ferguson-Miller, G. T. Babcock, *Chem. Rev.* **1996**, *96*, 2889–2900; d) E. Pidcoc, H. V. Obians, M. Abe, H.-C. Liang, K. D. Karlin, E. I. Solomon, *J. Am. Chem. Soc.* **1999**, *121*, 1299–1308; e) H. Willner, F. Aubke, *Angew. Chem.* **1997**, *109*, 2506–2530; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2402–2425.
- [4] K. Klier, *Langmuir* **1988**, *4*, 13–25.

- [5] W. Grünert, N. W. Hayes, R. W. Joyner, E. S. Shapiro, M. Rafiq, H. Siddiqui, G. N. Baeva, *J. Phys. Chem.* **1994**, *98*, 10832–10846.
- [6] a) G. Spoto, S. Bordiga, D. Scarano, A. Zecchina, *Catal. Lett.* **1992**, *13*, 39–44; b) G. Spoto, A. Zecchina, S. Bordiga, G. Ricchiardi, G. L. Martra, G. Leofanti, G. Petrini, *Appl. Catal. B* **1994**, *3*, 151–172.
- [7] The XANES spectrum of the sample before CO dosage (not reported) shows a prominent pre-edge peak due to the $1s \rightarrow 4p$ electronic transition, with an intensity of 0.88 in normalized adsorption and an inflection point at 8982.7 eV (-0.15 Ry in the scale of Figure 3). Note that the zero energy point (0.00 Ry) has been located by the program CONTINUUM^[22] at 8984.8 eV. Upon formation of $\text{Cu}^I(\text{CO})_3$ complexes (top curve in Figure 3) the peak loses intensity (0.42 in normalized adsorption) and shifts towards lower energies (8981.2 eV i.e. -0.26 Ry). For comparison, note that the first inflection point in Cu foil, Cu_2O and CuO model compounds are observed at 8979.0, 8980.9 and 8983.9 eV respectively (i.e. -0.43 , -0.29 and -0.07 Ry). A real energy calibration was achieved by measuring the absorption of a metal foil located after the sample, for more detail see: G. Turnes Palomino, P. Fiscaro, E. Giamello, S. Bordiga, C. Lamberti, A. Zecchina, *J. Phys. Chem. B* **2000**, *104*, 4064–4073.
- [8] C. Lamberti, S. Bordiga, F. Geobaldo, M. Salvalaggio, G. Spoto, A. Zecchina, G. Vlaic, M. Bellatreccia, *J. Phys. Chem. B* **1997**, *101*, 344–360.
- [9] L. Rodriguez-Santiago, M. Sierka, V. Branchadell, M. Sodupe, J. Sauer, *J. Am. Chem. Soc.* **1998**, *120*, 1545–1551; D. Nachtigallová, P. Nachtigall, M. Sierka, J. Sauer, *Phys. Chem. Chem. Phys.* **1999**, *1*, 2019–2026.
- [10] a) A. J. Lupinetti, S. Fau, G. Frenking, S. H. Strauss, *J. Phys. Chem. A* **1997**, *101*, 9551–9559; b) R. Ramprasad, W. F. Schneider, K. C. Hass, J. B. Adams, *J. Phys. Chem. B* **1997**, *101*, 1940–1949; c) M. Sodupe, V. Branchadell, M. Rosi, C. W. Bauschlicher, Jr., *J. Phys. Chem. A* **1997**, *101*, 7854–7859.
- [11] A. Zecchina, S. Bordiga, G. T. Palomino, D. Scarano, C. Lamberti, M. Salvalaggio, *J. Phys. Chem. B* **1999**, *103*, 3833–3844.
- [12] a) S. Bordiga, C. Lamberti, G. T. Palomino, F. Geobaldo, D. Arduino, A. Zecchina, *Microporous Mesoporous Mater.* **1999**, *30*, 129–135; b) G. Schulz, S. Ernst in *Handbook of Heterogeneous Catalysis*, Vol. 1 (Eds.: G. Ertl, H. Knözinger, J. Weitkamp), VCH, Weinheim, **1997**, pp. 374–387.
- [13] J. J. Rack, J. D. Webb, S. H. Strauss, *Inorg. Chem.* **1996**, *35*, 277.
- [14] For a brief overview on the $\tilde{\nu}(\text{CO})$ of different $\text{Cu}^I(\text{CO})$ complexes, see D. Scarano, S. Bordiga, C. Lamberti, G. Spoto, G. Ricchiardi, A. Zecchina, C. Otero Areán, *Surf. Sci.* **1998**, *411*, 272–285, or S. H. Strauss, *J. Chem. Soc. Dalton Trans.* **2000**, 1–6.
- [15] The intrazeolitic $\text{Cu}^I(\text{CO})$ complex is characterized by a single band at $\tilde{\nu}(\text{CO}) = 2157 \text{ cm}^{-1}$. This frequency compares well with that of $[\text{Cu}^I(\text{CO})]^+$ in superacidic media^[3] and of $[\text{Cu}(\text{CO})]^+[\text{Cl}]^-$ ($\tilde{\nu}(\text{CO}) = 2127 \text{ cm}^{-1}$, M. Håkanson, S. Janger, *Inorg. Chem.* **1990**, *29*, 5241–5243) and $[\text{Cu}(\text{CO})]^+[\text{HB}(3,5\text{-(CF}_3)_2\text{Pz}_3)]^-$, Pz = pyrazolyl, ($\tilde{\nu}(\text{CO}) = 2137 \text{ cm}^{-1}$, H. V. R. Dias, H.-L. Lu, *Inorg. Chem.* **1995**, *34*, 5380–5382).
- [16] The observation of an IR-active doublet ($\tilde{\nu}(\text{CO}) = 2151$ and 2178 cm^{-1}) indicates that the intrazeolitic $\text{Cu}^I(\text{CO})_2$ adducts have local C_{2v} symmetry, while the homogeneous counterpart in $[\text{Cu}^I(\text{CO})_2]^+ \text{AsF}_6^-$ ^[13] is linear ($D_{\infty h}$). Recently, Strauss et al. reported the stabilization of the dicarbonylcopper(I) ion by larger anions: $[\text{Cu}^I(\text{CO})_2]^+[\text{N}(\text{SO}_2\text{CF}_3)_2]^-$ and $[\text{Cu}^I(\text{CO})_2]^+[(1\text{-Bn-CB}_{11}\text{F}_{11})]^-$ have bent C_{2v} $\text{Cu}^I(\text{CO})_2$ moieties like those observed in ZSM-5: O. G. Polyakov, S. M. Ivanova, C. M. Gaudinski, S. M. Miller, O. P. Anderson, S. H. Strauss, *Organometallics* **1999**, *18*, 3769–3771; S. M. Ivanova, S. V. Ivanov, S. M. Miller, O. P. Anderson, K. A. Solntsev, S. H. Strauss, *Inorg. Chem.* **1999**, *38*, 3756–3757. The formation at room temperature of dicarbonyls indicates that there is a strong similarity between the chemistry towards CO of Cu^I in superacidic media (where they are in contact with extremely weak basic anions like AsF_6^-) and in Cu^I-ZSM-5 (where the role of counteranion is assumed by a zeolite anion). This result is in agreement with the fact that the conjugate acid H-ZSM-5 is very strong (like trifluoromethanesulfonic acid: C. Pazé, S. Bordiga, C. Lamberti, M. Salvalaggio, A. Zecchina, G. Bellussi, *J. Phys. Chem. B* **1997**, *101*, 4740–4751).
- [17] The IR spectrum of the $\text{Cu}^I(\text{CO})_3$ complex shown in Figure 1a (solid line) was obtained after the subtraction of the band at 2138 cm^{-1} ,

which is presumably due to liquidlike CO physisorbed in the zeolite channels (S. Bordiga, D. Scarano, G. Spoto, A. Zecchina, C. Lamberti, C. Otero Areán, *Vib. Spectrosc.* **1993**, *5*, 69–74). This band was previously wrongly attributed by us to a third component of the tricarbonyl adduct.^[6b, 8]

- [18] a) D. C. Koningsberger, R. Prins, *X-Ray Absorption. Principles, Applications, Techniques of EXAFS, SEXAFS and XANES*, Wiley, New York, **1988**; b) H. Bertagnolli, T. S. Ertl, *Angew. Chem.* **1994**, *106*, 16; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 45–66.
- [19] P. S. Braterman in *Metal Carbonyl Spectra in Organometallic Chemistry* (Eds.: P. M. Maitlis, F. G. A. Stone, R. West), Academic Press, London, **1975**.
- [20] The parallel IR investigation was fundamental, since it allowed the p_{CO} needed to obtain complete formation of the $\text{Cu}^I(\text{CO})_3$ complexes at 80 K to be determined and the XANES data for clusters having C_{3v} symmetry to be simulated.
- [21] a) A. Filippini, A. Di Cicco, C. R. Natoli, *Phys. Rev. B* **1995**, *52*, 15122–15134; b) A. Filippini, A. Di Cicco, *Phys. Rev. B* **1995**, *52*, 15135–15149.
- [22] a) C. R. Natoli, M. Benfatto, *J. Physique (Colloque C8)* **1986**, *47*, 11–23; b) T. A. Tyson, K. O. Hodgson, C. R. Natoli, M. Benfatto, *Phys. Rev. B* **1992**, *46*, 5997–6019.
- [23] J. G. Norman Jr., *Mol. Phys.* **1976**, *31*, 1191–1198.

Nickel-Catalyzed Generation of Schiff Base Aluminum Enolate Initiators for Controlled Methacrylate Polymerization**

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Many methods have been described that bring about the controlled polymerization of acrylate monomers^[1] including, for example, classical anionic techniques,^[2] screened anionic polymerization (SAP),^[3] group-transfer polymerization (GTP),^[4] catalytic chain transfer (CCT),^[5] reversible addition–fragmentation chain transfer (RAFT),^[6] atom transfer radical polymerization (ATRP),^[7] TEMPO-mediated free radical polymerization (TEMPO = 2,2,6,6-tetramethyl-1-piperidinoxyl),^[8] and coordination polymerization systems based on electrophilic metal centers such as samarium,^[9] zirconium,^[10] and aluminum.^[11] There continues to be great

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academic and commercial interest in the development of systems for the controlled polymerization of acrylate monomers, especially robust, commercially viable systems that will enhance the syndiotactic content to give higher softening temperatures in commodity polymers such as poly(methylmethacrylate) (PMMA).^[12]

For polymerizations mediated by electrophilic metals such as Sm, Zr, and Al, there is good evidence that metal enolates are the propagating species and, in the case of samarium, Yasuda and co-workers have isolated and structurally characterized a key enolate intermediate.^[9] Enolate species are also believed to play a central role in aluminum-mediated polymerizations, although direct evidence has been less forthcoming, and in general they appear to form less readily than their rare earth relatives.

In ground-breaking work on aluminum, Inoue and co-workers demonstrated that the porphyrin complex [Al(tpp)Me] (tpp = 5,10,15,20-tetraphenylporphyrinato), upon irradiation with visible light, forms a living (or “immortal”) polymerization system.^[11] The visible radiation has been shown to assist the formation of the active aluminum enolate initiator. However, the potential drawbacks of this system are twofold: 1) the use of a ligand that carries an intense chromophore can impart serious discolorations to the poly(acrylate) products; and 2) the requirement for a light source to facilitate the efficient formation of an enolate species in the case of [Al(tpp)Me]^[13] restricts potential commercial applications.

Here we describe a remarkably active and robust aluminum initiator system based upon the tetradentate ‘N₂O₂’ Schiff base ligand frame (‘N₂O₂’ = *N,N'*-ethylenebis(salicylideneimine)) [Eq. (1)] and a novel nickel-catalyzed approach to

generating the active aluminum enolate centers. The Schiff base ligand system was chosen for three principal reasons: 1) in general, Schiff base compounds are easily prepared by simple condensation procedures that are readily amenable to ligand backbone modification; 2) the resultant aluminum Schiff base complexes are significantly less colored than their porphyrinato analogues; and 3) the aluminum centers are anticipated to be more electrophilic in the Schiff base derivatives due to replacement of two nitrogen atoms by two oxygen atoms in the ligand core, which possibly offers polymerization centers of enhanced activity. Complexes of this type have been the focus of interest in other areas of catalysis^[14] and alkoxyaluminum derivatives have recently been exploited^[15, 16] in the stereoselective ring-opening polymerization (ROP) of lactide monomers.

The nickel-catalyzed approach to active enolate centers builds on the observation by Jeffery et al.^[17] that [Ni(acac)₂] can be used to catalyze the reaction of trimethylaluminum with α,β -unsaturated carbonyl species to give aluminum enolates in high yield. In this study, we focus on the Schiff base methylaluminum complex [Al(*t*Bu₄salen)Me] (**1**) (*t*Bu₄salen = 3,3',5,5'-tetra-*tert*-butyl-salen), a derivative with particularly good solubility in a range of solvents.^[18] The results of polymerization studies on methylmethacrylate are summarized in Table 1.

On its own, compound **1** is not a good initiator for MMA polymerization (entry 1), affording only 8 % conversion after 20 h, to give PMMA of high molecular weight and broad molecular weight distribution. Bis(2,6-di-*tert*-butyl-4-methylphenoxide)aluminummethyl (MAD) is widely used as a Lewis acid activator for MMA polymerizations,^[11b] and addition of three equivalents of MAD to **1** is found to increase the rate of reaction and conversion (entry 2), but it also dramatically increases the molecular weight of the resultant PMMA. Entries 3 and 4 show that neither MAD nor [Ni(acac)₂] on their own are capable of initiating the polymerization of MMA, although in combination these reagents give a highly active, albeit not well-controlled, polymerization system (entry 5). When [Ni(acac)₂] is combined with **1** in the absence of MAD, a poor initiating system is again obtained (entry 6).

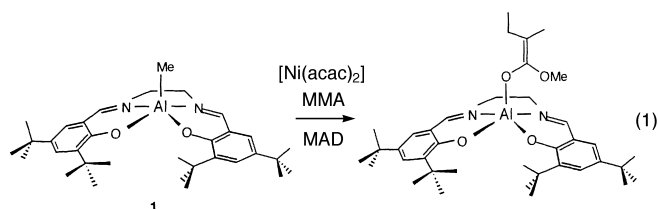


Table 1. Results of MMA polymerizations.^[a]

Entry	1	MAD	[Ni(acac) ₂]	Time	% Yield ^[b]	<i>M</i> _n ^[c]	<i>M</i> _w / <i>M</i> _n	% <i>r</i> _r ^[d]	% <i>mm</i> ^[d]
1	1	0	0	20 h	8	184 000	2.54	69	3
2	1	3	0	20 h	87	2 × 10 ⁶	2.86	72	3
3	0	3	0	20 h	0	–	–	–	–
4	0	0	1	20 h	0	–	–	–	–
5	0	3	1	< 2 min	95	118 300	1.63	72	2
6	1	0	1	20 h	7	229 400	1.85	71	2
7	1	3	1	< 2 min	92	24 700	1.17	69	3
8 ^[e]	1	3	1	< 2 mins	90	26 000	1.18	68	2
9 ^[f]	1	3	1	1 h	77	23 000	1.14	72	1
10 ^[g]	1	3	1	1 h	22	15 600	1.17	71	1
11 ^[g]	1	3	1	2 h	46	25 700	1.17	72	2
12 ^[g]	1	3	1	3 h	54	27 300	1.25	71	2
13 ^[g]	1	3	1	3.5 h	60	30 400	1.26	71	1
14 ^[g]	1	3	1	4 h	75	35 700	1.22	72	1

[a] Unless stated otherwise, conditions employed are: 1.00 g MMA (200 equiv, target *M*_n 20 000) in 2 mL CH₂Cl₂. [b] Yield after workup, polymer precipitated from acidified MeOH, washed 3 times with MeOH, then dried for 12 h (60 °C, 5 Torr). [c] Determined by GPC using monodisperse poly(styrene) standards. [d] Determined by ¹H NMR spectroscopy. [e] Reaction carried out in toluene. [f] Reaction carried out in THF. [g] 2.00 g MMA (400 equiv, *M*_n targeted at 100 % conversion = 40 000) in 4 mL THF.

It is only when $[\text{Ni}(\text{acac})_2]$ is combined with **1** and MAD in a three-component system that a highly active and well controlled polymerization results, affording PMMA of pre-determined molecular weight and narrow molecular weight distribution (entries 7 and 8). These observations suggest that the MAD reagent plays a dual role, one of activating the MMA monomer, the other of generating the active nickel catalyst responsible for enolate formation (see below).

Upon addition of the MMA solution (CH_2Cl_2 or toluene) to a 1:1:3 ratio of **1**, $[\text{Ni}(\text{acac})_2]$, and MAD, there is an immediate color change from pale green to bright orange, associated with the formation of a new nickel species along with the active aluminum initiator. There is a large exotherm and the reaction is complete inside two minutes, after which the solution color rapidly changes from orange to dark brown; the system is then no longer active. The syndiotactic (*rr*) content of the resultant PMMA is ca. 70% in all cases, and can be raised to 84% (< 1% *mm*) by carrying out the polymerization at -20°C . Pentad analyses on the carbonyl carbon signals in the ^{13}C NMR spectrum (triad test: $-4[rr][mm]/[mr]^2 = 0.7$ and $2[rr]/[mr] = 4.7$) suggests that the PMMA is formed by a chain-end control mechanism (see Supporting Information for more details).

Experiments in which the concentration of $[\text{Ni}(\text{acac})_2]$ is varied from 0.05 to 2 equivalents relative to $[\text{Al}(\text{tBu}_4\text{salen})\text{Me}]$ show no effect on the molecular weight or molecular weight distribution of the resultant PMMA, which confirms that the nickel component plays a catalytic role and that the polymerization is not nickel-centered. There is a linear relationship between M_n and $[\text{mon}]/[\textbf{1}]$ (Figure 1a), narrow molecular weight distribution PMMA being formed in each case, and varying the amount of MAD activator does not affect the molecular weight of the polymer formed, only the rate of reaction. This provides good evidence that the polymerization is centered at the Schiff base ligated aluminum, rather than the aluminum center of the activator.^[19]

The polymerization is significantly slower in THF (entry 9), attaining only 77% conversion after 1 h, presumably due to competition for the active site between monomer and THF. On reaching 100% conversion in THF, darkening of the solution is not observed, the system remains active and displays living characteristics. A plot of M_n versus percentage conversion (Figure 1b, Table 1, entries 10–14) reveals a linear relationship and narrow distribution product. A prepolymer solution generated in this manner can be used to bring about the formation of block copolymers. For example, addition of 200 equivalents of MMA to the initiator system followed by 141 equivalents of *n*-butylmethacrylate affords a PMMA-*b*-PBMA copolymer. Figure 2 shows the GPC traces for the PMMA prepolymer and the PMMA/PBMA block copolymer. A small amount of “dead” prepolymer can be seen as a low molecular weight tail to the GPC trace of the di-block product indicating that a small proportion of the prepolymer chains have died before re-initiation with BMA.

In order to probe further the role of $[\text{Ni}(\text{acac})_2]$, its reaction with the efficient alkylating agent $[\text{Me}_2\text{Al}(\text{OEt})]$ was studied. Upon mixing the aluminum and nickel species in CH_2Cl_2 , an immediate reaction was observed to give a brown solution reminiscent of the decomposition reaction following polymer-

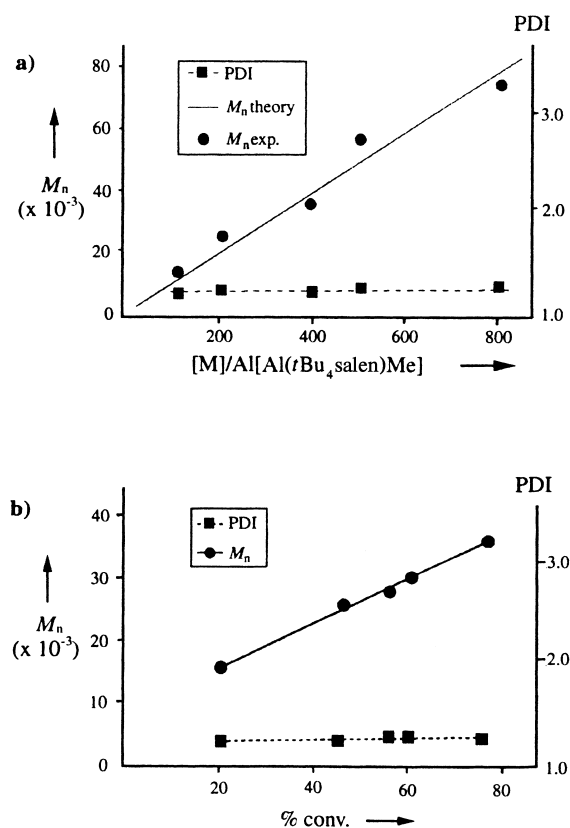


Figure 1. a) Plot of M_n versus $[\text{mon}]/[\text{tBu}_4\text{SalenAlMe}]$ (CH_2Cl_2 solution, $[\text{Ni}(\text{acac})_2]:[\text{Al}(\text{tBu}_4\text{salen})\text{Me}]:\text{MAD}:\text{MMA} = 1:1:3:x$, where $x = 100, 200, 400, 500, 800$); b) plot of M_n versus % conversion (THF solution, $[\text{Ni}(\text{acac})_2]:[\text{Al}(\text{tBu}_4\text{salen})\text{Me}]:\text{MAD} = 1:1:3$).

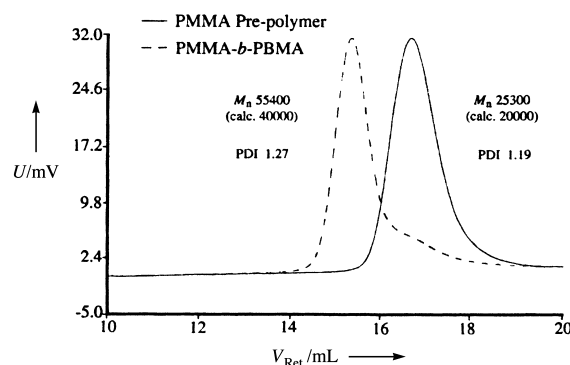
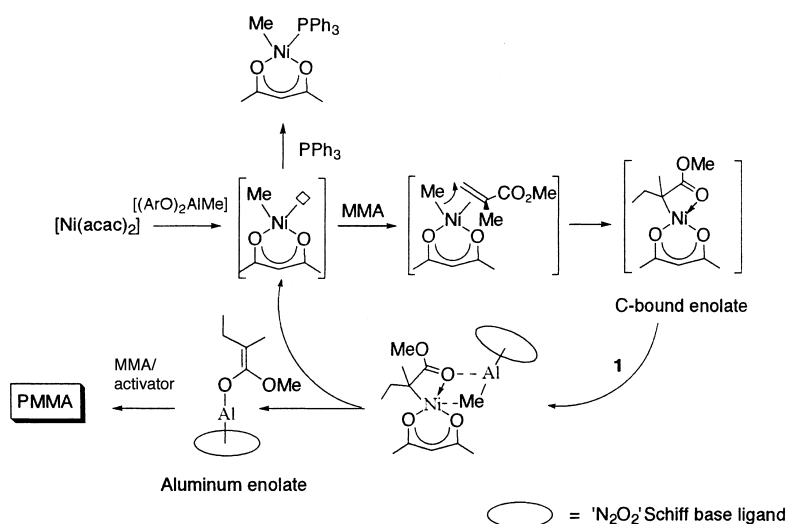


Figure 2. GPC traces for the PMMA prepolymer and the PMMA-PBMA di-block copolymer.

ization. When the same reaction was carried out in the presence of PPh_3 , the known complex $[\text{Ni}(\text{acac})(\text{Me})(\text{PPh}_3)]^{[20]}$ was formed, indicating that a key function of the activator is to abstract an acac ligand and to alkylate the nickel center which, in the presence of PPh_3 , is stabilized as the phosphane adduct (acac = acetylacetonato). The reaction with MAD, though more difficult to follow by NMR, is also believed to result in alkylation of the nickel center. In the absence of phosphane it is presumed that MMA will bind to the nickel center (Scheme 1), where it is then available to insert into the Ni–C(methyl) bond to give a carbon-bound enolate species. This species is apparently stable only in the presence of excess MMA. It is, therefore, probable that an



Scheme 1. Postulated mechanism for the role of nickel in the catalyzed formation of aluminum enolates.

additional equivalent of MMA binds to the nickel center, possibly in place of the carbonyl group (Scheme 1). To account for the catalytic dependence of the reaction on [Ni(acac)₂] and the fact that the polymerization is centered at the Al(*t*Bu₄salen) component, transfer of the enolate from nickel to aluminum with concomitant transfer of the methyl group from aluminum to nickel is proposed (Scheme 1). This generates the active aluminum enolate species for polymerization and reforms the "[Ni(acac)Me]" catalyst. In a separate study we have successfully synthesized the aluminum enolate species, [Al(*t*Bu₄salen){OC(OrBu)=CMe₂}], and shown that this is an efficient initiator for MMA polymerization. These results will be reported in due course.

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- [1] T. P. Davis, D. M. Haddleton, S. N. Richards, *Macromol. Chem. Phys.* **1994**, C34, 243.
- [2] a) K. Hatada, K. Kitayama, K. Ute, *Prog. Polym. Sci.* **1988**, 13, 189; b) S. Nakahama, A. Hirao, *Prog. Polym. Sci.* **1990**, 15, 299; c) W. Szwarc, *Adv. Polym. Sci.* **1983**, 49, 1.
- [3] D. G. H. Ballard, R. J. Bowles, D. M. Haddleton, S. N. Richards, R. Sellens, D. L. Twose, *Macromolecules* **1992**, 25, 5907.
- [4] O. W. Webster, W. R. Hertler, D. Y. Sogah, W. B. Farnham, T. V. Rajan Babu, *J. Am. Chem. Soc.* **1983**, 105, 5706.
- [5] a) A. F. Burczyk, K. F. O'Driscoll, G. L. Rempel, *J. Polym. Sci. Polym. Chem. Ed.* **1984**, 22, 3255; b) N. S. Enikolopyan, B. R. Smirnov, G. V. Ponomarev, I. M. Belgovskii, *J. Polym. Sci. Polym. Chem. Ed.* **1981**, 19, 879; c) A. H. Janowicz, *US-A Patent* 4694054, **1987**. [*Chem Abstr.* **1987**, 106, 157018.]
- [6] J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo, S. H. Thang, *Macromolecules* **1998**, 31, 5559.
- [7] a) C. Granel, P. Teyssié, P. DuBois, P. Jérôme, *Macromolecules* **1996**, 29, 8576; b) D. M. Haddleton, C. Waterson, P. J. Derrick, C. B. Jasieczek, A. Shooter, *Chem. Commun.* **1997**, 683; c) M. Kato, M. Kamigaito, M. Sawamoto, T. Higashimura, *Macromolecules* **1995**, 28, 1721; d) V. Percec, B. Barboiu, *Macromolecules* **1995**, 28, 7970; e) K. Matyjaszewski, *Curr. Opin. Solid State Mater. Sci.* **1996**, 1, 769, and references therein.
- [8] a) D. Mardare, K. Matyjaszewski, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **1993**, 34, 566; b) R. P. N. Veregin, M. K. Georges, P. M. Kazmaier, G. K. Hamer, *Proc. Am. Chem. Soc. Div. Polym. Mater. Sci. Eng.* **1993**, 68, 8.

- [9] H. Yasuda, H. Yamamoto, K. Yokota, S. Miyake, A. Nakamura, *J. Am. Chem. Soc.* **1992**, 114, 4908.
- [10] a) S. Collins, D. G. Ward, *J. Am. Chem. Soc.* **1992**, 114, 5460; b) K. Soga, H. Deng, T. Yano, T. Shiono, *Macromolecules* **1994**, 27, 7938.
- [11] a) M. Kuroki, T. Aida, S. Inoue, *J. Am. Chem. Soc.* **1987**, 109, 4737; b) M. Kuroki, T. Watanabe, T. Aida, S. Inoue, *J. Am. Chem. Soc.* **1991**, 113, 5903.
- [12] M. Chisholm, *Chem. Br.* **1998**, April, 33.
- [13] In later work, Inoue et al. have shown that thiolate derivatives such as [Al(tpp)(SnPr)] are active initiators in the absence of a light source: T. Adachi, H. Sugimoto, T. Aida, S. Inoue, *Macromolecules* **1993**, 26, 1238.
- [14] For recent examples, see: a) Cr: A. G. Dosseter, T. F. Jamison, E. N. Jacobsen, *Angew. Chem.* **1999**, 111, 2549; *Angew. Chem. Int. Ed.* **1999**, 38, 2398; b) Al: M. S. Sigman, E. N. Jacobsen, *J. Am. Chem. Soc.* **1998**, 120, 5315; c) Co: M. H. Wu, K. B. Hansen, E. N. Jacobsen, *Angew. Chem.* **1999**, 111, 2167; *Angew. Chem. Int. Ed.* **1999**, 38, 1212; d) Cu: L. A. Dakin, S. E. Schaus, E. N. Jacobsen, J. S. Panek, *Tetrahedron Lett.* **1998**, 39, 8947, and references therein.
- [15] T. M. Ovitt, G. W. Coates, *J. Am. Chem. Soc.* **1999**, 121, 4072.
- [16] C. P. Radano, G. L. Baker, M. R. Smith, *J. Am. Chem. Soc.* **2000**, 122, 1552.
- [17] a) E. A. Jeffery, A. Meisters, T. Mole, *J. Organomet. Chem.* **1974**, 74, 365; b) E. A. Jeffery, A. Meisters, T. Mole, *J. Organomet. Chem.* **1974**, 74, 373; c) E. A. Jeffery, A. Meisters, T. Mole, *Aust. J. Chem.* **1975**, 28, 801.
- [18] Synthesis and structure of **1**: D. A. Atwood, M. S. Hill, J. A. Jegier, D. Rutherford, *Organometallics* **1997**, 16, 2659.
- [19] Although polymerization can occur at the aluminum center of the MAD activator in the absence of [Al(*t*Bu₄salen)Me] (Table 1, entry 5), the polymerization is only controlled in the presence of the Schiff base complex.
- [20] a) K. Maruyama, T. Ito, A. Yamamoto, *J. Organomet. Chem.* **1978**, 155, 359; b) J. M. Huggins, R. G. Bergman, *J. Am. Chem. Soc.* **1979**, 101, 4410.

Reactive Intermediates on Metal Surfaces: A Ketene Monolayer on Single Crystal Platinum Generated by Photolysis of Pyridyl α -Diazoketones**

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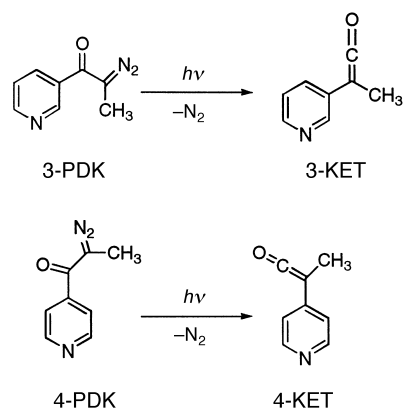
There is considerable current interest in understanding the chemistry of organic molecules as monolayers (saturated layer) on metal surfaces. Much of the effort in this regard has focused on long-chain alkyl thiols or disulfides on gold with

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the ultimate goals of being able to control the interfacial properties for use in wide ranging material and device applications.^[1] In contrast, examples of chemistry of smaller organic molecules as monolayers on metal and other electronic materials has not been as widely examined, although there is a large area of science which is directed at the adsorption of small molecules on metal surfaces. Our group's interest is in the study of photochemical reactions of molecules confined to metal surfaces and comparison of this reactivity to solution- and solid-phase transformations to try to gain a better understanding of the factors that control the reactions of organic molecules in these uniquely constrained environments. Considering the enormous amount of work in the area of self-assembled monolayers of long-chain alkyl thiols on gold, there are remarkably few studies reported on the photochemistry of these types of systems.^[2–8] Examples of photochemical conversions of small organic molecule monolayers chemisorbed on metal surfaces remains virtually unexplored,^[9–12] although photochemical conversions on semiconductor surfaces has led to many important discoveries.

Pyridine and substituted pyridines are a class of compounds that are known to form monolayers on platinum metal and their spectroscopic and electrochemical properties have been investigated in some detail.^[13–18] Here we report the characterization of 3- and 4-substituted pyridyl α -diazoketones (3-PDK and 4-PDK, respectively) as stable monolayers on single



crystal platinum (Pt (110)) using ultra high vacuum (UHV) reflection absorption infrared spectroscopy (RAIRS). Furthermore, photolysis of these monolayers by irradiation at wavelengths of 300–400 nm releases molecular nitrogen to yield a pyridylketene functionality (3- and 4-KET) at the interface through a photo-Wolff rearrangement.^[19] The IR spectroscopy of ketenes generated photochemically has been studied extensively in cryogenic matrices^[20] and by laser flash photolysis with time-resolved-infrared detection.^[21] To the best of our knowledge there is no example of the formation of a stable ketene generated photochemically on a metal surface, although small amounts of ketene itself have been observed by RAIRS on a metal surface through the thermal decomposition of acrolein.^[22] Thus, this study represents the first direct spectroscopic evidence of a platinum surface modified with a photoreactive α -diazocarbonyl monolayer and the subsequent selective formation of a stable ketene functionality in this type of environment. Diazocarbonyl and ketene

functionalities have versatile reactivities and has very significant application in research and technology;^[23, 24] we believe that knowledge of the interfacial reactivity in the present systems can be extended to provide a novel surface for template synthesis and modifications.

The diazoketones 3- and 4-PDK were synthesized by diazotization, using diazoethane, of the corresponding pyridoyl chlorides. Single crystal Pt (110) (1×2 reconstructed) was prepared and cleaned in a manner previously described and mounted in a UHV system equipped with an FTIR spectrometer, a quadrupole mass spectrometer, an Auger spectrometer with 3-grid retarding field analyzer for low-energy electron diffraction (LEED), a focused UV/Vis Hg-Xe arc lamp light source, and appropriate dosers.^[25] Monolayers of 3-PDK and 4-PDK were prepared by first dosing the clean Pt surface at 160 K to exposures corresponding to tens or even hundreds of monolayers. The resulting multilayer was then thermally desorbed by raising the temperature at 3 K s^{-1} . The maximum rate of desorption occurred at 263 K as determined by temperature programmed desorption (TPD) studies of masses indicative of the pyridyl substrates, namely m/z : 161 (M^+), 105 ($\text{C}_7\text{H}_7\text{N}^+$), and 78 ($\text{C}_5\text{H}_4\text{N}^+$). No further desorption of these high mass ions was observed at higher temperatures following desorption of the multilayer at 263 K. We infer from this that the remaining monolayer (saturated layer) could not be desorbed thermally before its decomposition at approximately 370 K. Thermal decomposition of the monolayer was determined by monitoring the release of N_2 (m/z : 28) from the surface. Dosing at low temperatures was used to ensure a high coverage, which is known to favor binding of the pyridyl nitrogen atom to Pt over an orientation where the aromatic ring lies flat on the surface.^[14–16] A second method for monolayer preparation consisted of dosing the clean Pt surface at 283 K, where multilayers do not form. This method yielded identical RAIR spectral features (see below) to that observed for monolayers produced by low temperature absorption followed by desorption of the multilayer. No CO signals are observed in the IR spectrum when the saturated layer was exposed to carbon monoxide, which indicates there are no available sites on the metal for its absorption.

Figure 1 shows the RAIR spectra of 3-PDK and 4-PDK on clean Pt (110) prepared using the second method and the corresponding transmission IR spectra for solutions of 3- and 4-PDK in CCl_4 . The remarkable similarity of the diagnostic^[26] antisymmetric (out-of-phase) $\text{C}=\text{N}=\text{N}$ and the $\text{C}=\text{O}$ absorptions between the solution-phase spectra of 3-PDK and 4-PDK and those obtained on the monolayer suggest that this functional group is stable on the Pt surface. The $\text{C}=\text{N}=\text{N}$ stretches in the monolayer occur at 2081 and 2085 cm^{-1} for 3- and 4-PDK, respectively, a slightly higher wavenumber than in solution (2071 and 2072 cm^{-1}). Slight shifts are expected when the pyridyl ring becomes more electron withdrawing as a result of the bonding of the nitrogen atom to Pt. The broadened $\text{C}=\text{O}$ region of the monolayer spectrum (maximum absorption 1605 and 1615 cm^{-1}) may indicate some interaction of the $\text{C}=\text{O}$ group with the surface, or may simply be a result of intermolecular interactions on the monolayer, as is observed generally on metal surfaces.^[27] Other absorptions arising from $\text{C}-\text{C}$ and $\text{C}-\text{H}$ stretches of

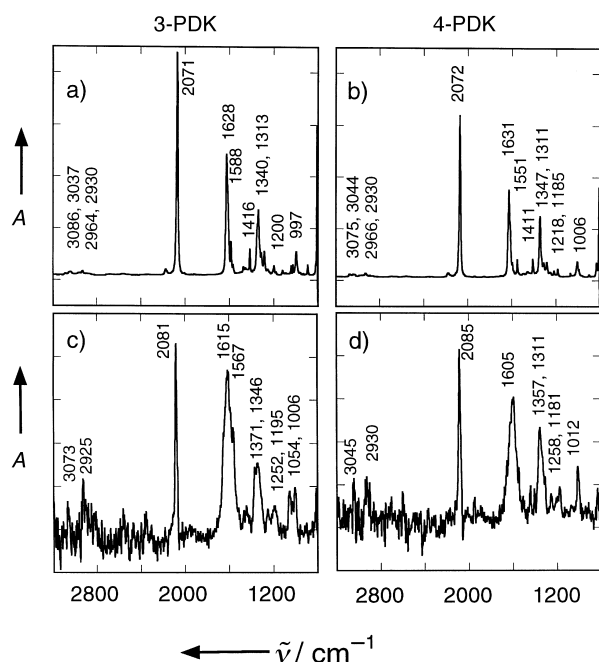


Figure 1. Comparison of the transmission IR spectra of solutions of a) 3-PDK and b) 4-PDK, measured in CCl_4 at 298 K (32 scans, 4 cm^{-1} resolution) with the corresponding RAIR spectra of c) 3-PDK and d) 4-PDK as monolayers on Pt (110) dosed and measured at 283 K (500 scans, 4 cm^{-1} resolution).

the pyridyl ring and ring breathing modes, as well as CH_3 umbrella modes are also observed in both the solution state and on the monolayer spectra. Key IR absorptions of both the transmission and RAIRS of 3-PDK and 4-PDK are included in Figure 1. Other researchers have shown that pyridine itself reorganizes at 300 K to form a α -pyridyl species, which is formed by the cleavage of an α -C–H bond.^[14] While we see no evidence for this type of species by a perturbation of the frequencies of the pyridyl ring absorptions relative to those measured at low temperature, we can not rule it out. This is because the region of the spectrum that is diagnostic is dominated by other absorptions in the diazoketone or is outside our available spectral window. Clearly there is no significant change in the diagnostic $\text{C}=\text{N}=\text{N}$ (2081 cm^{-1}) absorption or other main modes compared to the spectra at lower temperatures.

Compounds 3-PDK and 4-PDK form the respective ketene intermediates by the well known photo-Wolff rearrangement on photolysis with light of wavelength 300–400 nm.^[28] The ketene is not long lived in solution and reacts with a variety of nucleophilic substrates. The loss of molecular nitrogen upon photolysis of the Pt-3-PDK and Pt-4-PDK monolayers is monitored by mass spectroscopy (m/z : 14 and 28; Figure 2). These masses are only detected when the surface is being irradiated. Importantly, no peak at m/z 78 or 105, which correspond to pyridyl fragments, are observed and indicate that photolysis does not cause desorption of the pyridyl moiety. Partial RAIR spectra measured at various times upon irradiation of the Pt-3-PDK monolayer at 300–400 nm are shown in Figure 3. Particularly diagnostic of the photolysis is the decay of the $\text{C}=\text{N}=\text{N}$ antisymmetric absorption at 2081 cm^{-1} and the concomitant formation of an equally

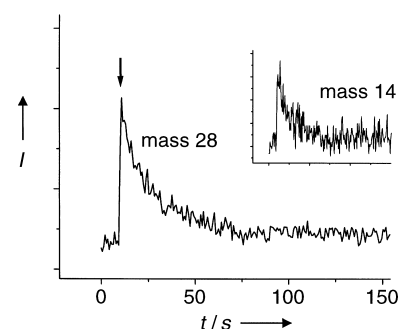


Figure 2. Mass spectrometer response monitoring the release of nitrogen gas (m/z : 28 and 14) from the diazoketone during the photolysis of 3-PDK monolayer on Pt (110). The arrow indicates when the surface was exposed to light.

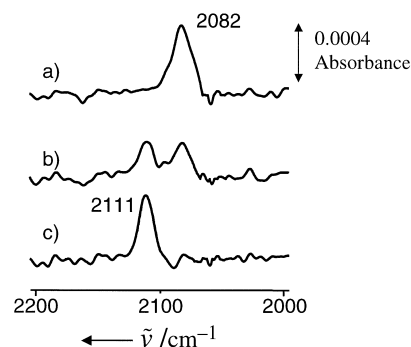


Figure 3. Partial RAIR spectra showing the changes in the 2000–2200 cm^{-1} region of the spectrum on photolysis of Pt-3-PDK: a) prior to photolysis, b) at intermediate photolysis time, and c) after complete photolysis.

intense absorption at 2111 cm^{-1} . The latter absorption continues to increase in magnitude during the photolysis and reaches a maximum when no further molecular nitrogen is liberated from the surface. This absorption is characteristic of a ketene $\text{C}=\text{C}=\text{O}$ antisymmetric (out-of-phase) stretch.^[19] Identical spectral features are observed in the photolysis of Pt-4-PDK. On the basis of the similarity, we assign the spectra obtained upon photolysis of Pt-3-PDK and Pt-4-PDK to Pt-3-KET and Pt-4-KET, respectively. Furthermore, conversion of the diazoketone to the ketene is high, based on the relative intensities of the $\text{C}=\text{N}=\text{N}$ and the $\text{C}=\text{C}=\text{O}$ absorptions before and after photolysis respectively.

Remarkably, the absorption at 2111 cm^{-1} is stable and showed no appreciable loss in intensity for 30 minutes after irradiation (measurements after this time are hindered by poorer baseline corrections). The spectrum is also stable to continued irradiation at wavelengths of 300–400 nm. However, when this surface is exposed in a separate step to an overlayer of methanol dosed at 140 K, the antisymmetric $\text{C}=\text{C}=\text{O}$ absorption disappears and changes in the intensity and shape in the $\text{C}=\text{O}$ portion of the RAIR spectrum are observed. This loss of the absorption at 2111 cm^{-1} with addition of a potential nucleophile provides further support for our assignment to the pyridylketene intermediate being generated on the metal surface.^[19] This aspect of the reactivity, which can be exploited for functional derivatization of the surface, is currently being investigated.

In conclusion, we report the first spectroscopic evidence of a Pt surface modified with a pyridyl diazoketone monolayer.

The stability of the pyridyl diazoketone on a Pt surface opens up the possibility of its use as a template for subsequent surface modifications using the rich chemistry of these functional groups. Furthermore, the diazoketone moiety of this monolayer can be selectively transformed to the remarkably stable pyridylketene functionality upon ultraviolet irradiation and loss of molecular nitrogen, presumably by a photo-Wolff reaction, analogous to the solution-phase chemistry. The spectroscopic evidence for the formation of the ketene suggests that its chemistry can also be used for subsequent surface modifications.

Experimental Section

3-PDK and 4-PDK: The 3- and 4-pyridyl diazoketones were synthesized by reaction of their respective 3- and 4-pyridoyl chlorides with diazoethane, in an analogous way to the synthesis of other aryl diazoketones.^[28] The substrates were purified by repeated chromatography on flash silica gel (80 g silica per 1 gram of diazoketone) with 75:25 ethyl acetate:hexanes.

3-PDK: ¹H NMR (300 MHz, CDCl₃, 25 °C, TMS): δ = 2.14 (s, 3H), 7.36 (m, 1H), 7.86 (m, 1H), 8.69 (m, 1H), 8.81 (m, 1H); ¹³C NMR (75 MHz, CDCl₃, 25 °C, TMS): δ = 9.2, 63.7, 123.3, 133.1, 134.6, 147.9, 151.9, 187.2; UV/Vis (MeOH) nm, (ε, M⁻¹cm⁻¹): 300.5 (6165), 255.5 (5924), 228.0 (5417); IR (CCl₄) cm⁻¹: 3086, 3037, 2964, 2930, 2071, 1628, 1588, 1416, 1340, 1313, 1200, 997; MS (70 eV): *m/z* (%): 161 (32) [M⁺], 133 (35), 105 (100), 104 (88), 78 (85), 51 (69); HR-MS: calcd: 161.0589; found: 161.0589.

4-PDK: ¹H NMR (300 MHz, CDCl₃, 25 °C, TMS): δ = 2.14 (s, 3H), 7.40 (dd, 4.4, 1.7 Hz, 1H), 8.7 (dd, 4.4, 1.7 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃, 25 °C, TMS): δ = 8.9, 63.7, 120.6, 144.0, 150.3, 187.5; UV/Vis (MeOH) nm, (ε, M⁻¹cm⁻¹): 304.5 (7558), 264 (6578), 220.4 (7000); IR (CHCl₃) cm⁻¹: 3075, 3044, 2966, 2930, 2072, 1631, 1551, 1411, 1347, 1311, 1218, 1185, 1006; MS (70 eV): *m/z* (%): 161 (29) [M⁺], 133 (35), 105 (74), 104(53), 78 (94), 51 (100); HR-MS: calcd: 161.0589; found: 161.0591.

The platinum single crystal Pt (110) was cut and polished using standard procedures, and cleaned before each dosing experiment by Ar⁺ sputtering, heating in oxygen, and annealing cycles. The RAIRS experiments were performed in an ultra high vacuum (UHV) chamber pumped to a base pressure below 3.8 × 10⁻¹⁰ mbar. The sample was heated with a current of up to 30 A and cooled with liquid nitrogen allowing temperature control between 100 and 1300 K. Samples of 3-PDK and 4-PDK were dosed on the Pt crystal with background pressures of 3.8 × 10⁻⁹ mbar. The chamber was equipped with a Hiden Analytical Limited 601 Quadrupole Mass Spectrometer for TPD experiments. A Mattson Galaxy Series 5000 FTIR spectrometer was used for RAIRS, and the RAIR spectra were measured at 283 K unless otherwise noted. The data was collected by focusing the IR source from the spectrometer through a KBr window onto the sample at grazing incidence and refocusing into a MCT detector.

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- [1] For a general introduction to self-assembled monolayers, see A. Ulman, *Chem. Rev.* **1996**, 96, 1533.
- [2] a) L. F. Rozsnyai, M. S. Wrighton, *J. Am. Chem. Soc.* **1994**, 116, 5993; b) E. W. Wollman, D. Kang, D. C. D. Frisbie, I. M. Lorkovic, M. S. Wrighton, *J. Am. Chem. Soc.* **1994**, 116, 4395.
- [3] a) M. O. Wolf, M. A. Fox, *Langmuir* **1996**, 12, 955; b) M. A. Fox, M. D. Wooten, *Langmuir* **1997**, 13, 7099; c) W. Li, V. Lynch, H. Thompson, M. A. Fox, *J. Am. Chem. Soc.* **1997**, 119, 7211; d) M. O. Wolf, M. A. Fox, *J. Am. Chem. Soc.* **1995**, 117, 1845.
- [4] a) T. Kim, R. M. Crooks, M. Tsen, L. Sun, *J. Am. Chem. Soc.* **1995**, 117, 3963; b) T. Kim, K. C. Chan, R. M. Crooks, *J. Am. Chem. Soc.* **1997**, 119, 189.
- [5] See for example: a) I. Willner, A. Doron, E. Katz, *J. Phys. Org. Chem.* **1998**, 11, 546, and references therein; b) M. Lahav, E. Katz, A. Doron, F. Patolsky, I. Willner, *J. Am. Chem. Soc.* **1999**, 121, 862.
- [6] G. J. Jocy, M. S. Workentin, *Chem. Commun.* **1999**, 839.
- [7] J. Jennane, T. Boutros, R. Giasson, *Can. J. Chem.* **1996**, 74, 2509.
- [8] J. Huang, D. A. Dahlgreen, J. C. Hemminger, *Langmuir* **1994**, 10, 626.
- [9] X.-L. Zhou, X.-Y. Zhu, J. M. White, *Surf. Sci. Rep.* (photochemistry at adsorbate/metal interface) **1991**, 13, 73, and references therein.
- [10] E. J. Lee, T. W. Bitner, J. S. Ha, J. S. M. J. Shane, M. J. Sailor, *J. Am. Chem. Soc.* **1996**, 118, 5375.
- [11] a) W. J. Dressick, C. S. Dulcey, J. H. Georger, Jr., J. M. Calvert, *Chem. Mater.* **1993**, 5, 148; b) C. S. Dulcey, J. H. Georger, Jr., V. Krauthamer, D. A. Stenger, T. L. Fare, J. M. Calvert, *Science* **1991**, 252, 551; c) C. S. Dulcey, J. H. Georger, Jr., M.-S. Chen, S. W. McElvany, C. E. O'Ferrall, V. I. Benzeza, J. M. Calvert, *Langmuir* **1996**, 12, 1638.
- [12] a) M. R. Linford, P. Fenter, P. M. Eisenberger, C. E. D. Chidsey, *J. Am. Chem. Soc.* **1995**, 117, 3145; b) M. R. Linford, C. E. D. Chidsey, *J. Am. Chem. Soc.* **1993**, 115, 12631.
- [13] a) V. H. Grassian, E. L. Muetterties, *J. Phys. Chem.* **1986**, 90, 5900; b) A. L. Johnson, E. L. Muetterties, J. Stohr, F. Sette, *J. Phys. Chem.* **1985**, 89, 4071.
- [14] a) S. Haq, D. A. King, *J. Phys. Chem.* **1996**, 100, 16957; b) M. Surman, S. R. Bare, P. Hofmann, D. A. King, *Surf. Sci.* **1987**, 179, 243.
- [15] Y. Gui, T. Kuwana, *J. Electroanal. Chem.* **1987**, 222, 321.
- [16] E. K. Krauskopf, L. M. Rice-Jackson, A. Wieckowski, *Langmuir* **1990**, 6, 970.
- [17] R. J. Forster, L. R. Faulkner, *J. Am. Chem. Soc.* **1994**, 116, 5444; R. J. Forster, L. R. Faulkner, *J. Am. Chem. Soc.* **1994**, 116, 5453.
- [18] a) D. Acevedo, H. D. Abruña, *J. Phys. Chem.* **1991**, 95, 9590; b) D. Acevedo, R. L. Bretz, J. D. Tirado, H. D. Abruña, *Langmuir* **1994**, 10, 1300; c) J. D. Tirado, D. Acevedo, R. L. Bretz, H. D. Abruña, *Langmuir* **1994**, 10, 1971.
- [19] a) T. Tidwell, *Ketenes*, Wiley, New York, **1995**; b) *The Chemistry of Ketenes, Allenes, and Related Compounds* (Ed.: S. Patai), Wiley, New York, **1980**.
- [20] For examples, see a) C. B. Moore, G. C. Pimentel, *J. Chem. Phys.* **1963**, 38, 2816; b) P. Visser, R. Zuhse, M. W. Wong, C. Wentrup, *J. Am. Chem. Soc.* **1996**, 118, 12598, and references therein.
- [21] For examples, see a) B. D. Wagner, B. R. Arnold, G. S. Brown, J. Lusztyk, *J. Am. Chem. Soc.* **1998**, 120, 1827, and references therein; b) Y. Wang, T. Yuzawa, H.-O. Hamaguchi, J. P. Toscano, *J. Am. Chem. Soc.* **1999**, 121, 2875.
- [22] J. C. de Jesus, F. Zaera, *J. Mol. Cat. A* **1999**, 138, 237. In addition, the adsorption and surface chemistry of ketene (CH₂C=O) dosed on metal surfaces, including Pt, has been examined in detail. In none of these studies was the ketene functionality observed directly. Instead it associates by di-σ bonding through the carbon atoms, as a bound acetyl, or decomposes to CO and CH₂, depending on temperature. For examples, see a) P. L. Radloff, C. M. Mitchell, J. M. Greenlief, J. M. White, C. A. Mims, *Surf. Sci.* **1987**, 183, 377; b) M. A. Henderson, Y. Zhou, J. M. White, C. A. Mims, *J. Phys. Chem.* **1989**, 93, 3688; c) B. Roop, B. S. A. Costello, C. M. Greenlief, J. M. White, *Chem. Phys. Lett.* **1988**, 143, 38; d) G. E. Mitchell, P. L. Radloff, J. M. Greenlief, M. A. Henderson, J. M. White, *Surf. Sci.* **1987**, 183, 403.
- [23] a) T. Ye, M. A. McKervey, *Chem. Rev.* **1994**, 94, 1091; b) B. B. Snider, *Chem. Rev.* **1988**, 88, 793; c) M. Regitz, G. Mass, G. *Diazo Compounds*, Academic Press, Orlando, Florida, **1986**.
- [24] a) A. Reiser, *Photoreactive Polymers: The Science and Technology of Resists*, Wiley, New York, **1989**; b) E. Reichmanis, L. F. Thompson, *Chem. Rev.* **1989**, 89, 1273; c) W. M. Moreau, *Semiconductor Lithography*, Plenum, New York, **1988**.
- [25] a) R. V. Kasza, J. G. Shapter, K. Griffiths, P. R. Norton, J. J. Sloan, *Surf. Sci.* **1994**, 321, L239. An Auger spectrum obtained using 3-grid LEED optics confirmed that the platinum was clean of C, O, and S; b) S. Serghini-Monim, L. L. Coatsworth, P. R. Norton, R. J. Puddephatt, *Rev. Sci. Instrum.* **1996**, 67, 3672.
- [26] D. Lin-Vien, N. B. Colthrup, W. G. Flately, J. G. Grasselli, *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, Academic Press, Boston, **1991**.
- [27] A. M. Bradshaw, E. Schweitzer, *Spectroscopy of Surfaces* (Eds. R. J. H. Clark, R. E. Hester), Wiley, Chichester, **1988**.
- [28] B. Eistert, *Synthesis with Diazomethane*, Interscience, New York, **1948**, p. 513.

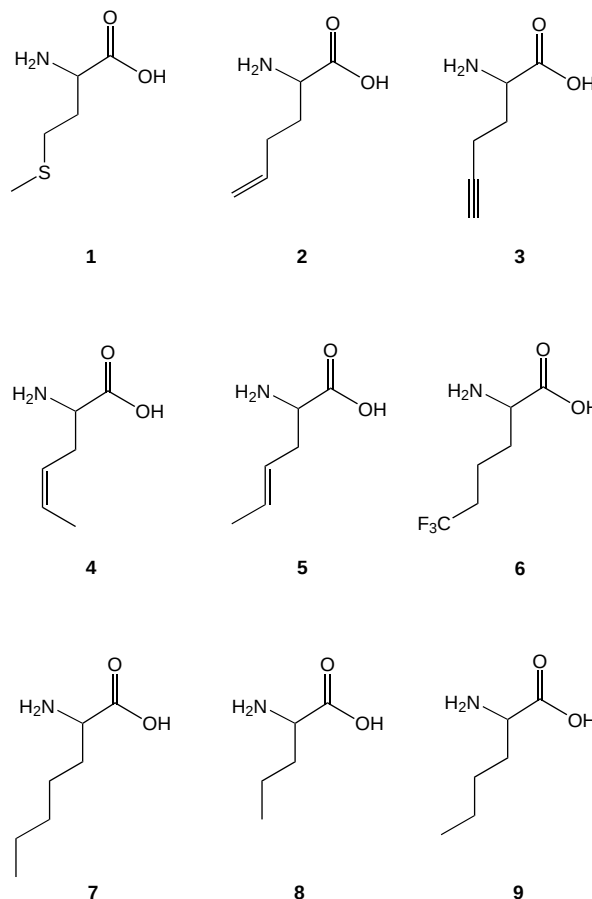
Expanding the Scope of Protein Biosynthesis by Altering the Methionyl-tRNA Synthetase Activity of a Bacterial Expression Host**

Kristi L. Kiick, Jan C. M. van Hest, and David A. Tirrell*

Expanding the scope of biological polymerizations to include non-natural monomers is an area of growing interest with important theoretical and practical consequences. Advances in DNA sequencing,^[1] DNA base pairing models,^[2] materials synthesis,^[3] and cell surface engineering^[4] have resulted from the recognition of non-natural monomers by the enzymes that control these polymerizations. Similar efforts to incorporate novel amino acids into proteins in vivo have relied on the ability of the translational apparatus to recognize amino acid analogues that differ in structure and functionality from the proteinogenic amino acids. The key determinant of the success of this method is recognition of analogues by the aminoacyl-tRNA synthetases (AARS), which safeguard the fidelity of amino acid incorporation into proteins. We and others have demonstrated the ability of the wild-type translational apparatus to use non-natural amino acids with fluorinated,^[5] unsaturated,^[6] electroactive,^[7] and other side chain functions.^[8] Nevertheless, the number of amino acid analogues shown conclusively to exhibit translational activity is small, and the chemical functionality that can be accessed by this method remains modest. Our most recent efforts have therefore been directed toward manipulating the activity of the AARS^[9] to expand the novel chemical and physical properties that can be engineered into proteins in vivo.^[10]

Our attention was drawn to methionyl-tRNA synthetase (MetRS) by reports of the replacement of methionine by ethionine, selenomethionine, telluromethionine, trifluoromethionine, and norleucine.^[11] Methionine (**1**) is an intriguing target for substitution by non-natural amino acids, as its hydrophobicity and polarizability make it an important amino acid for regulating protein structure and protein–protein

recognition processes;^[12] replacement of methionine by analogues may therefore permit purposeful manipulation of these events. We recently reported the replacement of methionine by the unsaturated methionine analogues homallylglycine (Hag, **2**) and homopropargylglycine (Hpg, **3**) in experiments utilizing the translational apparatus of wild-type *Escherichia coli* cells.^[13] We were unable to demonstrate the incorporation of *cis*- or *trans*-crotylglycine (Ccg, **4**, and Tcg, **5**, respectively), which was surprising given the reported antagonist activity of Ccg.^[14] We speculated that manipulation of the MetRS activity of the expression host might enable **4**, **5**, and additional methionine analogues to serve as substrates for bacterial protein biosynthesis.



The relative rates of activation of methionine and methionine analogues **2–5** by MetRS were characterized in vitro by the ATP–PP_i exchange assay.^[15] Figure 1 shows that analogues **2** and **3** are activated by MetRS, as anticipated on the basis of our in vivo experiments,^[13] although they cause exchange of PP_i at rates several-fold lower than methionine. Analogue **4** does not cause measurable exchange of PP_i by MetRS in vitro, which was expected since neither **4** nor **5** were indicated to be translationally active in vivo. Analogue **5**, however, is activated by MetRS, causing slow exchange of PP_i under the assay conditions used here. Given that k_{cat}/K_m for **5** is 4700-fold lower than that for methionine,^[13a] it is not surprising that **5** did not support measurable protein synthesis within the time frame of our in vivo experiments. This result

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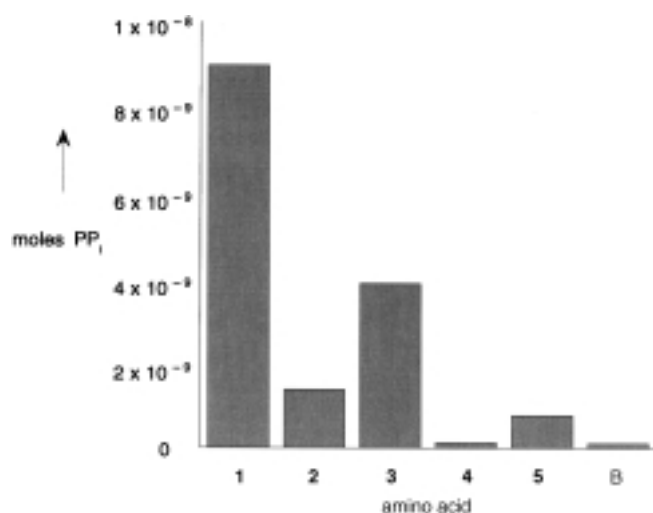


Figure 1. Activation of methionine and methionine analogues by MetRS as measured by ATP–PP_i exchange. The amount of PP_i exchanged in 20 min is shown for methionine (1) and for methionine analogues 2–5. The background (6) is given for a reaction mixture lacking both enzyme and amino acid.

suggested that increasing the MetRS activity of the expression host might allow efficient protein synthesis in cultures supplemented with 5. This strategy has not been employed previously for incorporating non-natural amino acids into proteins in vivo, but reports of in vivo misacylation of tRNA substrates by overexpressed AARS supported the viability of the approach.^[16]

To test this hypothesis, a bacterial host capable of over-expressing MetRS was produced by transforming *E. coli* strain B834(DE3),^[17] a methionine auxotroph, with repressor plasmid pREP4 and expression plasmid pQE15-MRS.^[18] The expression plasmid pQE15-MRS encodes MetRS under control of the *E. coli* promoter *metGp1* (GenBank accession number X55791)^[19] as well as the target protein murine dihydrofolate reductase (mDHFR) under control of a bacteriophage T5 promoter. The expression plasmid also encodes an N-terminal hexahistidine sequence for mDHFR which permits purification of the target protein by immobilized metal affinity chromatography.^[20] Furthermore, mDHFR contains eight methionine residues which can be replaced by methionine analogues. A control bacterial host, which produces only mDHFR and normal cellular levels of MetRS, was prepared by transforming B834(DE3) with pREP4 and pQE15.

Methionine analogues 2–9 were tested for translational activity in both bacterial hosts.^[21] Cultures of B834(DE3)/pQE15-MRS/pREP4 or B834(DE3)/pQE15/pREP4 in M9AA media^[22] were grown to an optical density (OD) of 0.90, and the cells were sedimented by centrifugation. Cells were washed three times with M9 medium and resuspended to an OD of 0.90 in M9 test media containing 19 amino acids plus 1) neither methionine nor analogue (negative control); 2) methionine (60 mg L⁻¹, positive control); or 3) an analogue of interest (60 mg L⁻¹). Expression of mDHFR was induced by addition of 0.4 mM isopropyl β-D-thiogalactopyranoside (IPTG), and protein synthesis was monitored after 4.5 h by SDS polyacrylamide gel electrophoresis (SDS-PAGE); accu-

mulation of the target protein mDHFR was taken as evidence for translational activity of the methionine analogue.

The target protein was not observed in the negative control culture of B834(DE3)/pQE15/pREP4, or in cultures supplemented with Ccg (4), 6,6,6-trifluoro-2-aminoheptanoic acid (6), 2-aminoheptanoic acid (7), or norvaline (8). In contrast, mDHFR was detected in both bacterial host cultures supplemented with methionine (1), Hag (2), Hpg (3), and norleucine (9), as indicated by the appearance of a protein band at the position expected for mDHFR in SDS-PAGE.

For the negative-control cultures and for cultures supplemented with Tcg, however, the behavior of the bacterial hosts differed (Figure 2). mDHFR was not detected in the

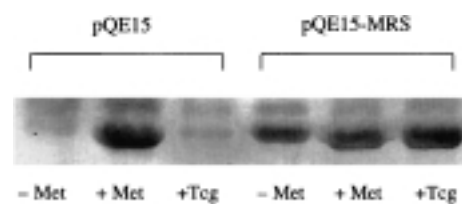


Figure 2. SDS-PAGE analysis of mDHFR synthesis by *E. coli* strains B834(DE3)/pQE15/pREP4 (designated pQE15) and B834(DE3)/pQE15-MRS/pREP4 (designated pQE15-MRS). Cultures (M9 medium plus 19 amino acids) were supplemented with nothing (–Met), methionine (+Met) or *trans*-crotylglycine (+Tcg, 5), as indicated.

B834(DE3)/pQE15/pREP4 culture supplemented with Tcg (5), whereas strong induction of mDHFR was observed for B834(DE3)/pQE15-MRS/pREP4 under the same conditions. Even the unsupplemented control culture of B834(DE3)/pQE15-MRS/pREP4 shows evidence of mDHFR synthesis, suggesting that introduction of pQE15-MRS does indeed increase the rate of activation of methionine in the modified host.^[23]

This supposition was confirmed by direct measurement of the MetRS activities of whole-cell lysates (Figure 3); B834(DE3)/pQE15-MRS/pREP4 exhibits a $V_{\max}$ value for

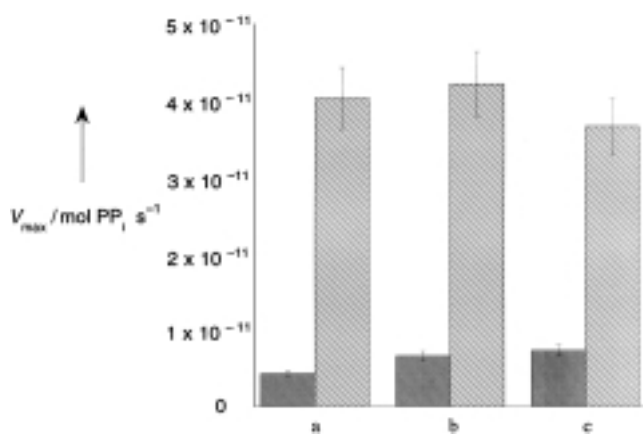


Figure 3. Activation rates of methionine by whole-cell lysates. Maximum ATP–PP_i exchange velocities, measured at a saturating concentration of methionine (750 μM), are shown for whole-cell lysates of B834(DE3)/pQE15/pREP4 and of B834(DE3)/pQE15-MRS/pREP4 (solid and striped columns, respectively). Rates were measured for cell lysates obtained from cultures prior to protein expression (a), cell lysates obtained from cultures supplemented with methionine during protein expression (b), and cell lysates obtained from cultures supplemented with Tcg (5) during protein expression (c).

methionine activation 5–10-fold higher than that observed for the control host B834(DE3)/pQE15/pREP4.^[24] These results show clearly that increasing the MetRS activity of the host is necessary and sufficient to observe translational activity of Tcg under convenient conditions *in vivo*. Protein yields (mDHFR-Tcg) of approximately 8.5 mg L⁻¹ were observed for B834(DE3)/pQE15-MRS/pREP4 cultures supplemented with Tcg (**5**), compared with yields of approximately 30 mg L⁻¹ for both B834(DE3)/pQE15-MRS/pREP4 and B834(DE3)/pQE15/pREP4 cultures supplemented with methionine (**1**). Amino acid analysis of protein containing Tcg shows a decrease in methionine content to 0.3 mol% from the expected value of 3.8 mol%. It is impossible to detect Tcg directly by amino acid analysis, owing to instability of the analogue under the analysis conditions. If, however, depletion of methionine is assumed to result from replacement by Tcg, the observed analysis corresponds to an overall extent of incorporation of the analogue of 91 ± 2%.

A direct assessment of the extent of incorporation of Tcg into mDHFR is provided by NMR spectroscopy.^[25] Comparisons of the 600 MHz ¹H NMR spectra (Figure 4) of mDHFR, Tcg, and mDHFR-Tcg indicate the appearance, in the mDHFR-Tcg spectrum (Figure 4c), of the Tcg vinylene protons at $\delta = 5.35$ (δ -CH) and $\delta = 5.60$ –5.70 (γ -CH). The signals at $\delta = 5.35$ and $\delta = 5.70$ occur at the same chemical-shift values as in free Tcg and are clearly due to incorporation of Tcg into mDHFR. That the signal at $\delta = 5.60$ arises from the γ -CH vinylene proton of Tcg is suggested by the fact that the integrated intensity of the signal at $\delta = 5.35$ equals the sum of the integrations of the signals at $\delta = 5.60$ and 5.70. This assignment is confirmed by 1D TOCSY (Total Correlation Spectroscopy) NMR experiments which indicate that the protons at both $\delta = 5.60$ and 5.70 are members of the same spin system (and therefore the same amino acid) as those at $\delta = 5.35$ (data not shown). More importantly, the 1D TOCSY experiments also show that the protons at $\delta = 5.35$ (and therefore those at $\delta = 5.60$ and 5.70) are associated with the spin system of the entire Tcg side chain.^[26] Integration of the spectrum suggests that five of the eight methionine positions (occupied by Tcg) are represented by the signal at $\delta = 5.60$; these protons must reside in a magnetically distinct environment from the protons at $\delta = 5.70$. These results unequivocally demonstrate the translational activity of Tcg in the host strain outfitted with elevated MetRS activity. Integration of the NMR spectrum indicates 90 ± 6% replacement of methionine.

Retention of the N-terminal (initiator) methionine in mDHFR is expected on the basis of the identity of the penultimate amino acid,^[27] so N-terminal sequencing provided a third means of assessing the extent of replacement of methionine by Tcg. Because Tcg is not degraded under the analysis conditions, it can be detected directly. Comparison of chromatograms of the N-terminal residues of mDHFR and mDHFR-Tcg (Figure 5) demonstrates that the methionine that normally occupies the initiator position of mDHFR (Figure 5a) is nearly completely replaced with Tcg (Fig-

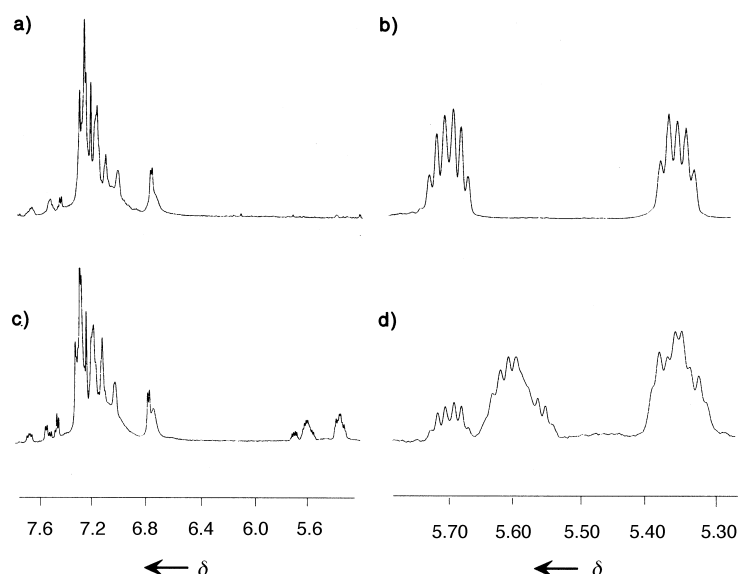


Figure 4. ¹H NMR spectra (599.69 MHz) of mDHFR (a), Tcg (b), and mDHFR-Tcg (c + d). Samples were dissolved at concentrations of approximately 10 mg mL⁻¹ in D₂O containing 2% [D₂]formic acid and spectra were recorded at 25°C overnight.

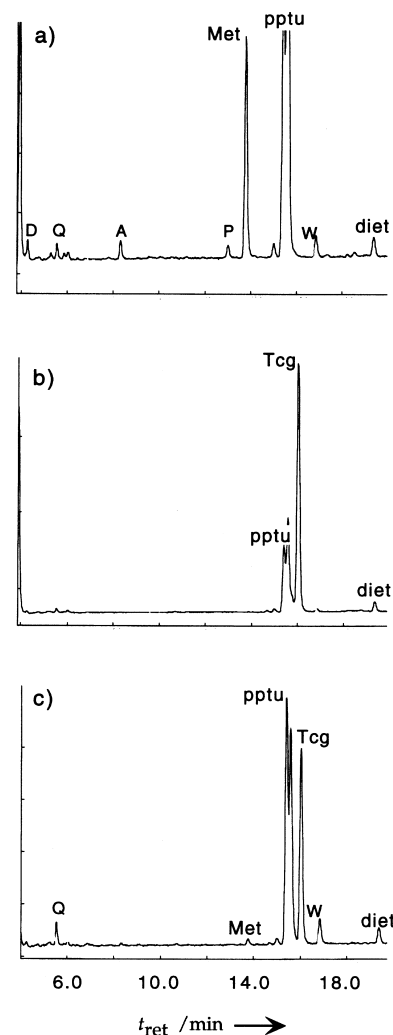


Figure 5. N-terminal sequencing results indicating occupancy of the initiator site in mDHFR-Tcg. Chromatograms are shown for the N-terminal residue of mDHFR (a), Tcg control (b), and the N-terminal residue of mDHFR-Tcg (c), as determined by Edman degradation.

ure 5b) in mDHFR-Tcg (Figure 5c).^[28] These results clearly indicate the incorporation of Tcg at the initiator site of mDHFR-Tcg and corroborate the NMR results. Integration of the peak areas corresponding to Tcg and to methionine indicates $96 \pm 2\%$ incorporation of the analogue at the initiator position.

The incorporation of Tcg into proteins in vivo constitutes the first example of broadening the amino acid substrate range of the *E. coli* translational apparatus through overproduction of MetRS in a bacterial host. The utilization of Tcg in all stages of protein synthesis (including initiation) indicates the appropriateness of targeting the AARS in studies aimed at in vivo incorporation of non-natural amino acids into proteins. Transport into the cell, recognition by methionyl-tRNA formylase, and recognition by the elongation factors and the ribosome are less likely to be limiting factors. These results indicate that this simple strategy—overexpression of AARS—may be used to modify proteins by incorporation of non-natural amino acids that are poor substrates for AARS and that would be essentially inactive in conventional expression hosts. Furthermore, overexpression of mutant forms of the AARS prepared by site-directed mutagenesis or directed evolution^[29] should provide additional strategies for incorporating non-natural amino acids into proteins in vivo.

The results reported here also suggest new opportunities for macromolecular synthesis through protein engineering. The versatile chemistry of the double bond^[30] can be used to control protein structure and function through chemical derivatization, an especially intriguing possibility in this case given the important role of methionine in protein–protein recognition processes. For example, ruthenium-catalyzed olefin metathesis^[31] of homoallylglycine^[32] and *o*-allylserine^[33] side chains has been used to produce covalently modified peptides. The incorporation of Tcg may be singularly useful in this regard as the internal olefin moiety is active in aqueous phase, ring closing metathesis reactions, whereas terminally unsaturated groups (such as those previously used to replace methionine in vivo) are not.^[34]

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- [1] F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463–5467.
- [2] a) M. J. Lutz, S. A. Benner, S. Hein, G. Breipohl, E. Uhlmann, *J. Am. Chem. Soc.* **1997**, *119*, 3177–3178; b) J. C. Morales, E. T. Kool, *Nat. Struct. Biol.* **1998**, *5*, 950–954.
- [3] a) W. H. Park, R. W. Lenz, S. Goodwin, *Macromolecules* **1998**, *31*, 1480–1486; b) Y. Doi, S. Kitamura, H. Abe, *Macromolecules* **1995**, *28*, 4822–4828.
- [4] a) K. J. Yarema, L. K. Mahal, R. E. Bruehl, E. C. Rodriguez, C. R. Bertozzi, *J. Biol. Chem.* **1998**, *273*, 31168–31179; b) L. K. Mahal, K. J. Yarema, C. R. Bertozzi, *Science* **1997**, *276*, 1125–1128.
- [5] a) E. Yoshikawa, M. J. Fournier, T. L. Mason, D. A. Tirrell, *Macromolecules* **1994**, *27*, 5471–5475; b) M. H. Richmond, *J. Mol. Biol.* **1963**, *6*, 284–294; c) D. B. Cowie, G. N. Cohen, E. T. Bolton, H. D. Robichon-Szulmajster, *Biochim. Biophys. Acta* **1959**, *34*, 39–46.
- [6] T. J. Deming, M. J. Fournier, T. L. Mason, D. A. Tirrell, *J. Macromol. Sci. Pure Appl. Chem.* **1997**, *A34*, 2143–2150.
- [7] S. Kothakota, T. L. Mason, D. A. Tirrell, M. J. Fournier, *J. Am. Chem. Soc.* **1995**, *117*, 536–537.

- [8] a) M. J. Dougherty, S. Kothakota, T. L. Mason, D. A. Tirrell, M. J. Fournier, *Macromolecules* **1993**, *26*, 1779–1781; b) T. Tuve, H. Williams, *J. Am. Chem. Soc.* **1957**, *79*, 5830–5831; c) D. B. Cowie, G. N. Cohen, *Biochim. Biophys. Acta* **1957**, *26*, 252–261.
- [9] a) N. Sharma, R. Furter, P. Kast, D. A. Tirrell, *FEBS Lett.* **2000**, *467*, 37–40; b) M. Ibba, H. Hennecke, *FEBS Lett.* **1995**, *364*, 273–275.
- [10] Chemical aminoacylation methods circumvent the AARS altogether (S. M. Hecht, B. L. Alford, Y. Kuroda, S. Kitano, *J. Biol. Chem.* **1978**, *253*, 4517–4520; C. J. Noren, S. J. Anthony-Cahill, M. C. Griffith, P. G. Schultz, *Science* **1989**, *244*, 182–188; J. D. Bain, C. G. Glabe, T. A. Dix, A. R. Chamberlin, *J. Am. Chem. Soc.* **1989**, *111*, 8013–8014) but suffer from low protein yields. Alteration of the AARS activities of the cell through introduction of heterologous synthetases (D. R. Liu, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4780–4785; R. Furter, *Protein Sci.* **1998**, *7*, 419–426; D. R. Liu, T. J. Magliery, M. Pastnak, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10092–10097) is also possible. The relative simplicity of the in vivo approach, its capacity for multisite substitution, and its high synthetic efficiency make it the method of choice whenever possible.
- [11] a) H. Duestel, E. Daub, V. Robinson, J. F. Honek, *Biochemistry* **1997**, *36*, 3404–3416; b) N. Budisa, B. Steipe, P. Demange, C. Eckerskorn, J. Kellermann, R. Huber, *Eur. J. Biochem.* **1995**, *230*, 788–796; c) W. A. Hendrickson, J. R. Horton, D. M. LeMaster, *EMBO J.* **1990**, *9*, 1665–1672.
- [12] a) T. Yuan, A. M. Weljie, H. J. Vogel, *Biochemistry* **1998**, *37*, 3187–3195; b) H. L. Schenck, G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 12487–12494.
- [13] a) J. C. M. van Hest, K. L. Kiick, D. A. Tirrell, *J. Am. Chem. Soc.* **2000**, *122*, 1282–1288; b) J. C. M. van Hest, D. A. Tirrell, *FEBS Lett.* **1998**, *428*, 68–70.
- [14] C. G. Skinner, J. Edelson, W. Shive, *J. Am. Chem. Soc.* **1961**, *83*, 2281–2286.
- [15] The fully active, truncated form of MetRS was purified from overnight cultures of JM101 cells carrying the plasmid pGG3. The enzyme was purified by size exclusion chromatography as previously described (P. Mellot, Y. Mechulam, D. LeCorre, S. Blanquet, G. Fayat, *J. Mol. Biol.* **1989**, *208*, 429–443). Activation of methionine analogues by MetRS was assayed at 25°C by using the amino acid dependent ATP–PP_i exchange reaction as previously described (G. Ghosh, H. Pelka, L. H. Schulman, *Biochemistry* **1990**, *29*, 2220–2225). Assays to determine if the methionine analogues 2–5 are recognized by MetRS were conducted in solutions 75 nM in enzyme and 5 mM in the *L*-isomer of the analogue with a reaction time of 20 min. Kinetic parameters for analogue 5 were obtained with an enzyme concentration of 50 nM and analogue concentrations of 100 μM to 10 mM. Parameters for methionine were obtained by using concentrations ranging from 10 μM to 1 mM. *K_m* values for methionine were similar to those previously reported (24 ± 2 μM), though the measured *k_{cat}* value was somewhat lower than the literature value (13.5 s^{−1}) (H. Y. Kim, G. Ghosh, L. H. Schulman, S. Brunie, H. Jakubowski, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11553–11557). Kinetic constants were calculated by nonlinear regression analysis.
- [16] a) S. Li, N. V. Kumar, U. Varshney, U. L. RajBhandary, *J. Biol. Chem.* **1996**, *271*, 1022–1028; b) J. M. Sherman, M. J. Rogers, D. Soll, *Nucleic Acids Res.* **1992**, *20*, 2847–2852; c) U. Varshney, U. L. RajBhandary, *J. Bacteriol.* **1992**, *174*, 7819–7826; d) R. Swanson, P. Hoben, M. Sumner-Smith, H. Uemura, L. Watson, D. Soll, *Science* **1988**, *242*, 1548–1551.
- [17] Novagen, Inc., Madison, WI, USA.
- [18] A gene encoding a mutant MetRS was removed from plasmid pBSM547W305F (D. Fourmy, Y. Mechulam, S. Brunie, S. Blanquet, G. Fayat, *FEBS Lett.* **1991**, *292*, 259–263) by treatment with restriction enzymes *Sac*I and *Kpn*I. The *Sac*I/*Kpn*I fragment (2450 bp) was ligated into the cloning vector pUC19-NheI, which was constructed to permit the cohesive ends of the mutant MetRS gene to be changed to *Nhe*I. The MetRS gene with *Nhe*I cohesive ends was then ligated into the unique *Nhe*I site of the plasmid pQE15 (Qiagen, Inc., Valencia, CA, USA) to yield plasmid pQE15-W305F. Transformation of pQE15-W305F into a *recA*-positive cell strain resulted in genetic recombination of the mutant MetRS gene with the chromosomal copy of the wild-type MetRS gene, yielding plasmid pQE15-MRS. Expression plasmid pQE15-MRS and repressor plasmid pREP4 were

transformed into the expression host B834(DE3) to yield B834(DE3)/pQE15-MRS/pREP4. Plasmid DNA from all B834(DE3)/pQE15-MRS/pREP4 cultures used for protein expression experiments was sequenced to confirm that it encoded wild-type MetRS.

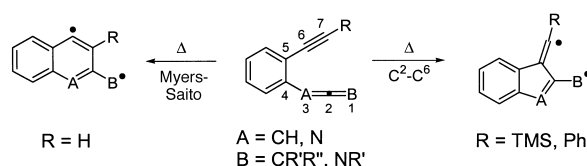
- [19] F. Dardel, M. Panvert, G. Fayat, *Mol. Gen. Genet.* **1990**, 223, 121–133.
- [20] *The Qiagen Expressionist*, Qiagen, Inc., Valencia, CA, **2000**, p. 68.
- [21] Methionine analogues were synthesized by alkylation of diethylacetamidomalonate, as previously described.^[13]
- [22] The M9AA medium was prepared by supplementing sterile M9 medium with 60 mg mL⁻¹ of each of the amino acids, 1 mM MgSO₄, 0.2 wt % glucose, 1 mg mL⁻¹ thiamine chloride, and 1 mg mL⁻¹ calcium chloride. The antibiotics ampicillin and kanamycin were added at concentrations of 200 mg L⁻¹ and 35 mg L⁻¹, respectively.
- [23] B834(DE3)/pQE15-MRS/pREP4 cells, which overexpress MetRS, have sufficient MetRS activity to synthesize measurable levels of protein from the very low intracellular levels of methionine in the negative-control culture. Interestingly, AARS overexpression is induced by amino acid starvation in some Gram-positive bacteria, presumably to permit continued protein synthesis (D. Luo, J. Leautey, M. Grunberg-Manago, H. Putzer, *J. Bacteriol.* **1997**, 179, 2472–2478). B834(DE3)/pQE15/pREP4 cultures, which lack the increased MetRS activity, do not show background expression of protein in negative-control cultures.
- [24] ATP-PP_i exchange assays were conducted by using the methods described in ref. [15] A 50-μL aliquot of whole-cell lysate with a normalized OD₆₀₀ of 20 was prepared by one freeze–thaw cycle and added to the assay mixture to yield a final volume of 150 μL. A saturating concentration of methionine (750 μM) was used to determine the maximum exchange velocity for each cell lysate.
- [25] ¹H NMR spectra were recorded by using a Varian Inova NMR spectrometer with proton acquisition at 599.69 MHz. Spectra were recorded at 25°C overnight. A simple presaturation pulse was used for water suppression.
- [26] 1D TOCSY NMR spectra were recorded on a Varian Inova NMR spectrometer with proton acquisition at 599.69 MHz. A 1D TOCSY pulse sequence (D. Uhrin, P. N. Barlow, *J. Magn. Reson.* **1997**, 126, 248–255) with selective irradiation of the signal at δ = 5.35 (E. Kupce, J. Boyd, I. D. Campbell, *J. Magn. Reson. Ser. B.* **1995**, 106, 300–303) was used to identify which protons belonged to the spin system of that signal. The selectivity of the pulse is demonstrated in a separate, simple 1D experiment in which the selective pulse is applied alone; no other resonances are observed in the spectrum under these conditions. Observation after a mixing time of 60 ms, however, showed the protons at δ = 5.60 and 5.70, indicating that those protons are members of the same spin system (and therefore the same amino acid residue) as those corresponding to the resonance at δ = 5.35. The α-carbon and side chain β- and γ-carbon protons are also observed at chemical shift values characteristic of the free amino acid (δ = 4.3 (α-CH), 2.5 (β-CH₂), and 1.6 (δ-CH₃)).
- [27] P. H. Hirel, J. M. Schmitter, P. Dessen, G. Fayat, S. Blanquet, *Proc. Natl. Acad. Sci. USA* **1989**, 86, 8247–8251.
- [28] The signal corresponding to methionine elutes at 13.8 min while that corresponding to Tcg elutes at 16.0 min. The large peaks which elute at approximately 15.4 min correspond to piperidylphenylthiourea (pptu), a product of the analysis resulting from the buffer, and the small peak at 19.4 min corresponds to diethylphthalate (diet), an internal standard.
- [29] a) F. H. Arnold, J. C. Moore, *Adv. Biochem. Eng. Biotechnol.* **1997**, 58, 1–14; b) F. H. Arnold, *Chem. Eng. Sci.* **1996**, 51, 5091–5102.
- [30] B. M. Trost, I. Fleming, *Comprehensive Organic Synthesis*, Pergamon Press, Oxford, **1991**.
- [31] a) D. M. Lynn, B. Mohr, R. H. Grubbs, *J. Am. Chem. Soc.* **1998**, 120, 1027–1028; b) E. L. Dias, T. N. SonBinh, R. H. Grubbs, *J. Am. Chem. Soc.* **1997**, 119, 3887–3897.
- [32] T. D. Clark, M. R. Ghadiri, *J. Am. Chem. Soc.* **1995**, 117, 12364–12365.
- [33] H. E. Blackwell, R. H. Grubbs, *Angew. Chem.* **1998**, 110, 3469–3472; *Angew. Chem. Int. Ed.* **1998**, 37, 3281–3284.
- [34] T. A. Kirkland, D. M. Lynn, R. H. Grubbs, *J. Org. Chem.* **1998**, 63, 9904–9909.

A Highly Efficient Triplet Analogue of a Thermal Biradical Cyclization—The Photochemical C²–C⁶ Cyclization of Enyne-Heteroallenes**

Michael Schmittel,* David Rodríguez, and Jens-Peter Steffen

Dedicated to Professor Harald Günther on the occasion of his 65th birthday

Cycloaromatizations of enediynes^[1] (Bergman cyclization) and enyne–allenes^[2] (Myers–Saito cyclization) have received great interest over the last decade because the resultant biradicals constitute key intermediates in the mode of action of natural enediyne antitumor antibiotics.^[3] While the above cyclizations may be regarded as electrocyclic reactions^[4] that lead to aromatic biradicals, the proper choice of substituents at the alkyne terminus (Scheme 1) has allowed us^[5] to steer the regioselectivity of thermal enyne–(hetero)-allene biradical cyclizations^[6–8] away from the Myers–Saito path and instead to the C²–C⁶ path, which leads to (hetero)-benzofulvene biradicals.



Scheme 1. Biradical intermediates of the thermal C²–C⁷ (Myers–Saito) and C²–C⁶ cyclization reactions. TMS = Si(CH₃)₃.

As the novel C²–C⁶ cyclization currently is the focus of theoretical,^[9] DNA cleavage,^[10] and synthetic studies (for example, towards the synthesis of the kinamycin^[11] and the neocryptolepine^[12] families), it appeared important to develop a photochemical variant—as for the Bergman cyclization^[13]—which should allow a direct access to the intermediate biradicals.^[14] Herein, we now report for the first time on the photochemical reactions of enyne–carbodiimides and enyne–ketenimines constituting the first triplet analogues of a thermal biradical cyclization.

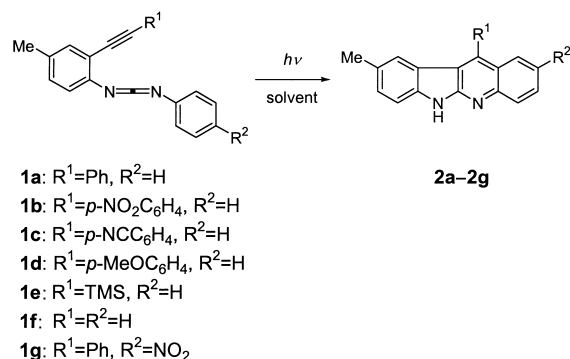
We have become aware of the photochemical cyclization when the enyne–carbodiimide **1a** partially formed indoloquinoline **2a** after prolonged exposure to sunlight (several

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days). Consequently, the photochemical behavior of various substituted enyne–carbodiimides **1** was examined (Scheme 2). Photolyses of degassed solutions of **1** under different



Scheme 2. Photocyclization of enyne–carbodiimides **1a–1g**.

conditions (see Tables 1 and 2) were carried out in a Rayonet RPR-100 Photochemical Reactor at four different wavelengths (254, 300, 350, and 419 nm). Conversion of the reactant was monitored by thin-layer chromatography (TLC). Depending on their solubility, indoloquinolines **2** were isolated by filtration or column chromatography.

Table 1. Photoreactivity of enyne–carbodiimides **1** under direct irradiation.^[a]

Substrate	Solvent	Wave-length λ [nm]	Reaction time [min]	Conversion of 1 [%]	Yield ^[b] of 2 [%]
1a	toluene	300	90	< 5	–
1b	<i>n</i> -hexane	419	60	100	95
1b	toluene	300	≈ 8	100	quantitative
1b	<i>n</i> -hexane	300	≈ 5	100	92
1c	toluene	300	540	100	91
1d	toluene	300	90	< 5	–
1f	toluene	300	90	< 5	–
1g	toluene	300	60	100	96

[a] $c = 4–8$ mm. [b] Yield of isolated product.

Table 2. Triplet-sensitized photoreaction of enyne–carbodiimides **1**.^[a]

Substrate	Solvent	Wave-length λ [nm]	Reaction time [min]	Conversion of 1 [%]	Yield ^[b] of 2 [%]
1a	<i>n</i> -hexane	254	360	31	19
1a	benzene	254	380	62	60
1a	toluene	254	40	100	96
1a	acetone	300	60	100	89
1d	toluene	254	120	100	93
1e	toluene	254	630	100	66 (2e), 31 (2f)
1f	toluene	254	360	100	94

[a] $c = 4–18$ mm. [b] Yield of isolated product.

When the enyne–carbodiimide is substituted by an electron-withdrawing group either at the alkyne (**1b**, **1c**) or carbodiimide (**1g**) terminus, direct irradiation at 300 nm or longer wavelengths yields >90% of **2** (Table 1). Clean conversion is also shown by the occurrence of four isosbestic points in the UV/Vis investigation (Figure 1). Moreover, all

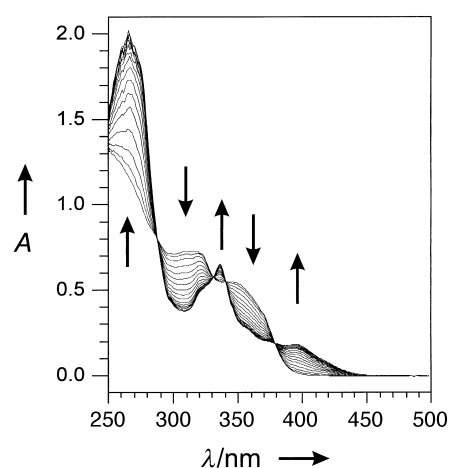


Figure 1. Spectral changes during photolysis of **1b** ($50 \mu\text{M}$ in *n*-hexane) at 350 nm. Between the consecutively recorded spectra, the irradiation time was 3 seconds; complete photocyclization was achieved within 50 seconds.

other systems can also be efficiently cyclized at shorter wavelengths when irradiation is carried out in a solvent that can act as a triplet photosensitizer (triplet energy^[15] $E_T = 83 \text{ kcal mol}^{-1}$ (toluene), 84 kcal mol^{-1} (benzene), 82 kcal mol^{-1} (acetone); Table 2).

The above observations provide some evidence for a triplet cyclization. To further strengthen our mechanistic assignment, the photochemical cyclization of **1a** was effected in presence of triplet sensitizers of varying E_T in hexane (Table 3). While strong sensitizers ($E_T > 60 \text{ kcal mol}^{-1}$) work very well, the

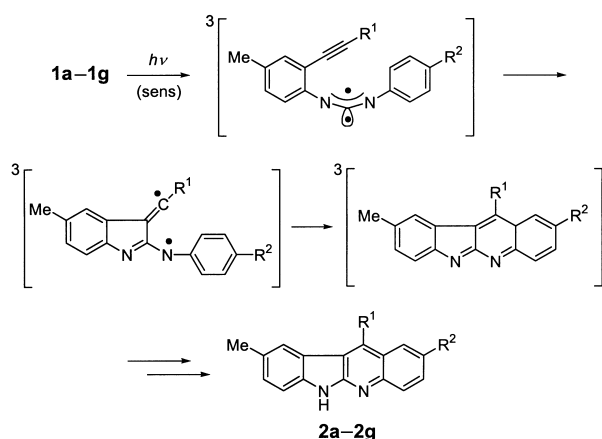
Table 3. Triplet-sensitized photoreaction of enyne–carbodiimide **1a** in *n*-hexane.^[a]

Sensitizer	E_T ^[b] [kcal mol ^{−1}]	Wave-length λ [nm]	Re-action time [min]	Conver-sion of 1a [%]	Yield ^[c] of 2a [%]
acetophenone ^[d]	74	350	60	100	88
acetophenone ^[e]	74	350	360	94	85
acetophenone ^[d]	74	300	40	100	90
benzophenone ^[f]	69	350	25	100	83
naphthalene ^[f]	61	254	60	100	81
diacetyl ^[f]	57	254	60	20	15
benzil ^[f, g]	54	254	60	< 5	–
no sensitizer	0	254	60	< 5	–

[a] $c = 5–11$ mm. [b] Triplet energy.^[15] [c] Yield of isolated product. [d] *n*-Hexane/acetophenone = 10/1. [e] 1 equiv (based on **1a**). [f] 20 equiv (based on **1a**). [g] Not completely soluble.

efficiency of the cyclization with benzil as a sensitizer ($E_T = 54 \text{ kcal mol}^{-1}$) is close to zero, as it is without sensitizer. Moreover, a triplet quencher is expected to interfere with any triplet cyclization. Indeed, photochemical cyclization of **1a** in toluene as well as in *n*-hexane could be completely suppressed in the presence of 1,4-diphenyl-1,3-butadiene, a triplet quencher with $E_T = 42 \text{ kcal mol}^{-1}$.^[15]

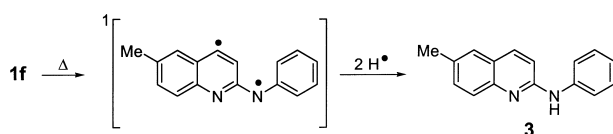
The mechanistic tests above clearly indicate that cyclization is taking place via triplet intermediates. Hence, we favor the following mechanistic proposal (Scheme 3) that is additionally supported by several independent facts: 1) allenes^[16] and heteroallenes^[17] are readily excited to the triplet state; 2) PM3



Scheme 3. Proposed mechanism for the triplet-sensitized cyclization of **1a-1g**. (sens) = sensitizer.

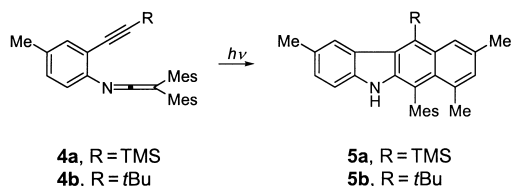
calculations^[18] indicate that the triplet diazabenzofulvene biradical is more stable than the singlet species,^[19] and 3) the regioselective 5-*exo-dig* cyclization of triplet biradicals is well established.^[20]

Furthermore, a strong argument for a triplet cyclization can be derived from the clean photochemical transformation **1f** → **2f** (94 %, Table 2). An intermediate singlet biradical can definitely be ruled out for this reaction, since under pure thermal conditions, i.e. via singlet biradical intermediates, **1f** forms aminoquinoline **3** along the Myers-Saito pathway (Scheme 4).^[6b]



Scheme 4. Thermal cyclization of **1f**.^[6b]

To establish the photocyclization as a general route to triplet heterobenzofulvene biradicals we have additionally subjected the stable enyne-ketenimines **4a** and **4b** to the conditions applied above (Scheme 5, Table 4). Notably, the cyclization can equally be effected but the yields of **5**^[21] are reduced due to formation of polymeric material.



Scheme 5. Photocyclization of enyne-ketenimines **4a** and **4b**. Mes = 2,4,6-(CH₃)₃C₆H₂.

Table 4. Irradiation of enyne-ketenimines **4a** and **4b**.^[a]

Substrate	Solvent	Wave-length λ [nm]	Reaction time [min]	Conversion of 4 [%]	Yield ^[b] of 5 [%]
4a	toluene	254	270	100	50 ^[c]
4a	<i>n</i> -hexane	254	360	54	14 ^[c]
4b	toluene	254	300	100	62 ^[c]

[a] $c = 6-8$ mm. [b] Yield of isolated product. [c] Besides **5**, only polymeric material was obtained.

In conclusion, we have found two remarkably efficient photocyclizations of enyne-ketenimides and enyne-ketenimines. As evidence for triplet intermediates in the Bergman case is rather poor,^[13b] the above cyclizations constitute the first unequivocal triplet analogues of thermal biradical cyclizations. Because of the very good yields and the high 5-*exo-dig* regioselectivity, these cyclizations not only contrast the Myers-Saito pathway (see R¹ = H in **1f**) but also open the way for photoactive prodrugs towards the neocryptolepine^[12] family. Moreover, while the lack of a photochemical Myers-Saito cyclization still precludes a time-resolved study of the Myers-Saito biradical, the present triplet photoreaction appears to be ideal for flash photolysis or matrix isolation^[22] and for characterizing (hetero)benzofulvene biradicals. A mechanistic study along this line will be reported in due course.

Experimental Section

Photolysis of 1a in toluene: A solution of **1a** (23.0 mg, 74.5 μ mol) in degassed toluene (13 mL, $c = 5.7$ mm) was placed in a quartz flask under a nitrogen atmosphere and irradiated with 16 lamps for 40 minutes (Rayonet RPR-100 photochemical reactor lamp, 253.7 nm, water cooling to $20 \pm 5^\circ\text{C}$). Reaction control by TLC showed the complete disappearance of **1a**. The solution was concentrated in vacuo and the remaining residue was purified by column chromatography (silica gel, *n*-hexane/ethyl acetate 3/1), to yield **2a** as a yellow solid (22.0 mg, 71.3 μ mol, 96 %) that was recrystallized from ethanol.

2a: Yellow prisms; m.p. 242.5–243.5 $^\circ\text{C}$ (ethanol); ¹H (600 MHz, CDCl₃): $\delta = 2.28$ (s, 3H), 6.84 (s, 1H), 7.17 (d, ³J(H,H) = 7.9 Hz, 1H), 7.39 (d, ³J(H,H) = 7.9 Hz, 1H), 7.39 (dd, ³J(H,H) = 8.4, 6.3 Hz, 1H), 7.53–7.55 (m, 2H), 7.65–7.70 (m, 3H), 7.74 (dd, ³J(H,H) = 8.3, 6.3 Hz, 1H), 7.76 (d, ³J(H,H) = 8.4 Hz, 1H), 8.26 (d, ³J(H,H) = 8.3 Hz, 1H), 12.29 (br. s, 1H; NH); ¹³C (50 MHz, CDCl₃): $\delta = 21.39, 110.54, 116.81, 121.24, 122.94, 123.30, 123.76, 126.36, 126.61, 128.61, 128.97$ (2 signals), 129.12, 129.24, 129.40, 136.46, 139.43, 142.98, 145.68, 153.20; IR (KBr): $\tilde{\nu} = 3440, 3145, 3056, 2916, 2853, 1600, 1485, 1442, 1382, 1355, 1294, 1250, 1230, 1150, 1128, 1071, 799, 756, 702$ cm⁻¹; elemental analysis calculated for C₂₂H₁₆N₂: C 85.69, H 5.23, N 9.08; found: C 85.27, H 5.50, N 9.44.

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- a) R. R. Jones, R. G. Bergman, *J. Am. Chem. Soc.* **1972**, *94*, 660–661; b) T. P. Lockhart, P. B. Comita, R. G. Bergman, *J. Am. Chem. Soc.* **1981**, *103*, 4082–4090.
- a) A. G. Myers, E. Y. Kuo, N. S. Finney, *J. Am. Chem. Soc.* **1989**, *111*, 8057–8059; b) R. Nagata, H. Yamanaka, E. Okazaki, I. Saito, *Tetrahedron Lett.* **1989**, *30*, 4995–4998.
- Reviews: a) K. K. Wang, *Chem. Rev.* **1996**, *96*, 207–222; b) J. W. Grissom, G. U. Gunawardena, D. Klingberg, D. Huang, *Tetrahedron* **1996**, *52*, 6453–6518.
- I. Morao, F. P. Cossio, *J. Org. Chem.* **1999**, *64*, 1868–1874.
- a) M. Schmittel, M. Strittmatter, S. Kiau, *Tetrahedron Lett.* **1995**, *36*, 4975–4978; b) M. Schmittel, M. Strittmatter, K. Vollmann, S. Kiau, *Tetrahedron Lett.* **1996**, *37*, 999–1002; c) M. Schmittel, M. Strittmatter, S. Kiau, *Angew. Chem.* **1996**, *108*, 1952–1954; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1843–1845; d) M. Schmittel, M. Keller, S. Kiau, M. Strittmatter, *Chem. Eur. J.* **1997**, *3*, 807–816; e) B. Engels, C. Lennartz, M. Hanrath, M. Schmittel, M. Strittmatter, *Angew. Chem.* **1998**, *110*, 2067–2070; *Angew. Chem. Int. Ed.* **1998**, *37*, 1960–1963.
- a) M. Schmittel, J.-P. Steffen, M. Á. Wencesla Ángel, B. Engels, C. Lennartz, M. Hanrath, *Angew. Chem.* **1998**, *110*, 1633–1635; *Angew. Chem. Int. Ed.* **1998**, *37*, 1562–1564; b) M. Schmittel, J.-P. Steffen, B. Engels, C. Lennartz, M. Hanrath, *Angew. Chem.* **1998**, *110*, 2531–2533; *Angew. Chem. Int. Ed.* **1998**, *37*, 2371–2373.

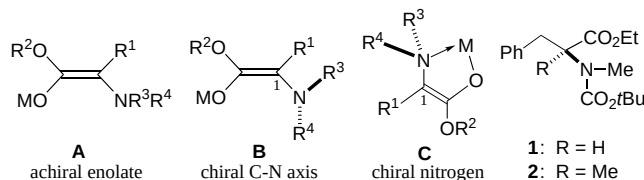
- [7] a) C. Shi, K. K. Wang, *J. Org. Chem.* **1998**, *63*, 3517–3520; b) C. Shi, Q. Zhang, K. K. Wang, *J. Org. Chem.* **1999**, *64*, 925–932.
- [8] See also Moore cyclization: a) L. D. Foland, J. O. Karlsson, S. T. Perri, R. Schwabe, S. L. Xu, S. Patil, H. W. Moore, *J. Am. Chem. Soc.* **1989**, *111*, 975–989; b) H. W. Moore, B. R. Yersa, *Chemtracts* **1992**, 273–313.
- [9] a) B. Engels, M. Hanrath, *J. Am. Chem. Soc.* **1998**, *120*, 6356–6361; b) P. R. Schreiner, M. Prall, *J. Am. Chem. Soc.* **1999**, *121*, 8615–8627.
- [10] a) M. Schmittel, S. Kiau, T. Siebert, M. Strittmatter, *Tetrahedron Lett.* **1996**, *37*, 7691–7694; b) M. Schmittel, M. Maywald, M. Strittmatter, *Synlett* **1997**, 165–166.
- [11] Ó de Frutos, A. M. Echavarren, *Tetrahedron Lett.* **1997**, *38*, 7941–7942.
- [12] M. Alajarin, P. Molina, A. Vidal, *J. Nat. Prod.* **1997**, *60*, 747–748.
- [13] a) R. L. Funk, E. R. R. Young, R. M. Williams, M. F. Flanagan, T. L. Cecil, *J. Am. Chem. Soc.* **1996**, *118*, 3291–3292; b) A. Evenzahav, N. J. Turro, *J. Am. Chem. Soc.* **1998**, *120*, 1835–1841; c) T. Kaneko, M. Takahashi, M. Hirama, *Angew. Chem.* **1999**, *111*, 1347–1349; *Angew. Chem. Int. Ed.* **1999**, *38*, 1267–1268.
- [14] W. Sander, *Acc. Chem. Res.* **1999**, *32*, 669–676.
- [15] a) J. G. Calvert, J. N. Pitts Jr., *Photochemistry*, Wiley, New York, **1966**; b) *Einführung in die Photochemie* (Ed.: H. G. O. Becker), 2nd ed., Thieme, Stuttgart, **1983**; c) K. Nakatani, K. Adachi, K. Tanabe, I. Saito, *J. Am. Chem. Soc.* **1999**, *121*, 8221–8228.
- [16] a) J. D. Price, R. P. Johnson, *J. Org. Chem.* **1991**, *56*, 6372–6376; b) W. C. Shakespeare, R. P. Johnson, *J. Org. Chem.* **1991**, *56*, 6377–6379; c) R. D. J. Froese, G. L. Lange, J. D. Goddard, *J. Org. Chem.* **1996**, *61*, 952–961.
- [17] a) L. A. Singer, P. D. Bartlett, *Tetrahedron Lett.* **1964**, 1887–1892; b) J. H. Boyer, P. J. A. Frints, *Tetrahedron Lett.* **1968**, 3211–3212; c) L. A. Singer, G. A. Davis, R. L. Knutsen, *J. Am. Chem. Soc.* **1972**, *94*, 1188–1193.
- [18] J. J. P. Stewart, *J. Comput. Chem.* **1989**, *10*, 221–264.
- [19] Calculations on a higher level of theory have commenced in cooperation with B. Engels (Würzburg, Germany).
- [20] W. C. Agosta, P. Margaretha, *Acc. Chem. Res.* **1996**, *29*, 179–182.
- [21] Product **5** is formed through the loss of a methyl group from the intermediate, a formally Diels–Alder product. According to preliminary studies, this is also a photochemical process which will be detailed in the full paper to come.
- [22] a) W. Sander, H. Wandel, G. Bucher, J. Gräfenstein, E. Kraka, D. Cremer, *J. Am. Chem. Soc.* **1998**, *120*, 8480–8485; b) R. Marquardt, A. Balster, W. Sander, E. Kraka, D. Cremer, J. G. Radziszewski, *Angew. Chem.* **1998**, *110*, 1001–1005; *Angew. Chem. Int. Ed.* **1998**, *37*, 955–958.

A Chiral Nonracemic Enolate with Dynamic Axial Chirality: Direct Asymmetric α -Methylation of α -Amino Acid Derivatives**

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The structure of enolates was long believed to be achiral because all four substituents are on the same plane as the enolate double bond. For example, enolates generated from

α -amino acid derivatives are seemingly achiral when substituents R^1 – R^4 are achiral (**A**). However, we proposed that some enolate structures are intrinsically chiral.^[1–3] As shown in **B** an enolate with axial chirality along the C1–N axis is expected if R^3 is different from R^4 . An enolate with a chiral nitrogen atom is shown in **C**, where tight coordination of the



nitrogen atom to a metal cation creates a stereogenic nitrogen atom.^[4] Racemization of these chiral enolates takes place so readily through simple C1–N bond rotation that the chirality is not static, but dynamic. These enolates can exist in chiral nonracemic forms for a limited time at low temperatures. We describe here experimental evidence for a chiral nonracemic enolate with dynamic axial chirality, as exemplified in **B**. Asymmetric α -methylation of various α -amino acid derivatives can occur in a highly enantioselective manner through the intrinsically chiral enolate intermediate.^[5–7]

We have previously reported that phenylalanine derivative **1** undergoes asymmetric α -methylation by treatment with lithium 2,2,6,6-tetramethylpiperide followed by methyl iodide to give **2** in 82% *ee* and 40% yield.^[2] This is the second example of the retention of chiral information of optically active α -amino acid derivatives during their α -alkylation. The first one was reported by Seebach and Wasmuth.^[6a] Although the transformation of **1** into **2** was noteworthy in that asymmetric induction was realized without using any external chiral sources, such as chiral auxiliaries or chiral ligands, there were significant drawbacks: 1) low chemical yield, 2) low generality of asymmetric induction among α -amino acids, and 3) difficulty in removing the *N*-methyl protective group. The mechanism of the novel asymmetric induction was ambiguous. We further examined the present strategy in order to develop a more efficient process and to elucidate the mechanism. We anticipated that the choice of R^3 and R^4 in **B** or **C** would have the key role for the asymmetric induction, so we screened the substituents at the nitrogen atom of phenylalanine. We found that substrates possessing *t*-butoxycarbonyl (Boc) and methoxymethyl (MOM) groups at the nitrogen atom gave satisfactory results. Treatment of *N*-Boc-*N*-MOM-phenylalanine derivative **3** with potassium hexamethyldisilazide (KHMDs) in toluene:THF (4:1) at -78°C for 30 min followed by methyl iodide afforded α -methylated product **4** in 96% yield and 81% *ee* (Table 1, entry 1).^[8] Similarly, α -methylation of histidine derivative **5** gave **6** in 83% yield and 93% *ee* (entry 2). The α -amino acid derivatives **7**, **9**, and **11** with aromatic side chains, as well as those with aliphatic side chains, **13** and **15**, gave α -methylated products **8**, **10**, **12**, **14**, and **16**, respectively, in 78–95% yields and *ee* values of 76–87% by a similar treatment (entries 3–7). Removal of the protective groups of **4**, **8**, **14**, and **16** was readily accomplished in one step by treatment with 6M

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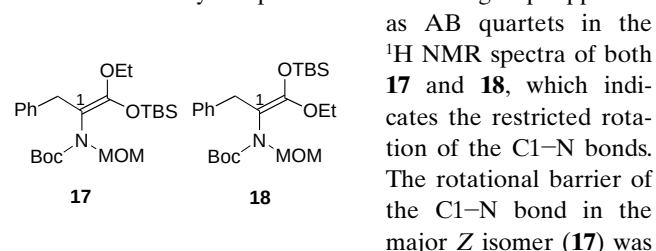
Table 1. Asymmetric α -methylation of α -amino acid derivatives.^[a]

$ \begin{array}{c} \text{R} \\ \\ \text{H} \text{---} \text{C} \text{---} \text{CO}_2\text{Et} \\ \\ \text{N} \text{---} \text{CH}_2\text{OMe} \\ \\ \text{CO}_2\text{tBu} \end{array} \xrightarrow[\text{b) MeI / THF:toluene (1:4), -78}^\text{a) KHMDS} \begin{array}{c} \text{R} \\ \\ \text{Me} \text{---} \text{C} \text{---} \text{CO}_2\text{Et} \\ \\ \text{N} \text{---} \text{CH}_2\text{OMe} \\ \\ \text{CO}_2\text{tBu} \end{array} $							
Entry	R	Substrate ^[b]	Product	Yield [%]	<i>ee</i> [%] ^[c]	$[\alpha]_D^{20}$ (c in CHCl ₃)	Configuration ^[d]
1	PhCH ₂	3	4	96	81	−89 (1.2)	<i>S</i>
2		5	6	83	93	−43 (1.1)	[e]
3		7	8	94	79	−81 (1.0)	<i>S</i>
4		9	10	95	80	−96 (1.0)	<i>S</i>
5		11	12	88	76	−64 (0.9)	[e]
6	Me ₂ CH	13	14 ^[f]	81	87	+8.5 (1.2) ^[g]	<i>S</i>
7	Me ₂ CHCH ₂	15	16 ^[f]	78	78	+20 (0.5) ^[g]	<i>S</i>

[a] The substrate was treated with 1.1 equiv of KHMDS at -78°C for 30 min (for **3**, **5**, **7**, **9**, and **11**) or 60 min (for **13** and **15**) followed by 10 equiv of methyl iodide for 16–17 h at -78°C . See the Supporting Information for the experimental procedure and physical data. [b] The *ee* value of each substrate is $>99\%$. [c] Determined by HPLC using columns with chiral stationary phases: **4**: Chiralpack AD, 2% *i*PrOH in hexane; **6**, **8**: Chiralpack AD, 5% EtOH in hexane; **10**, **12**: Chiralpack AD, 5% *i*PrOH in hexane; **14** (benzoate): Chiralpack AS, 3% *i*PrOH in hexane; **16** (benzoate): Chiralpack AD, 1% *i*PrOH in hexane. [d] Absolute configuration of the corresponding α -methyl- α -amino acid. [e] Not determined. [f] Obtained as an inseparable mixture with the substrate. The yield was determined on the basis of the ratio of signals observed in the 400 MHz ^1H NMR spectra. Complete separation was achieved with the corresponding *N*-benzoyl derivative. [g] Optical rotation of the corresponding *N*-benzoyl derivative.

aqueous HCl to give the corresponding α -methyl- α -amino acids in 51–86% yields.^[9] The α -methylation was shown to proceed with retention of stereochemistry. The degree of asymmetric induction in the α -methylation was comparable among several amino acids. This observation implies that MOM and Boc groups at the nitrogen atom have a decisive effect on the stereochemical course of the reaction.

The structure and chiral properties of the intermediate enolate were investigated to clarify the mechanism of the present asymmetric induction. Treatment of **3** with KHMDS (1.1 equiv) in toluene:THF (4:1) at -78°C for 30 min followed by addition of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBS triflate) gave the *Z* enol silyl ether **17** and its *E* isomer **18** in a 2:1 ratio in combined yields of 83%.^[10] The methylene protons of the MOM groups appeared



as AB quartets in the ^1H NMR spectra of both **17** and **18**, which indicates the restricted rotation of the C1–N bonds. The rotational barrier of the C1–N bond in the major *Z* isomer (**17**) was determined to be 16.8 kcal mol^{−1} (365 K) by variable-temperature NMR measurements in [D₈]toluene (400 MHz ^1H NMR, $J_{\text{AB}} = 9.9$ Hz, $\Delta\nu_{\text{AB}} = 228.4$ Hz, $T = 365$ K). The restricted bond rotation brings about axial chirality in **17** (chiral C1–N axis), as shown in **D**.^[11] The half-life of racemization in **17** was estimated from the rotational barrier to be 5×10^{-4} s at 92°C or approximately 7 days at -78°C .^[12] This value implies that the corresponding potassium enolate could also exist in an axially chiral form with a relatively long half-life to racemization at low temperatures.

We then investigated the behavior of the enolate intermediate toward racemization. When **3** was treated with KHMDS for 24 h at -78°C , the reaction of the resulting enolate with methyl iodide gave **4** (84% yield) with 36% *ee* (81% *ee* after 30 min base treatment, Table 1, entry 1). When the enolate was prepared and maintained at -78°C for 30 min then kept at -40°C for 30 min, its reaction with methyl iodide at -78°C produced **4** (88% yield) with 5% *ee*. These results clearly indicate that racemization of the enolate intermediate occurs. The barrier to racemization was determined through the periodic quenching of the enolate intermediate generated at -78°C with methyl iodide. A graph of the logarithm of the relative *ee* values of **4** as a function of time for base-treatment of **3** (Figure 1) indicates a very good

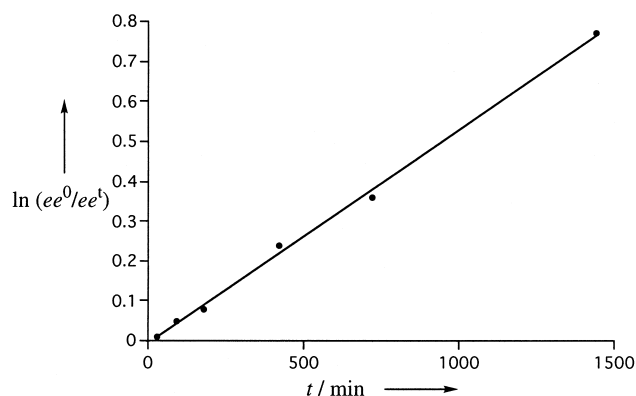
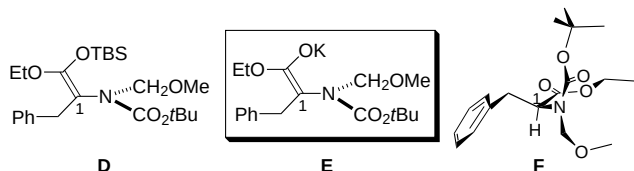


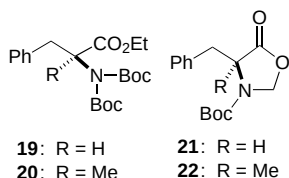
Figure 1. Plot of the logarithm of the relative *ee* value of **4** ($\ln ee^0/ee^t$) versus time (*t*) for base treatment of **3**. ee^0 = the *ee* value of **4** obtained by the reaction of the enolate immediately after its generation from **3** with methyl iodide. ee^t = the *ee* value of **4** obtained by the treatment of **3** with KHMDS for the time indicated followed by addition of methyl iodide. See Supporting Information for experimental details.

linear relationship exists ($r=0.999$), although the enolate is a 2:1 mixture of the *Z* and *E* forms. This observation suggests that the rates of racemization of the *Z* and *E* enolates are very close to each other.^[13] The barrier was calculated from the slope ($2k=5.34 \times 10^{-4} \text{ min}^{-1}$) to be $16.0 \text{ kcal mol}^{-1}$ at -78°C , which matches well with the rotational barrier of the C1–N bond of **17**. This result suggests that the chirality of the potassium enolate intermediate also originates in the restricted rotation of the C1–N bond. We conclude that a chiral nonracemic enolate with dynamic axial chirality (**E**) is the



origin for the present asymmetric induction. The half-life of racemization of the chiral enolate was 22 h at -78°C , which is long enough for the chiral enolate to undergo asymmetric methylation. The stereochemical course (retention) of α -methylation of **3** may be explained by assuming: 1) deprotonation occurs from the stable conformer **F**^[14] where the C1–H bond is eclipsed by the N–C(MOM) bond to produce enantiomerically enriched chiral enolate (**E**)^[15] and 2) the electrophile (methyl iodide) approaches from the sterically less demanding face (MOM) of the enolate double bond of **E**.

Support for this novel mechanism was obtained from the reactions of **19** and **21**. The di-Boc derivative **19** (>99% *ee*) and methylene acetal derivative **21** (>99% *ee*) gave racemic **20** (95% yield) and **22** (95% yield), respectively, upon α -methylation. These results are consistent with the conclusions



above, since the enolates generated from **19** and **21** are not expected to be axially chiral along the C–N axis.^[16, 17]

In conclusion, a chiral nonracemic enolate with dynamic axial chirality was shown to be a crucial intermediate for

direct asymmetric α -methylation of α -amino acid derivatives. The racemization barrier of the chiral enolate was 16 kcal mol^{-1} and the half-life was 22 h at -78°C . Likewise, some enolates with a restricted bond rotation should have axial chirality with an intrinsic barrier to racemization. Since the rotational barrier is controllable by introducing substituents or protective groups, asymmetric induction based on the present strategy would have further applicability in enolate chemistry.

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- [4] Formation of a stereogenic nitrogen atom by coordination with a metal cation has been claimed: D. Sato, H. Kawasaki, I. Shimada, Y. Arata, K. Okamura, T. Date, K. Koga, *J. Am. Chem. Soc.* **1992**, *114*, 761–763.
- [5] Asymmetric reactions through chiral enolate intermediates are described in the reviews: a) D. Seebach, A. R. Sting, M. Hoffmann, *Angew. Chem.* **1996**, *108*, 2880–2921; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2708–2748; b) J. Clayden, *Angew. Chem.* **1997**, *109*, 986–988; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 949–951; c) T. Wirth, *Angew. Chem.* **1997**, *109*, 235–237; *Angew. Chem. Int. Ed. Engl.* **1997**, *37*, 225–227.
- [6] For examples of asymmetric α -substitution of α -amino acid derivatives without employing external chiral sources, see a) D. Seebach, D. Wasmuth, *Angew. Chem.* **1981**, *93*, 1007; *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 971; b) B. Beagley, M. J. Betts, R. G. Pritchard, A. Schofield, R. J. Stoodley, S. Vohra, *J. Chem. Soc. Chem. Comm.* **1991**, 924–925; c) M. J. Betts, R. G. Pritchard, A. Schofield, R. J. Stoodley, S. Vohra, *J. Chem. Soc. Perkin 1* **1999**, 1067–1072.
- [7] A chiral nonracemic enolate has been claimed as a possible intermediate of an asymmetric rearrangement: T. Gees, W. B. Schweizer, D. Seebach, *Helv. Chim. Acta.* **1993**, *76*, 2640–2653.
- [8] A reaction of **3** is briefly described in reference [3].
- [9] Conversion of **10** into α -methyl-3-(3,4-dihydroxyphenyl)alanine (α -methyl dopa) was accomplished in the following three-step sequence, since the treatment of **10** with 6M HCl gave the corresponding tetrahydroisoquinoline derivative: 1) TMSBr/Me₂S, 2) 1M NaOH, 3) 47% aq HBr.
- [10] **17** and **18** were separately isolated in 56% and 27% yield, respectively. Each of them exists as a mixture of *N*-Boc *E/Z* isomers (4:1 for **17** and 5:1 for **18**).
- [11] The absolute configuration of **D** and **E** is shown provisionally.
- [12] The half-life at -78°C was roughly estimated on the assumption that $\Delta S^\ddagger$ of the restricted bond rotation is nearly zero.
- [13] The *Z* and *E* enolate intermediates should afford α -methylated products of the same absolute configuration, since the 2:1 geometric mixture of enolates gave the product of 81% *ee* in 96% yield (Table 1, entry 1).
- [14] The most stable conformation **F** of **3** was generated by molecular modeling search with the MM3* force field using MacroModel V6.0: a) G. Chang, W. C. Guida, W. C. Still, *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386; b) I. Kolossváry, W. C. Guida, *J. Am. Chem. Soc.* **1996**, *118*, 5011–5019.
- [15] The difference in the potential energies between **F** and a conformer where the C1–H bond is eclipsed by the N–C(Boc) bond, which will give the enantiomer of **E**, is estimated to be $1.0 \text{ kcal mol}^{-1}$. The difference may increase at the transition state for deprotonation as a result of the enhanced steric interactions in the presence of a base.
- [16] The 2,3-dihydroxazole ring in the potassium enolate generated from **21** and KHMDS is supposed to be nearly planar.
- [17] A mixed aggregate of an achiral enolate with an unde protonated starting material was considered as another possible intermediate for asymmetric induction. This was eliminated by a crossover experiment between **3** and **7**: A 1:1 mixture of racemic **3** and (*S*)-**7** (>99% *ee*) was α -methylated according to the procedure in Table 1 and afforded racemic **4** (79% yield) and (*S*)-**8** (74% *ee*, 79% yield), respectively.

[1] T. Kawabata, K. Yahiro, K. Fuji, *J. Am. Chem. Soc.* **1991**, *113*, 9694–9697.

[2] T. Kawabata, T. Wirth, K. Yahiro, H. Suzuki, K. Fuji, *J. Am. Chem. Soc.* **1994**, *116*, 10809–10810.

[3] K. Fuji, T. Kawabata, *Chem. Eur. J.* **1998**, *4*, 373–397.

Generation of Heteroatom-Substituted Carbene Complexes of Iridium by Double C–H Activation of Ether and Amine Substrates**

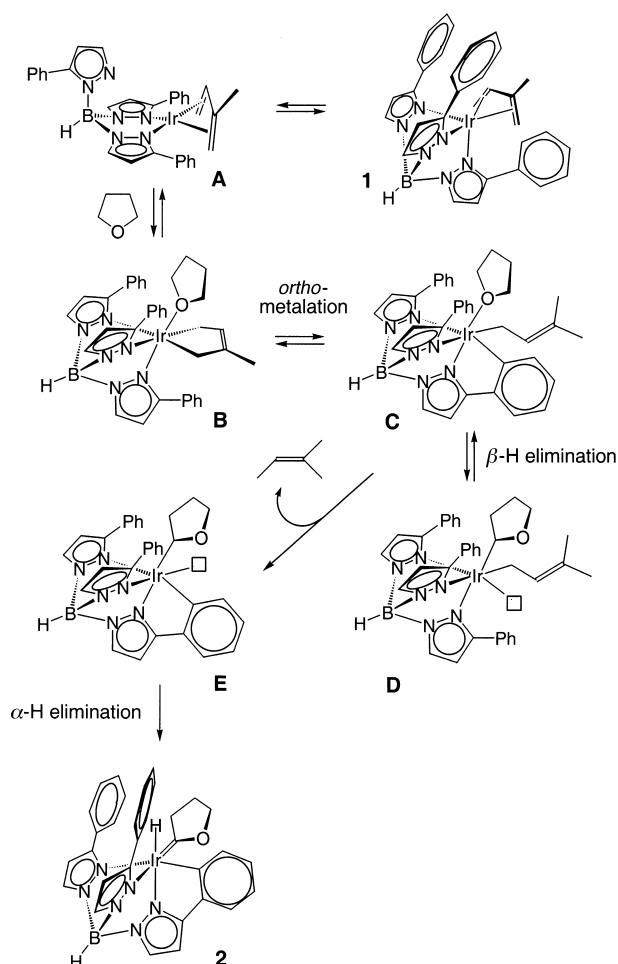
Christian Slugovc,* Kurt Mereiter, Swiatoslaw Trofimenko, and Ernesto Carmona*

Dedicated to Professor José Barluenga on the occasion of his 60th birthday

The selective activation of C–H bonds of hydrocarbons and other common organic substrates (such as ethers, amines, and nitriles) is a far-reaching objective of current research in organometallic chemistry.^[1] A very valuable reaction is the regioselective activation of two α -C–H bonds of an ether or amine functionality, RCH_2X ($\text{X} = \text{OR}'$, NR_2'), since it constitutes an unused synthetic route to Fischer-type carbenes, $\text{M} = \text{C}(\text{R})\text{X}$.^[2] Despite its potential applications, this double C–H activation^[3, 4] is still a rarely observed process. Herein, we wish to report that $[\text{Ir}(\kappa^3\text{-Tp}^{\text{Ph}})(\eta^4\text{-isoprene})]$ (**1**; $\text{Tp}^{\text{Ph}} = \text{hydridotris}(3\text{-phenylpyrazol-1-yl})\text{borate}$), when treated with substrates such as anisole (MeOPh), *N,N*-dimethylaniline (Me_2NPh), tetrahydrofuran ($\text{C}_4\text{H}_8\text{O}$), ethyl phenyl ether (EtOPh), or even diethyl ether (Et_2O), generates heteroatom-substituted carbene complexes.^[4–7] Most significantly, and at variance with somewhat related Cp^*Ir systems ($\text{Cp}^* = \eta^5\text{-Me}_5\text{C}_5$),^[7] α -elimination from the activated ether or amine substrate takes place for every case investigated, even if β -hydrogen atoms are available.

Recently, it has been shown that $[\text{IrTp}^{\text{Me}_2}(\eta^4\text{-H}_2\text{C}=\text{C}(\text{R})\text{-C}(\text{R})=\text{CH}_2)]$ compounds ($\text{R} = \text{H}$, Me ; $\text{Tp}^{\text{Me}_2} = \text{hydridotris}(3\text{-methylpyrazol-1-yl})\text{borate}$) give Ir^{III} adducts of composition $[\text{IrTp}^{\text{Me}_2}(\sigma^2\text{-CH}_2\text{-C}(\text{R})=\text{C}(\text{R})\text{-CH}_2)(\text{L})]$ when treated with a Lewis base L .^[8] Taking advantage of the possibility of precoordination of a substrate and, having additionally demonstrated that $[\text{IrTp}^x(\text{R})(\text{R}')]^+$ centers (R , $\text{R}' = \text{H}$, alkyl, aryl; $x = \text{Me}$, Ph) activate two α -C–H bonds of THF,^[4b, c, 9] **1** was heated in the presence of THF (80 °C, 4 h).^[10] High yields (about 90 %) of a cyclic carbene **2** derived from a molecule of

THF were obtained. As can be seen, not only were two α -C–H bonds of THF cleaved but also one of the Tp^{Ph} -phenyl substituents underwent cyclometalation (see Supporting Information for characterization). One of the THF hydrogen atoms formed the hydride functionality of **2** (Scheme 1; the reaction pathway is discussed in full later), the other two activated hydrogens formally added in a 1,4-fashion to the original η^4 -diene moiety of **1**, which therefore acted as a sacrificial hydrogen-atom acceptor and leaving group^[11] (evolved as 2-methyl-2-butene according to NMR evidence).



Scheme 1. Proposed reaction pathway to **2**.

As a further test for the applicability of **1** in this C–H bond activation chemistry, its reactions with MeOPh and Me_2NPh (both 80 °C, 4 h) were investigated. As shown in Scheme 2, the former led to the formation of another cyclic carbene complex **3** (97% yield by NMR), whereas the second provided a mixture of two *N*-substituted carbene derivatives, **4a** and **4b**, in an approximately 2:1 ratio (NMR). This proportion did not vary upon prolonged heating (toluene, 120 °C, 24 h). It is worth noting that the preferential formation of **4a** and the seemingly exclusive one of **3** hints that, in terms of phenyl group activation, the putative less-strained metalation of the ring attached to the carbene prevails over that of the Tp^{Ph} -phenyl substituent.^[9]

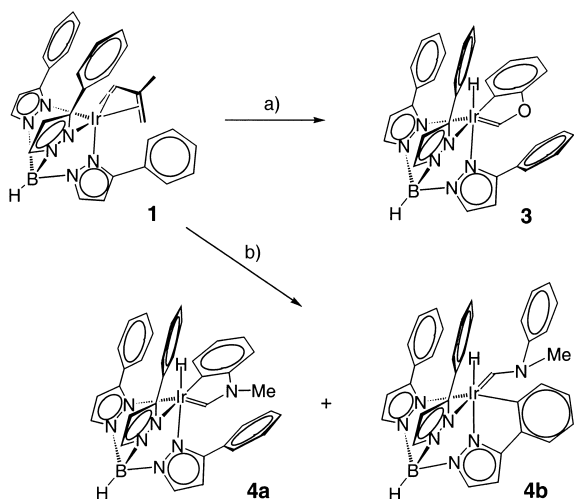
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Scheme 2. Reaction conditions: a) MeOPh, 4 h, 80 °C, toluene; b) Me₂NPh, 4 h, 80 °C, toluene. The isoprene ligand is liberated as 2-methyl-2-butene.

Characterization of **3**, **4a**, and **4b** was made by elemental analysis, NMR- and IR spectroscopies (see Supporting Information) and was confirmed in the case of **4a** by a single crystal structure determination (Figure 1).^[12] Some relevant bonding parameters included in the Figure legend compare

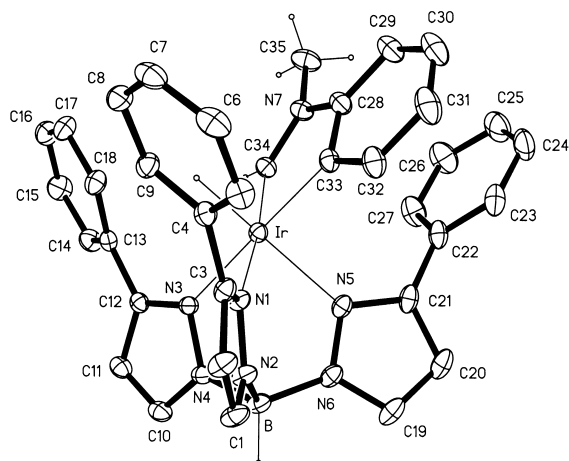


Figure 1. ORTEP plot of **4a** (most hydrogen atoms are omitted for clarity; thermal ellipsoids are at the 20% probability level). Selected bond lengths [Å] and angles [°]: Ir–H 1.63(3), Ir–C33 2.015(3), Ir–C34 1.930(3), Ir–N1 2.169(2), Ir–N3 2.177(2), Ir–N5 2.200(2), N7–C34 1.320(4), N7–C35 1.475(4); C34–Ir–C33 79.2(1), C34–Ir–N1 171.9(1), C33–Ir–N1 98.4(1), C34–Ir–N3 98.0(1), C33–Ir–N3 176.3(1), N1–Ir–N3 84.8(1), N1–Ir–N5 87.5(1), N3–Ir–N5 85.8(1).

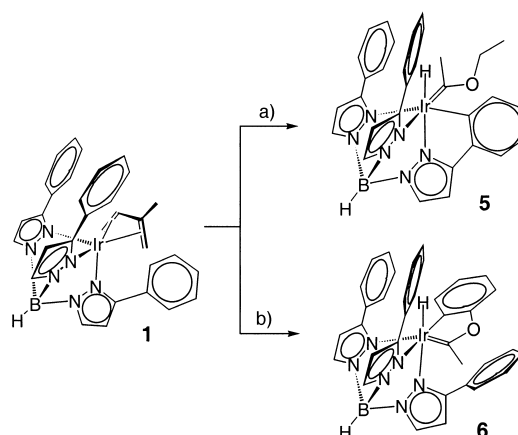
well with related data reported in the literature.^[4c, 7b, 13] Compound **4b** was identified as the isomer featuring a κ^4 -Tp^{Ph} ligand and a nonmetalated =C(H)NMePh fragment, as detected by ¹³C NMR spectroscopy (the two characteristic peaks for 3-phenylpyrazol-1-yl metalation at 122.4 and 120.8 ppm were observed).^[9] Because of a slow rotation around the carbene carbon–nitrogen bond, the ¹H NMR data show broadened signals for the hydride and for all hydrogen atoms of the carbene ligand. Low temperature NMR resulted in resolution of the signals associated with the hydride,

methyl, and carbene moieties into two peaks in the corresponding region (500 MHz, $T_c = 27 \pm 1$ °C), so that the possibility of a reversible α -H migration reaction^[7b] responsible for the broadening of the signals can be excluded.

In view of the complexity of this system which, as has already been pointed out, comprises three C–H activation steps, rationalizing the formation of **2–4** is not a simple task. Perusal of the literature^[8, 14] suggests that the reaction presumably proceeds initially by opening one pyrazolyl arm to give the tetracoordinated, 16-electron species **A** (Scheme 1), which is subsequently trapped by THF and rearranges to the formally Ir^{III} species **B**. Metalation of a Tp^{Ph}-phenyl substituent (to give **C**) could precede reversible β -C–H activation from the ether,^[15] until eventually the **C/D** equilibrium mixture would eliminate 2-methyl-2-butene irreversibly to give **E**, which, by α -H elimination, forms the product **2**.

In agreement with the assumptions implied in Scheme 1, treatment of **1** with [D₈]THF gives [D_{8.2}]**2**, in which the hydride NMR signal is absent in the ¹H spectrum but apparent in the ²H spectrum (signal intensity = 1 relative to the functionalized THF-deuterium atoms; that is, about 1.2 equivalents of deuterium became incorporated into one Tp^{Ph}-phenyl ring). That means, the final step in the formation of **2** is the α -C–H activation, specifically, an α -H elimination reaction. For the analogous transformations of **1** to produce **3** and **4** (assuming that this conclusion can be extended to these systems) there exists no alternative to the β -elimination pathway, whereas in the case of **2**, α -H elimination prevails over β -H elimination.^[6] To ascertain whether the rigid, cyclic nature of the ether has any influence on this result, reactions with the more flexible Et₂O molecule were investigated.

Heating a solution of **1** in Et₂O (60 °C, 12 h) gave the hydride ethoxy carbene complex **5** (Scheme 3), which displays a ¹H NMR signal at $\delta = -22.8$ (hydride) and a ¹³C signal at $\delta = 274.8$ (carbene; see Supporting Information). It is evident that α -H elimination also competes favorably with β -H elimination in this system. A comparable activation of Et₂O has been reported recently for platinum.^[16] However, the somewhat related compound [IrCp*(Me)(OTf)(PMe₃)] (Tf = SO₂CF₃), whilst activating THF in a manner similar to **1**, reacts with Et₂O to give [IrCp*(H)(η^2 -H₂C=C(H)-OEt)]⁺



Scheme 3. Reaction conditions: a) neat Et₂O, 12 h, 60 °C; b) EtOPh, 10 h, 60 °C, toluene; or EtOPh, 2 h, 110 °C, toluene.

instead, presumably by means of β -H elimination.^[7] As shown also in Scheme 3, activation of EtOPh by **1** (toluene, 110 °C, 2 h) gave compound **6** (see Supporting Information). It thus appears that the very important steric demands of the Tp^x ligand (as compared with the somewhat less bulky cyclopentadienyl ligand, Cp^x) and its rigid nature, which tends to enforce six-fold coordination to the metal center, favors α -H elimination over β -H elimination in the transformations studied here.

In summary, we have shown that the Ir^I compound **1** effects the regioselective, double C–H activation of a variety of ether and amine substrates by allowing their precoordination to the metal center. The formation of Fischer-type carbenes in this cascade activation (cyclometallation- β -elimination (or vice versa)- α -elimination) is made possible by the presence of an adequate hydrogen-accepting leaving group. The Tp^x- and Cp^x-Ir systems reported herein include O and N heteroatoms. Comparative studies on the facility of these fundamental organometallic reactions (α - and β -eliminations) on closely related Tp^x- and Cp^x-Ir systems without these heteroatoms that could alter the relative energies of the transition states and intermediates,^[17] are presently in progress and will be reported in due course.

Experimental Section

1: To a suspension of [(IrCl(coc)₂)₂] (175 mg, 0.20 mmol) in CH₂Cl₂ (6 mL), 2-methylbutadiene (0.3 mL, excess) was added at room temperature to give a colorless solution. A solution of TITp^{Ph} (253 mg, 0.40 mmol) in CH₂Cl₂ (6 mL) was then added. Stirring the reaction mixture for 4 h at room temperature resulted in the precipitation of TlCl. After filtration, the clear solution was evaporated to dryness. The crude product (orange powder) was purified via column chromatography (neutral aluminum oxide 90% activated, eluents PE (petroleum ether):Et₂O = 15:1, eluted as a pale violet band). Compound **1** was obtained upon changing the eluents to PE:Et₂O = 4:1 and it eluted as a yellow band. Removing the solvent and drying in vacuum gave pure **1**. Yield: 198 mg (71%). Elemental analysis: calcd for C₃₂H₃₀BIrN₆ (%): C 54.8, H 4.3, N 12.0; found: C 54.4, H 4.1, N 12.2.

Compounds **2–6** can be prepared similarly by treating **1** with an excess of the corresponding ether or amine. As a representative example the synthesis of **5** is given: A solution of **1** (70 mg, 0.10 mmol) in Et₂O was heated at 60 °C for 12 h. The solution was evaporated to dryness. Purification by flash chromatography (neutral aluminum oxide 90% activated, eluents PE:Et₂O = 2:1, eluted as a yellow band) resulted in pure **5**. Yield: 58 mg (82%). Elemental analysis: calcd for C₃₁H₃₀BIrN₆O (%): C 52.8, H 4.3, N 11.9; found: C 53.0, H 4.6, N 11.7.

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- [1] a) S. S. Stahl, J. A. Labinger, J. E. Bercaw, *Angew. Chem.* **1998**, *110*, 2298; *Angew. Chem. Int. Ed.* **1998**, *37*, 2181; b) B. A. Arndtsen, R. G. Bergman, T. A. Mobley, T. H. Peterson, *Acc. Chem. Res.* **1995**, *28*, 154; c) J. C. W. Lohrenz, H. Jacobsen, *Angew. Chem.* **1996**, *108*, 1403; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1305.
- [2] a) F. Zaragoza Dörwald, *Metal Carbenes in Organic Synthesis*, Wiley-VCH, Weinheim, **1999**; b) K. H. Dötz, H. Fischer, P. Hofmann, R. R. Kreissl, U. Schubert, K. Weiss, *Transition Metal Carbene Complexes*, VCH, Weinheim, **1993**.
- [3] For gas phase Fe^I-mediated 1,1-elimination of H₂ from a methoxy group, see: T. Prüsse, A. Fiedler, H. Schwarz, *J. Am. Chem. Soc.* **1991**, *113*, 8335.
- [4] a) H. Werner, B. Weber, O. Nürnberg, J. Wolf, *Angew. Chem.* **1992**, *104*, 1079; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1025; b) O. Boutry, E. Gutiérrez, A. Monge, M. C. Nicasio, P. J. Pérez, E. Carmona, *J. Am. Chem. Soc.* **1992**, *114*, 7288; c) E. Gutiérrez-Puebla, A. Monge, M. C.

- Nicasio, P. J. Pérez, M. L. Poveda, E. Carmona, *Chem. Eur. J.* **1998**, *4*, 2225.
- [5] a) A. Sen, M. Lin, L.-C. Kao, A. C. Hutson, *J. Am. Chem. Soc.* **1992**, *114*, 6385; b) Z.-W. Li, H. Taube, *J. Am. Chem. Soc.* **1994**, *116*, 11 584.
- [6] R. R. Schrock, S. W. Seidel, N. C. Mösch-Zanetti, K.-Y. Shih, M. B. O'Donoghue, W. M. Davis, W. M. Reiff, *J. Am. Chem. Soc.* **1997**, *119*, 11 876.
- [7] a) B. A. Arndtsen, R. G. Bergman, *Science*, **1995**, *270*, 1970; b) H. F. Luecke, B. A. Arndtsen, P. Burger, R. G. Bergman, *J. Am. Chem. Soc.* **1996**, *118*, 2517.
- [8] E. Gutiérrez-Puebla, A. Monge, M. Paneque, M. L. Poveda, V. Salazar, E. Carmona, *J. Am. Chem. Soc.* **1999**, *121*, 248.
- [9] C. Slugovc, K. Mereiter, S. Trofimenko, E. Carmona, *Chem. Commun.* **2000**, 121.
- [10] The related compound [Ir(κ^4 (N,N',N'',C^{Ph})-Tp^{Ph})(Et)(η^2 -C₂H₄)] activates THF to give **2** but with the other ether or amine substrates employed in this work the reaction failed due to the formation of [Ir(κ^5 (N,N',N'',C^{Ph},C^{Ph'})-Tp^{Ph})(η^2 -C₂H₄)]^[9].
- [11] C. Six, B. Gabor, H. Görls, R. Mynott, P. Philipps, W. Leitner, *Organometallics*, **1999**, *18*, 3316.
- [12] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-139166. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [13] D. M. Heinekey, J. M. Millar, T. F. Koetzle, N. G. Payne, K. W. Zilm, *J. Am. Chem. Soc.* **1990**, *112*, 909, and references therein.
- [14] U. E. Bucher, A. Currao, R. Nesper, H. Rüegger, L. M. Venanzi, E. Younger, *Inorg. Chem.* **1995**, *34*, 66.
- [15] Y. Kataoka, M. Imanishi, T. Yamagata, K. Tani, *Organometallics* **1999**, *18*, 3563.
- [16] M. W. Holtcamp, J. A. Labinger, J. E. Bercaw, *J. Am. Chem. Soc.* **1997**, *119*, 848.
- [17] J. Jaffart, R. Mathieu, M. Etienne, J. E. McGrady, O. Eisenstein, F. Maseras, *Chem. Commun.* **1998**, 2011.

DesVI: A New Member of the Sugar N,N-Dimethyltransferase Family Involved in the Biosynthesis of Desosamine**

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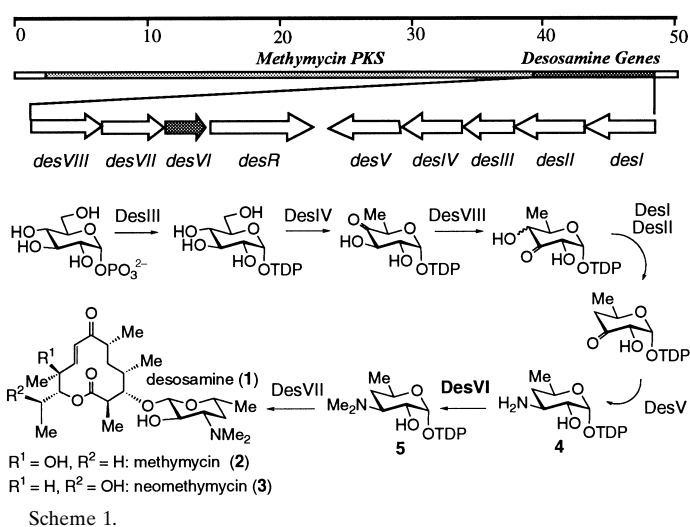
Methylation catalyzed by S-adenosylmethionine (AdoMet) dependent enzymes is one of the most common reactions occurring in biological systems. While a large number of methyltransferases which catalyze methylation at carbon,

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oxygen, sulfur, and nitrogen atoms are known, only a few are capable of catalyzing *N,N*-dimethylation.^[1] Our knowledge of these enzymes is scarce as most of them have never been isolated and fully characterized. Recently, genetic studies of the biosynthesis of desosamine (**1**), a structural component of the antibiotics methymycin (**2**), neomethymycin (**3**), pikromycin, and narbomycin produced by *Streptomyces venezuelae*,^[2] have led to the tentative assignment of *desVI* in the methymycin/pikromycin biosynthetic gene cluster as a candidate for encoding a new *N,N*-dimethyltransferase.^[3] The DesVI protein presumably acts on the C-3 amino group of sugar substrate **4** to give TDP-desosamine (**5**, see Scheme 1). The fact that disruption of the *desVI* gene in the genome of *S. venezuelae* resulted in the formation of a series of macrolides carrying an amino sugar devoid of *N,N*-dimethylation provided initial evidence to indicate that *desVI* indeed encodes an *N,N*-dimethyltransferase.^[4]

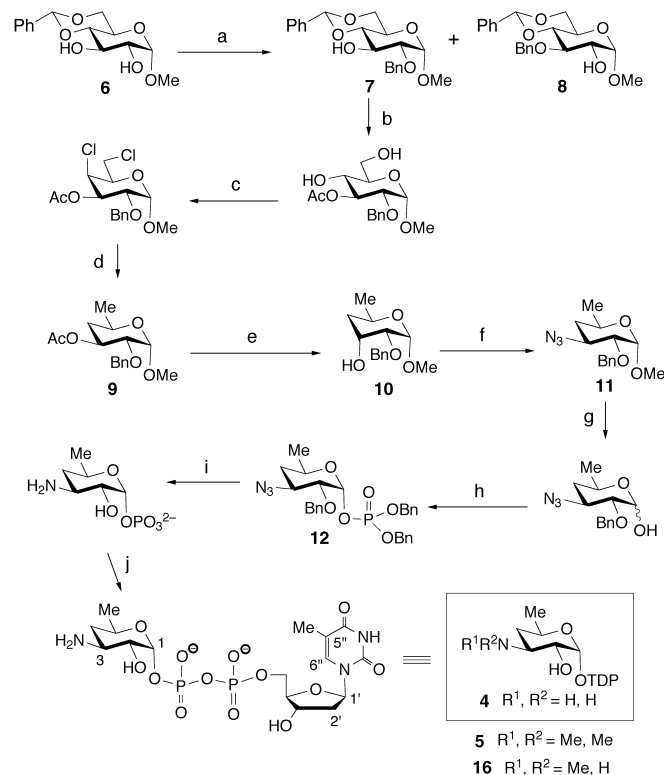


To further verify the proposed role of the *desVI* gene, we have now expressed and purified its encoded product, and characterized the catalytic function of this protein. In this paper, we report chemical evidence which establishes the role of DesVI enzyme as the methyltransferase catalyzing the *N,N*-dimethylation, the last step in the biosynthesis of desosamine. The *N,N*-dimethylation of the C-3 amino group in **4** rendered the product **5** more susceptible to protonation, a modification essential for enhancing the binding affinity of the resulting macrolide antibiotic toward its negatively charged 23S ribosomal RNA target.^[5]

To study the function of DesVI, the *desVI* gene was amplified by the polymerase chain reaction (PCR) and cloned into the *NdeI/EcoRI* sites of the expression vector pET28b(+). The resulting plasmid was used to transform *Escherichia coli* BL21(DE3) cells, and the recombinant strain was grown at 30 °C in LB (Luria-Bertani) medium. Induction of protein expression was achieved by the addition of 0.1 mM isopropyl β -D-thiogalactoside (IPTG). The DesVI enzyme, tagged with His₆ at its N terminus, was purified to near homogeneity using Ni–nitrilotriacetic acid (Ni–NTA) resin (Qiagen), and was found to be stable in phosphate

buffers containing 0.1 mM dithiothreitol (DTT) and 0.1 mM AdoMet. Further analysis using size-exclusion chromatography (Superdex 200 resin) revealed that DesVI exists as a homodimer with a subunit molecular mass of 28.1 kDa.^[6] The UV/Vis spectrum of the purified enzyme is transparent above 300 nm.

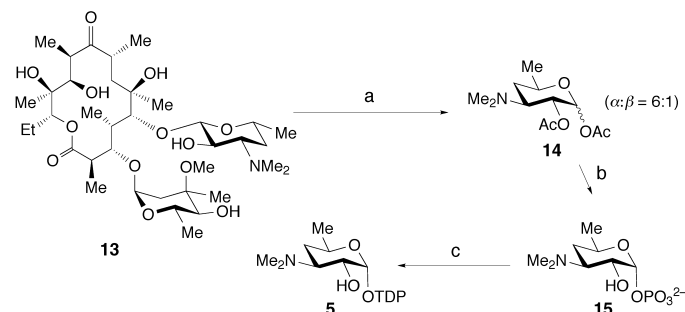
The predicted substrate of DesVI, **4**, was synthesized from methyl 4,6-*O*-benzylidene-glucoside (**6**) as depicted in Scheme 2. Preparation of the first key intermediate **9** was



initiated by a selective monobenylation mediated by bis-(tributyltin) oxide (**7:8** = 8:1),^[7] followed by acetylation, deprotection, dichlorination, and reduction with an overall yield of 14 %. After hydrolysis, the configuration of 3-OH was inverted by a Swern oxidation and NaBH₄ reduction (77 % yield from **9**). The amino group, masked as an azide moiety, was introduced at C-3 of **10** under Mitsunobu conditions to give **11** in 81 % yield. Subsequent demethylation was effected by BBr₃ at low temperature (–78 to 0 °C), and the product was benzylphosphorylated to give **12** (34 % yield). The final steps involved hydrogenation of **12** with Pd(OH)₂, Degussa type,^[8] and coupling of the resulting product with TMP-morpholidate.^[9] Compound **4** was purified by a Bio-P2

column (eluted with 25 mM NH_4HCO_3), and then MonoQ fast protein liquid chromatography (FPLC; linear gradient elution from 0 to 0.5 M NH_4HCO_3).^[10]

To facilitate confirmation of the enzymatic product structure, a convenient protocol to make the expected product, TDP-desosamine (**5**), was also developed. As delineated in Scheme 3, the preparation began with the acid hydrolysis of



Scheme 3. a) 1. 6 N HCl, H_2O , EtOH, reflux; 2. Ac_2O , H_2SO_4 ; b) 1. H_3PO_4 , P_2O_5 ; 2. 2 M LiOH; c) TMP-morpholidate, 1*H*-tetrazole, pyridine.

the commercially available erythromycin B (**13**).^[11] The released desosamine was directly acetylated to give **14** in 89% yield. The crude product was relatively labile at room temperature; however, it was rendered more stable after neutralization by dissolving in dichloromethane and washing with ammonium hydroxide and water. Direct phosphorylation of **14** using crystalline phosphoric acid followed by alkaline hydrolysis with lithium hydroxide afforded desosamine 1-phosphate (**15**) as a mixture of α - and β -anomers. The crude mixture was loaded onto a Dowex-50W column (cyclohexylammonium ion form) and eluted with water. The desired α -anomer of **15** was obtained in 12% yield after further purification by a Bio-P2 column (eluted with water). The subsequent coupling with TMP-morpholidate and the purification of the desired product **5** followed a procedure which was analogous with the synthesis of **4**.^[12]

To test whether DesVI is the desired methyltransferase, the purified DesVI (20 μg) was incubated with **4** (0.06 μmol), AdoMet (0.5 μmol), and DTT (2 mM) at 27 °C for 3 h in 50 mM potassium phosphate buffer (50 μL , 10% glycerol, pH 7.5). The incubation mixture was analyzed by HPLC with an Adsorbosphere SAX column (5 μ , 4.6 $\times$ 250 mm) using a linear gradient from 140 to 320 mM potassium phosphate buffer (pH 3.5) over 20 min. Detection was at 267 nm, and the flow rate was 1.0 mL min⁻¹. It was found that greater than 80% of **4**, which has a retention time of 6.5 min, was consumed and a new peak appeared at 17.0 min. A subsequent large scale incubation of 1.8 mg of DesVI, **4** (5.7 μmol), AdoMet (30 μmol), and DTT (2 mM) in 1 mL of the same buffer at 25 °C for 3 h allowed the isolation and purification of this new compound. Spectral characterization confirmed that the purified product is indeed the desired TDP-desosamine (**5**). When the reaction was quenched (boiled for 2 min) at an earlier time, an intermediate with a retention time of 9.3 min, presumably the monomethylated species **16**, could also be detected. The percentage conversions of **4** to **16** and **5** were estimated based on the integration of the corresponding

HPLC peaks, and by fitting these data to the rate laws for an irreversible unimolecular consecutive reaction (**4** $\rightarrow$ **16** $\rightarrow$ **5**) led to the constants of 0.005 and 0.82 min⁻¹ for the mono- and dimethylation steps, respectively. Thus, our results clearly demonstrate that DesVI is the required methyltransferase in the biosynthesis of desosamine, and it alone catalyzes the *N,N*-dimethylation of the 3-amino group of **4** in a stepwise manner.

In summary, the above results have clearly established DesVI as a new member of the small family of enzymes that are capable of catalyzing *N,N*-dimethylation.^[1] It should be pointed out that the deduced sequence of DesVI reveals significant similarity to those of EryCVI from the erythromycin cluster of *Saccharopolyspora erythraea* (62% identity),^[3] OleM1 from the oleandomycin cluster of *Streptomyces antibioticus* (61%),^[13] TylM1 from the tylosin cluster of *Streptomyces fradiae* (60% identity),^[14] SnoX from the nogalamycin cluster of *Streptomyces nogalater* (55% identity),^[15] RdmD from the rhodomycin cluster of *Streptomyces purpurascens* (57% identity),^[16] and SrmX from the spiramycin cluster of *Streptomyces ambifaciens* (57% identity).^[17] The catalytic role of TylM1 as an AdoMet-dependent *N,N*-dimethyltransferase has recently been verified.^[18] The fact that DesVI also acts on the amino group of a sugar substrate in a similar manner provides convincing evidence suggesting similar roles for these proteins in their respective biosynthetic pathways. The *N,N*-dimethylation of amino sugars could be a convenient tool to modify their chemical properties due to the changes in size, hydrogen-bonding capacity, and propensity for protonation of the amino group. Therefore, with the addition of DesVI and the expectation that other similar methyltransferases will be added in the future to the arsenal of biosynthetic genes, the ability to prepare designed glycoconjugate analogues with novel tailored biological activities should be enhanced significantly.

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- [1] a) T. Kodaki, S. Yamashita, *Eur. J. Biochem.* **1989**, *185*, 243–251; b) J. D. Gary, W.-J. Lin, M. C. Yang, H. R. Herschman, S. Clarke, *J. Biol. Chem.* **1996**, *271*, 12585–12594; c) M. Zalacain, E. Cundliffe, *J. Bacteriol.* **1989**, *171*, 4254–4260.
- [2] a) M. N. Donin, J. Pagano, J. D. Dutcher, C. M. McKee, *Antibiot. Annu.* **1953–1954**, *1*, 179–185; b) C. Djerassi, J. A. Zderic, *J. Am. Chem. Soc.* **1956**, *78*, 6390–6395.
- [3] a) S. Gaissner, G. A. Bohm, J. Cortés, P. F. Leadlay, *Mol. Gen. Genet.* **1997**, *256*, 239–251; b) R. G. Summers, S. Donadio, M. J. Staver, E. Wendt-Pienkowski, C. R. Hutchinson, L. Katz, *Microbiology* **1997**, *143*, 3251–3262; c) Y. Xue, L. Zhao, H.-w. Liu, D. H. Sherman, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12111–12116.
- [4] L. Zhao, D. H. Sherman, H.-w. Liu, *J. Am. Chem. Soc.* **1998**, *120*, 10256–10257.
- [5] H. Sakakibara, S. Omura, *Macrolide Antibiotics*, Chemistry, Biology and Practice (Ed.: S. Omura), Academic Press, New York, **1984**, Chapter 3, pp. 85–125.
- [6] The molecular mass of each subunit calculated, based on the translated DesVI sequence together with its His₆ tag, is 28,110 Da.
- [7] F. Dasgupta, P. J. Garegg, *Synthesis* **1994**, 1121–1123.
- [8] R. Wischnat, R. Martin, C.-H. Wong, *J. Org. Chem.* **1998**, *63*, 8361–8365.
- [9] V. Wittmann, C.-H. Wong, *J. Org. Chem.* **1997**, *62*, 2144–2147.
- [10] Spectral data for **4**: ¹H NMR (500 MHz, D₂O) δ = 1.24 (3H, d, *J* = 6.3 Hz, 5-Me), 1.50 (1H, ddd, *J* = 12.7, 12.3, 11.7 Hz, 4-H_{ax}), 1.93 (3H, s, 5'-Me), 2.27 (1H, ddd, *J* = 12.7, 4.0, 3.0 Hz, 4-H_{eq}), 2.40 (2H, m, 2'-H), 3.59 (1H, ddd, *J* = 12.2, 10.7, 4.0 Hz, 3-H), 3.74 (1H, dt, *J* = 10.7,

- 2.9 Hz, 2-H), 4.20 (3H, m, 4'-H, 5'-H), 4.29 (1H, ddq, $J = 11.7, 6.3, 3.0$ Hz, 5-H), 4.62 (1H, dt, $J = 5.8, 3.0$ Hz, 3'-H), 5.63 (1H, dd, $J = 7.3, 3.4$ Hz, 1-H), 6.35 (1H, t, $J = 6.8$ Hz, 1'-H), 7.71 (1H, s, 6''-H); ^{13}C NMR (125 MHz, D_2O) $\delta = 11.6, 19.5, 35.3, 38.5, 48.9, 65.4, 69.5$ (d, $J = 6.6$), 70.9, 84.9, 85.0, 85.3 (d, $J = 10.1$), 95.0 (d, $J = 8.0$), 111.7, 137.3, 151.7, 166.5; ^{31}P NMR (121 MHz, D_2O) $\delta = -12.4$ (d, $J = 20.9$), -10.5 (d, $J = 20.9$); high-resolution fast atom bombardment (FAB) MS: calcd for $\text{C}_{16}\text{H}_{28}\text{N}_3\text{O}_{13}\text{P}_2$ [$M^+ + 2\text{H}$] 532.1097, found [$M^+ + 2\text{H}$] 532.1098.
- [11] E. H. Flynn, M. V. Sigal, Jr., P. E. Wiley, K. Gerzon, *J. Am. Chem. Soc.* **1954**, 76, 3121–3131.
- [12] Spectral data for **5**: ^1H NMR (500 MHz, D_2O) $\delta = 1.24$ (3H, d, $J = 6.4$ Hz, 5-Me), 1.62 (1H, dt, $J = 12.7, 11.7$ Hz, 4- H_{ax}), 1.90 (3H, s, 5''-Me), 2.12 (1H, dt, $J = 12.7, 3.4$ Hz, 4- H_{eq}), 2.3–2.4 (2H, m, 2'-H), 2.83 (6H, s, NMe_2), 3.76 (1H, dt, $J = 11.7, 3.4$ Hz, 3-H), 3.88 (1H, dt, $J = 11.2, 2.9$ Hz, 2-H), 4.1–4.2 (3H, m, 4'-H, 5'-H), 4.28 (1H, ddq, $J = 11.7, 6.4, 3.4$ Hz, 5-H), 4.59 (1H, dt, $J = 5.4, 3.4$ Hz, 3'-H), 5.64 (1H, dd, $J = 7.1, 3.1$ Hz, 1-H), 6.33 (1H, t, $J = 6.8$ Hz, 1'-H), 7.70 (1H, s, 6''-H); ^{13}C NMR (125 MHz, D_2O) $\delta = 11.6, 19.7, 29.1, 38.5, 38.6$ (broad, NMe_2), 62.0, 65.5 (d, $J = 5.1$ Hz), 65.8, 66.7 (d, $J = 8.1$), 70.9, 84.9, 85.2 (d, $J = 10.1$), 95.2 (d, $J = 6.0$), 111.7, 137.3, 151.7, 166.5; ^{31}P NMR (121 MHz, D_2O) $\delta = -12.4$ (d, $J = 20.8$), -10.6 (d, $J = 20.8$); high-resolution FAB MS: calcd for $\text{C}_{18}\text{H}_{30}\text{N}_3\text{O}_{13}\text{P}_2$ [$M^+ + 2\text{H}$] 560.1410, found [$M^+ + 2\text{H}$] 560.1400.
- [13] C. Olano, A. M. Rodriguez, J. M. Michel, C. Mendez, M. C. Raynal, J. A. Salas, *Mol. Gen. Genet.* **1998**, 259, 299–308.
- [14] A. R. Gandecha, S. L. Large, E. Cundliffe, *Gene* **1997**, 184, 197–203.
- [15] K. Ylihanko, J. Tuikkanen, S. Jussila, L. Cong, P. Mäntsälä, *Mol. Gen. Genet.* **1996**, 251, 113–120.
- [16] J. Niemi, P. Mäntsälä, *J. Bacteriol.* **1995**, 177, 2942–2945.
- [17] M. Geistlich, R. Losick, J. R. Turner, R. N. Rao, *Mol. Microbiol.* **1992**, 6, 2019–2029.
- [18] H. Chen, Z. Guo, H.-w. Liu, *J. Am. Chem. Soc.* **1998**, 120, 9951–9952.

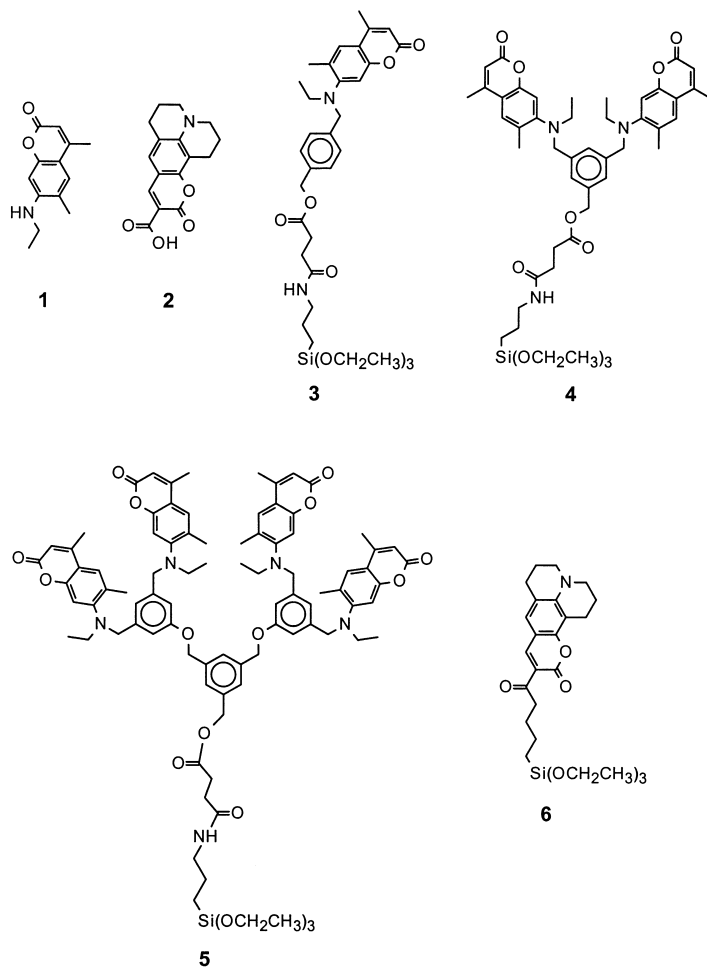
Surface-Confined Light Harvesting, Energy Transfer, and Amplification of Fluorescence Emission in Chromophore-Labeled Self-Assembled Monolayers**

Lysander A. J. Chrisstoffels, Alex Adronov, and Jean M. J. Fréchet*

Light harvesting and energy transfer have received considerable attention in the literature because of their important role in natural photosynthesis. These processes involve the use of a light-absorbing antenna (donor) moiety that is capable of

transferring the absorbed energy to a nearby secondary energy acceptor species. The energy-transfer process is influenced by the spatial relationship of the donor and acceptor chromophores, and recently researchers have sought to mimic the efficient arrangements found in nature. These include chromophore-functionalized polymers,^[1] dendrimers,^[2] supported Langmuir–Blodgett films,^[3] thin films,^[4] and microspheres.^[5] Here, we report the use of self-assembled monolayers (SAMs) to align the donor and acceptor chromophores and facilitate intermolecular energy transfer. Intermolecular interactions between adjacent adsorbates have previously been applied to improve the chemical stability of monolayers^[6] and to facilitate surface-directed polymerization^[7] or surface-confined photodimerization.^[8] We extend the exploration of these interactions to include long-range photo-induced Förster energy transfer^[9] between donor and acceptor chromophores on SAMs. This process involves a through-space dipole–dipole interaction that does not occur by “hopping” through bonds. Energy transfer can thus be facilitated by assembly of donor and acceptor chromophores as mixed monolayers.

We have recently reported efficient light harvesting by chromophore-labeled dendrimers in which light is funneled through space by Förster energy transfer from multiple coumarin-2 (**1**) donor dyes at the dendrimer periphery to a single coumarin-343 (**2**) acceptor chromophore.^[10] These dyes were also chosen for our chromophore-labeled monolayers



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since they provide good spectral overlap between the donor emission (380–450 nm) and acceptor absorption bands (400–455 nm), have a low probability of self-quenching, and high fluorescence quantum yields.^[10] Moreover, since these dyes also have large transition dipole moments and high extinction coefficients, the Coulombic mechanism is expected to dominate over orbital overlap contributions, such as electron exchange.^[10–12]

Donor chromophore adsorbates (**3–5**) and acceptor chromophore adsorbate **6** were synthesized for the preparation of mixed siloxane monolayers on silicon wafers. Full synthetic details are given in the Supporting Information. A schematic representation of a mixed SAM of coumarin-2-containing donor (**4**) and coumarin-343-containing acceptor (**5**) dyes is given in Figure 1. Surface-oxidized n-type Si(100) wafers were

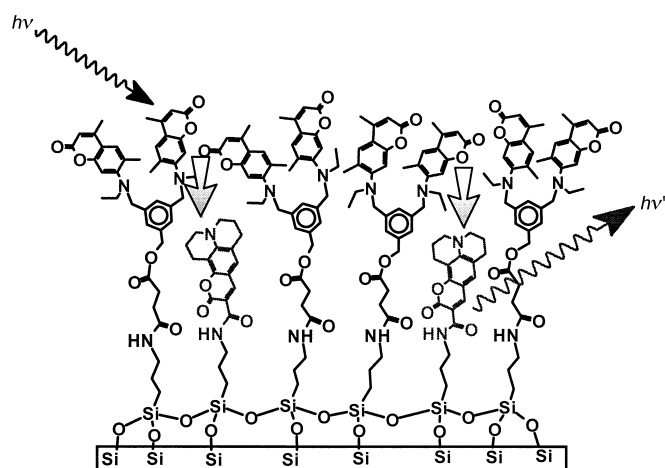


Figure 1. Schematic representation of Förster energy transfer within a mixed monolayer of donor (**4**) and acceptor (**6**) chromophores on a silicon wafer.

used as substrates,^[13, 14] since they do not affect the excited state of the adsorbed chromophores. As a consequence of its sensitivity, front-face fluorescence spectroscopy is suitable for the characterization of the small amounts of material present in monolayers^[15] and for the study of their light-harvesting and energy-transfer properties.

Chromophore-labeled monolayers were prepared by immersing the cleaned and surface-oxidized wafers in a solution of adsorbate **3**, **4**, **5**, or **6** (25 mM) in EtOH (containing 1 vol % H₂O and 0.5 vol % H₃CCOOH) for 20 minutes. Subsequently, the wafers were rinsed and heated at 70 °C under high vacuum (5 min) to chemisorb the adsorbate chromophores onto the surface as polysiloxanes. Any physisorbed material was removed by repeated rinsing with chloroform and ethanol. Contact-angle goniometry using the sessile drop method^[16] showed that the original contact angle ($\theta = 15^\circ$) of the clean wafer was changed to 56°, 50°, and 49° for donor chromophores **3**, **4**, and **5**, respectively, and to 55° for acceptor chromophore **6**. These values are in agreement with those reported for coumarin adsorbates assembled on gold ($\theta = 58^\circ$).^[17] Additionally, optical-ellipsometry measurements provided an estimate of the monolayer thickness. Monolayers of acceptor **6** had thicknesses in the range of 6–7 Å, whereas film thicknesses for donor adsorbates **3–5** were on the order

of 10–13 Å. The measured thicknesses are slightly less than expected based on the size of the adsorbates, which is indicative of sub-monolayer formation. Front-face fluorescence spectroscopy confirmed that the emission of freshly cleaned wafers was negligible when irradiated at wavelengths of 300–500 nm. Self-assembled monolayers of the individual chromophores **3**, **4**, **5**, or **6** on silicon showed large emission intensities when irradiated at their chromophore-specific excitation wavelengths (Figure 2). The fluorescence spectra

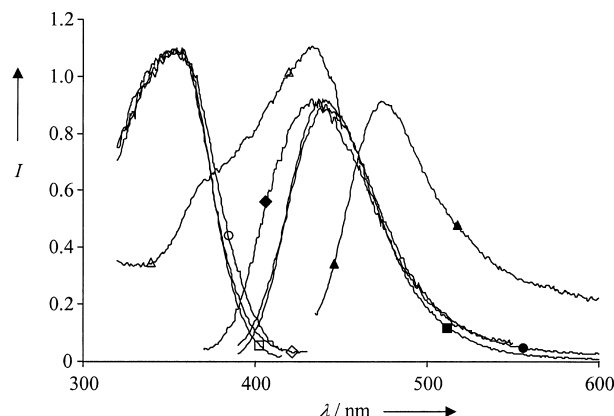


Figure 2. Normalized excitation and emission spectra from monolayers of chromophores **3** (●: $\lambda_{\text{ex}} = 350$ nm; ○: $\lambda_{\text{em}} = 420$ nm), **4** (◆: $\lambda_{\text{ex}} = 350$ nm; ◇: $\lambda_{\text{em}} = 420$ nm), **5** (■: $\lambda_{\text{ex}} = 350$ nm; □: $\lambda_{\text{em}} = 420$ nm), **6** (▲: $\lambda_{\text{ex}} = 425$ nm; △: $\lambda_{\text{em}} = 480$ nm).

of adsorbates **3–5** are very similar, which indicates that the photophysical properties of the coumarin-2 chromophores on the surface are essentially unaffected by the linker between the chromophores and the silane moiety. This effect was also observed in the fluorescence spectra of adsorbates **3–5** in toluene solutions (10^{-6} mol L⁻¹), where excitation of donor chromophores **3**, **4**, and **5** gave a maximum emission intensity at $\lambda_{\text{em}} = 412$, 416, and 420 nm, respectively ($\lambda_{\text{ex}} = 350$ nm). The maximum emission of adsorbate **6** in toluene was observed at $\lambda_{\text{em}} = 447$ nm ($\lambda_{\text{ex}} = 420$ nm). The chromophore-labeled monolayers show emissions red-shifted by 15–35 nm from those of the reference toluene solutions, which is in agreement with previous observations for other monolayers.^[15] Fluorescence spectroscopy is a very sensitive diagnostic technique for monitoring the assembly process since the monolayers exhibit the characteristic fluorescence spectra of the coumarin chromophores.^[15]

The observed emission from SAMs of donor **5** is negligible in the range of $\lambda_{\text{em}} = 440$ –550 nm when irradiated with light with a wavelength of 420 nm relative to its emission when irradiated at $\lambda_{\text{ex}} = 350$ nm (Figure 3). Thus, acceptor adsorbate **6** can be excited selectively at $\lambda_{\text{ex}} = 420$ nm in mixed monolayers. It is therefore possible to evaluate the energy-transfer and light-harvesting properties of mixed monolayers by comparing the observed emission at $\lambda_{\text{em}} = 480$ nm after excitation of the layer at $\lambda_{\text{ex}} = 350$ nm and at $\lambda_{\text{ex}} = 420$ nm.

It was expected that the photophysical properties of mixed chromophore monolayers would be dependent on both the chromophore composition and the branched nature of the donor adsorbate. Therefore, mixed monolayers were prepared with different donor–acceptor combinations in various

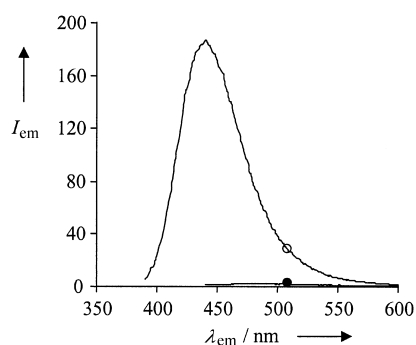


Figure 3. Emission spectra from a monolayer of adsorbate **5** after excitation at $\lambda_{\text{ex}} = 350$ nm ($\circ$) and $\lambda_{\text{ex}} = 420$ nm ($\bullet$); I_{em} in 10^3 counts s^{-1} .

molar ratios r ($r = (\text{moles of donor adsorbate})/(\text{moles of acceptor adsorbate})$ before immersion of the substrates). The influence of the dendritic nature of the donor adsorbate on the energy-transfer process becomes apparent from a comparison of the mixed monolayers of **3** and **6** ($r = 4$, $\theta = 56^\circ$) and **5** and **6** ($r = 1$, $\theta = 51^\circ$). Dendritic donor **5** contains four coumarin-2 chromophores linked to a triethoxysilane moiety through a branched linker, whereas linear donor **3** contains a single coumarin-2 chromophore per triethoxysilane moiety. Although the molar ratio of donor:acceptor dyes for both adsorbate solutions is 4:1 prior to monolayer formation, the resulting mixed monolayers exhibit very different photophysical properties (Figure 4).^[18]

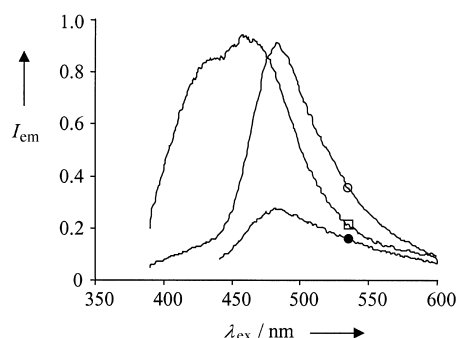


Figure 4. Normalized emission spectra from mixed monolayers of **3** and **6** ($\square$), and **5** and **6** ($\circ$) after excitation at $\lambda_{\text{ex}} = 350$ nm. The emission spectrum ($\bullet$) was obtained after excitation of the monolayer formed between **5** and **6** at $\lambda_{\text{ex}} = 420$ nm. The normalization factor for spectra $\circ$ and $\bullet$ is the same.

The donor emission from mixed monolayers of **5** and **6** ($r = 1$) is completely quenched, which demonstrates an efficient energy transfer. This result indicates that all the donor and acceptor chromophores are aligned on the surface well within the Förster radius. However, a large unwanted donor emission remains from mixed monolayers of adsorbates **3** and **6** ($r = 4$), which indicates that in this case the donor–acceptor distances are too large. A significant influence of the interchromophoric distance upon the transfer efficiency within the monolayer is expected since the energy-transfer efficiency decreases as the inverse sixth power of this distance.^[9] We have recently calculated the Förster radius for this pair of chromophores in toluene to be $R_0 = 42$ Å.^[11] This value is an order of magnitude higher than the inter-adsorbate distance in a polysiloxane

monolayer of alkyl chains (4.4 Å).^[13] The inter-chromophoric distance on the monolayer is determined by both the surface coverage and the distribution of the dyes on the surface. This, in turn, is affected by the formation of dimeric and trimeric oligosiloxanes in solution prior to adsorption.^[19, 20] Kinetic analysis suggests that the molar amount of dimers and trimers containing no acceptor chromophores is high for solutions containing donor and acceptor chromophores at a molar ratio greater than two.^[21] Adsorption of the resulting oligomers on the surface may lead to the formation of donor-rich domains affecting the interchromophoric distance. As a result, incomplete quenching of the donor emission at higher molar ratios is expected. These experiments clearly demonstrate the effectiveness of a dendritic linker in mixed monolayers to increase the relative amount of donor chromophore while preserving a random distribution of adsorbates on the surface.

Amplified emission by energy transfer from the donor to acceptor chromophores was demonstrated by excitation of mixed monolayers of **5** and **6** ($r = 1$) either at 350 nm (Figure 4, curve $\circ$) or at 420 nm (Figure 4, curve $\bullet$). Since the acceptors quench all donor emission, the emission at 480 nm solely emanates from the acceptor chromophores, which enables a direct comparison of the emission intensities. It is clear that a much higher acceptor emission is observed after energy transfer by excitation of donor adsorbate **5** relative to direct excitation of the acceptors. Mixed monolayers of **5** and **6** of lower molar ratios were also prepared ($r = 0.5, 0.25$). In these cases, the donor emission was completely quenched and only a small amplification of the acceptor emission was observed. The amplification decreased significantly when lower r values were used.

The above findings were confirmed by our observations with mixed monolayers of **4** and **6**. The fluorescence emission spectra of the corresponding layers were measured after irradiation of the layers at $\lambda_{\text{ex}} = 350$ nm. The fluorescence emission from SAMs obtained from a solution of $r = 2$ ($\theta = 57^\circ$) and $r = 3$ ($\theta = 51^\circ$) are depicted in Figure 5. Mixed

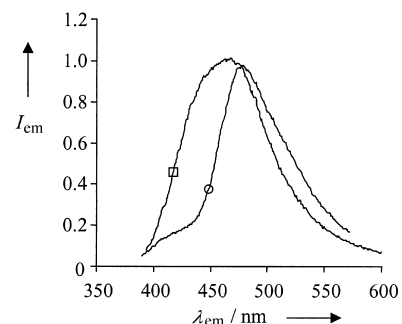


Figure 5. Normalized emission spectra from mixed monolayers of **4** and **6** after excitation at $\lambda_{\text{ex}} = 350$ nm ($r = 3$: $\square$, $r = 2$: $\circ$).

monolayers of **4** and **6** with $r = 3$ exhibit a broad emission in the range of 420 nm–500 nm as a result of the combined emission of both the donor and acceptor chromophores (Figure 5, curve $\square$). The energy transfer is not optimal, as the donor emission is not completely quenched by the acceptors. However, when monolayers from solutions of **4** and **6** with $r = 2$ were irradiated with light with a wavelength of 350 nm a

large emission from the acceptors at 480 nm was observed, while the donor emission at 420 nm was almost completely quenched (Figure 5, curve ○). The above experiments clearly reveal that simply varying the molar ratio r changes the light-harvesting properties of the SAMs. Since the donor emission for monolayers of **4** and **6** with $r=2$ are fully quenched the amplification of emission was also studied. The observed emission upon excitation at 350 nm was almost twice as intense as the emission observed after direct excitation of the acceptor dyes at 420 nm.

Excitation of the donor chromophores in monolayers from solutions of **4** and **6** at low molar ratio ($r=0.5$) did show complete quenching of the donor emission. However, the acceptor emission at 480 nm was greater when the acceptors were directly excited at 420 nm and amplified emission was not observed. In this case, the donor chromophores on the monolayer are too sparsely dispersed to provide an efficient light-harvesting antenna.

In conclusion, efficient energy transfer within the xy plane of self-assembled monolayers of mixed chromophoric adsorbates on silicon wafers has been demonstrated. Large amplification of the acceptor emission was achieved using dendritic chromophores since the emission resulting from through-space energy transfer far exceeds that possible by direct chromophore excitation. Our reported data demonstrate that intermolecular energy transfer between donor and acceptor chromophores, or more generally between any donor–quencher pair, may be utilized as a physical transducer for fluorescence-sensor devices.^[22] In addition, monolayer alignment of chromophores may provide easy access to other systems for energy transfer, such as for the cascade-type of energy transfer.^[23]

Experimental Section

Fluorescence spectra were recorded under N_2 on a SPEX/ISA Fluorolog 3.22 equipped with double excitation and double emission monochromators and a digital photon-counting photomultiplier with slit widths of 3 nm bandpass on excitation and 5 nm on emission. Emission from the wafers was detected using front-face fluorescence at an angle of 22.5° to the excitation beam. Repeated measurements (5 times) showed no decrease in emission intensity. Measurements in toluene were carried out as described previously.^[10]

Polished n-type Si(100) wafers were cleaned/oxidized prior to monolayer formation.^[14] The adsorbates (**25** mm) were dissolved in aqueous EtOH (1 vol % H_2O and 0.5 vol % H_3CCOOH) for 5 min before immersing the freshly cleaned wafers for 15 min. A small amount of $CHCl_3$ (15 vol %) was added to solubilize adsorbate **5**. The wafers were rinsed with $CHCl_3$ (3 mL), dried under a gentle flow of N_2 , heated in a vacuum oven at 70 °C, and washed with $CHCl_3$ ($3 \times$) and EtOH ($3 \times$) to remove any physisorbed adsorbate. Contact angles were determined on a Krüss Model G10 goniometer at room temperature and ambient relative humidity using the sessile drop method;^[16] averages of measurements on three different areas of the wafer are reported. The thickness of the monolayer was determined using a Gaertner L116A ellipsometer equipped with a 632.8 nm He-Ne laser. Monolayer thicknesses were determined assuming a refractive index (n_f) of 1.5, and with the incident and reflected light making a 70° angle to the surface normal. The data represents averages of at least ten different measurements on three different areas of the wafer.

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- [1] a) M. Nowakowska, V. P. Foyle, J. E. Guillet, *J. Am. Chem. Soc.* **1993**, *115*, 5975–5981; b) K. Hisada, I. Shinzaburo, M. Yamamoto, *Langmuir* **1995**, *11*, 996–1000.
- [2] a) G. Denti, S. Campagna, S. Serroni, M. Ciano, V. Balzani, *J. Am. Chem. Soc.* **1992**, *114*, 2944–2950; b) C. Devadoss, P. Bharathi, J. S. Moore, *J. Am. Chem. Soc.* **1996**, *118*, 9635–9644; c) G. M. Stewart, M. A. Fox, *J. Am. Chem. Soc.* **1996**, *118*, 4354–4360; d) M. R. Shortreed, S. F. Swallen, Z.-Y. Shi, W. Tan, Z. Xu, C. Devadoss, J. S. Moore, R. Kopelman, *J. Phys. Chem. B* **1997**, *101*, 6318–6322; e) D.-L. Jiang, T. Aida, *Nature* **1997**, *388*, 454–456; f) A. Bar-Haim, J. Klafter, *J. Phys. Chem. B* **1998**, *102*, 1662–1664; g) M. Plevoets, F. Vögtle, L. DeCola, V. Balzani, *New J. Chem.* **1999**, *23*, 63–69.
- [3] a) N. Ohta, N. Tamai, T. Kuroda, T. Yamazaki, Y. Nishimura, I. Yamazaki, *Chem. Phys.* **1993**, *177*, 591–600; b) D. Papoutsis, V. Bekiari, E. Stathatos, P. Lianos, A. Laschewesky, *Langmuir* **1995**, *11*, 4355–4360; c) H. Huesmann, G. Striker, D. Möbius, *Langmuir* **1997**, *13*, 4929–4932; d) E. Vuorimaa, H. Lemmetyinen, P. Ballet, M. van der Auweraer, F. C. de Schryver, *Langmuir* **1997**, *13*, 3009–3015; e) A. K. Dutta, H. Lavoie, K. Ohta, C. Salesse, *Langmuir* **1997**, *13*, 801–807; f) T. Morita, S. Kimura, Y. Imanishi, *Langmuir* **1998**, *14*, 171–175; g) J. Matsui, M. Mitsuishi, T. Miyashita, *Macromolecules* **1999**, *32*, 381–386; h) P. J. Dutton, L. Conte, *Langmuir* **1999**, *15*, 613–617.
- [4] a) D. M. Kaschak, T. E. Mallouk, *J. Am. Chem. Soc.* **1996**, *118*, 4222–4223; b) D. M. Kaschak, J. T. Lean, C. C. Warakaska, G. B. Saupe, H. Usami, T. E. Mallouk, *J. Am. Chem. Soc.* **1999**, *120*, 3435–3445; c) J. Feng, M. A. Winnik, A. Siemiarz, *J. Polym. Sci.* **1998**, *36*, 1115–1128; d) M. Mabuchi, S. Ito, M. Yamamoto, T. Miyamoto, A. Schmidt, W. Knoll, *Macromolecules* **1998**, *31*, 8802–8808.
- [5] a) M.-T. Charreyre, A. Yekta, M. A. Winnik, T. Delair, C. Pichot, *Langmuir* **1995**, *11*, 2423–2428; b) F. Caruso, E. Donath, H. Mohwald, *J. Phys. Chem. B* **1998**, *102*, 2011–2016.
- [6] a) S.-W. Tam-Chang, H. A. Biebuyck, G. M. Whitesides, N. Jeon, R. G. Nuzzo, *Langmuir* **1995**, *11*, 4371–4392; b) R. S. Clegg, J. E. Hutchison, *Langmuir* **1996**, *12*, 5239–5343.
- [7] a) D. N. Batchelder, S. D. Evans, T. L. Freeman, L. Häussling, H. Ringsdorf, H. Wolf, *J. Am. Chem. Soc.* **1994**, *116*, 1050–1053; b) T. Kim, K. C. Chan, R. M. Crooks, *J. Am. Chem. Soc.* **1997**, *119*, 189–193; c) R. M. McCarley, R. J. Willicut, *J. Am. Chem. Soc.* **1998**, *120*, 9296–9304; d) J. F. Ford, T. J. Vickers, C. K. Mann, J. B. Schlenoff, *Langmuir* **1996**, *12*, 1944–1946; e) S. Inoaka, D. M. Collard, *Langmuir* **1999**, *15*, 3752–3758.
- [8] a) M. A. Fox, M. D. Wooten, *Langmuir* **1997**, *13*, 7099–7105; b) M. A. Fox, W. Li, M. Wooten, A. McKerron, J. K. Whitesell, *Thin Solid Films* **1998**, *327*, 477–480.
- [9] a) T. Förster, *Z. Naturforsch. A* **1949**, *4*, 319–327; b) B. W. Van der Meer, G. Coker III, S.-Y. Simon Chen, *Resonance Energy Transfer, Theory and Data*, VCH, Weinheim, **1994**.
- [10] a) S. L. Gilat, A. Adronov, J. M. J. Fréchet, *Angew. Chem.* **1999**, *111*, 1519–1524; *Angew. Chem. Int. Ed.* **1999**, *38*, 1422–1427; b) S. L. Gilat, A. Adronov, J. M. J. Fréchet, *J. Org. Chem.* **1999**, *64*, 7474–7484.
- [11] A. Adronov, S. L. Gilat, J. M. J. Fréchet, K. Ohta, F. V. R. Neuwahl, G. R. Fleming, *J. Am. Chem. Soc.* **2000**, *122*, 1175–1185.
- [12] B. P. Krueger, G. D. Scholes, G. R. Fleming, *J. Phys. Chem. B* **1998**, *102*, 5378–5386.
- [13] A. Ulman, *Chem. Rev.* **1996**, *96*, 1533–1554.
- [14] D. C. Tully, K. Wilder, J. M. J. Fréchet, A. R. Trimble, C. F. Quate, *Adv. Mater.* **1999**, *11*, 314–318.
- [15] a) M. Ayadim, J. L. Habib Jiwan, A. P. De Silva, J. P. Soumillion, *Tetrahedron Lett.* **1996**, *37*, 7039–7042; b) D. S. Karpovich, G. J. Blanchard, *Langmuir* **1996**, *12*, 5522–5524; c) Y. Hayashi, K. Ichimura, *Langmuir* **1996**, *12*, 831–835; d) D. L. Pilloud, C. Moser, K. S. Reddy, P. L. Dutton, *Langmuir* **1998**, *14*, 4809–4818; e) S. Reese, M. A. Fox, *J. Phys. Chem. B* **1998**, *102*, 9820–9824; f) K. Moteshare, D. C. Myles, *J. Am. Chem. Soc.* **1998**, *120*, 7328–7336; g) J. C. Horne, Y. Hung, G.-Y. Liu, G. J. Blanchard, *J. Am. Chem. Soc.* **1999**, *121*, 4419–4426.
- [16] J. Xu, W. J. Choyke, J. T. Yates Jr., *J. Appl. Phys.* **1997**, *82*, 6289–6292.
- [17] W. Li, V. Lynch, H. Thompson, M. A. Fox, *J. Am. Chem. Soc.* **1997**, *119*, 7211–7217.

- [18] Reabsorption of donor emission by neighboring acceptors instead of Förster energy transfer is considered to be nominal because of the 2-dimensional alignment of donor and acceptor chromophores as a mixed monolayer. The emission spectra from a mixed monolayer of **3** and **6** ($r=4$) were measured after small changes of the incidence angle α of the excitation beam ($\Delta\alpha < 20^\circ$). Only minor changes in the emission spectra were observed and the relative emission intensity from the donor and acceptor chromophores remained the same.
- [19] B. Arkles, *CHEMTECH*, **1977**, 7, 766.
- [20] The assembly of alkoxyxilanes is a two-step process that involves pretreatment of the adsorbates prior to adsorption on the substrate. Initially, siloxane dimers and trimers are formed in the adsorbate solution by an acid-catalyzed condensation. Upon immersion of the silicon wafer, these oligomers adsorb onto the silanol surface by hydrogen bonding and become covalently bound to the surface by curing the wafers. The adsorption of the chromophore oligomers from solution occurs randomly. Therefore, the chromophore composition on the monolayer may not be the same as in solution.
- [21] The donor (D) and acceptor (A) distribution in the siloxane dimers and trimers was calculated by reaction kinetics from the molar amount of D and A in solution. It is assumed that the reaction rate for siloxane formation is not affected by its substituents and that the reaction is pseudo zero order in acid. The simulated mixture composition was analyzed at various reaction times. The relative distribution of D and A varies slightly with reaction time and rate, and ranges for the oligomer distribution are given. A mixture of mole ratio $r=D/A=4$ contains 70–76 mol % dimers and trimers without acceptor (DDD, DD) and 19–20 mole % dimers and trimers having one acceptor (AD, ADD, DAD). Only 30–33 mol % of DD and DDD oligomers are formed for a mixture of mole ratio $r=1$ and 32–33 mol % of dimers and trimers contain one acceptor.
- [22] Chromophore-labeled SAMs have recently been reported as fluorescence-based chemical sensors: a) M. Lösche, *Curr. Opin. Solid State Mater. Sci.* **1997**, 2, 546–556; b) R. Potyrailo, R. C. Conrad, A. D. Ellington, G. M. Hieftje, *Anal. Chem.* **1998**, 70, 3419–3425; c) X. Fang, X. Liu, S. Schuster, W. Tan, *J. Am. Chem. Soc.* **1999**, 121, 2921–2922; d) M. M. Sekar, P. D. Hampton, T. Buranda, G. P. Lopez, *J. Am. Chem. Soc.* **1999**, 121, 5135–5141; e) S. Flink, F. C. J. M. van Veggel, D. N. Reinhoudt, *Chem. Commun.* **1999**, 2229–2230.
- [23] M. Beggren, A. Dodabalapur, R. E. Slusher, Z. Bao, *Nature* **1997**, 389, 466–469.

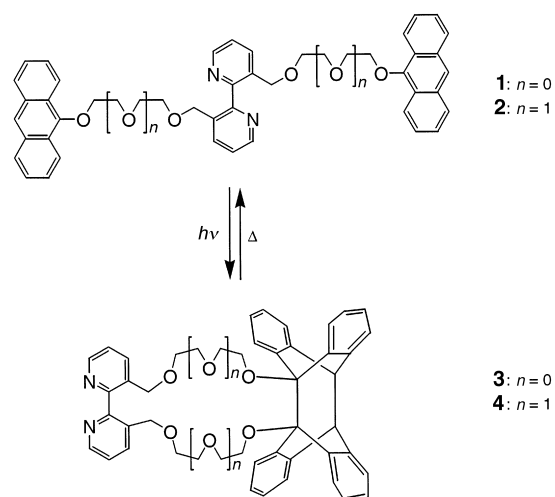
An Anthracene-Based Photochromic System That Responds to Two Chemical Inputs**

Gordon McSkimming, James H. R. Tucker,*
Henri Bouas-Laurent, and Jean-Pierre Desvergne*

Photochromic molecules, compounds that interconvert between one form and another upon the action of light,^[1] play an important role in approaches towards the develop-

ment of photo-active switches and logic devices that operate at the molecular level.^[2] There are now a number of examples of multi-functional photochromic systems that respond to a separate external stimulus, be it physical or chemical, in addition to a light stimulus.^[2c, f] One such example uses a spirobenzopyran unit linked to a crown ether that only exhibits thermally irreversible photochromism in the presence of metal cations.^[2f] We have previously reported multi-functional photochromic systems containing bis(anthracene) crown ether molecules that complex s-block cations in addition to undergoing reversible intramolecular photocyclization reactions.^[3] Other photochromic systems are based on the same photocycloaddition.^[4] Here we report photochemical studies on compounds **1**, **2**, and **5**, which are related to other ditopic ligands^[5] in that they contain a 2,2'-bipyridyl (bipy) unit for binding transition metals and two sidearms containing ethereal oxygen atoms for binding alkali metals.

Irradiation of acetonitrile solutions of **1** and **2** at 369 nm results in the formation of their $[4\pi+4\pi]$ photocycloadducts **3** and **4** (Scheme 1),^[6] as evidenced by the disappearance of the



Scheme 1. Schematic representation of the formation of crown ether photocycloadducts **3** and **4** from **1** and **2**, respectively.

¹L_a UV absorption band and by NMR spectroscopic studies (see Experimental Section). Data from studies carried out on **1** and **2** and their mercury(II) complexes^[5a] [Hg(**1**)Cl₂] and [Hg(**2**)Cl₂], in the absence and presence of excess NaClO₄, are displayed in Table 1. It is clear from the reaction quantum

Table 1. Intramolecular photocycloaddition quantum yields (Φ_R) for the formation of **3** and **4** from **1** and **2** (ca. 10^{-5} M), respectively, in the absence and in the presence of an excess of anhydrous NaClO₄ (ca. 10^{-2} M) in degassed MeCN (369 nm) at 298 K.

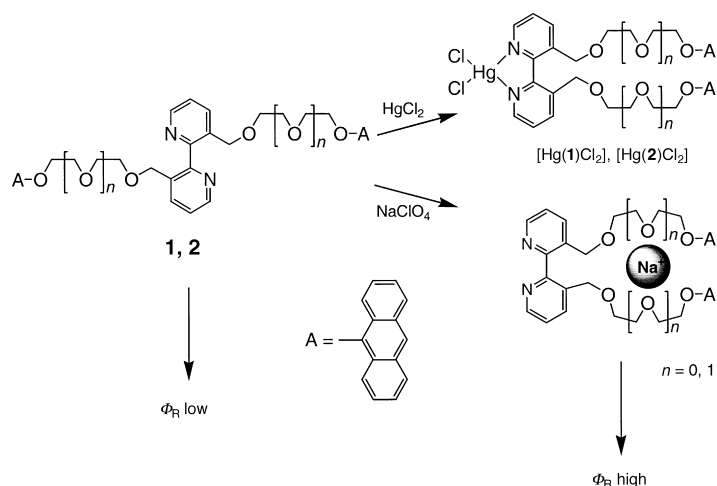
Ligand	IN ₁	IN ₂	Φ_R (369nm)	OUTPUT
1	–	–	0.19	low
1	Na ⁺	–	0.29	high
1	–	Hg ^{II}	0.26	high
1	Na ⁺	Hg ^{II}	0.32	high
2	–	–	0.23	low
2	Na ⁺	–	0.43	high
2	–	Hg ^{II}	0.37	high
2	Na ⁺	Hg ^{II}	0.43	high

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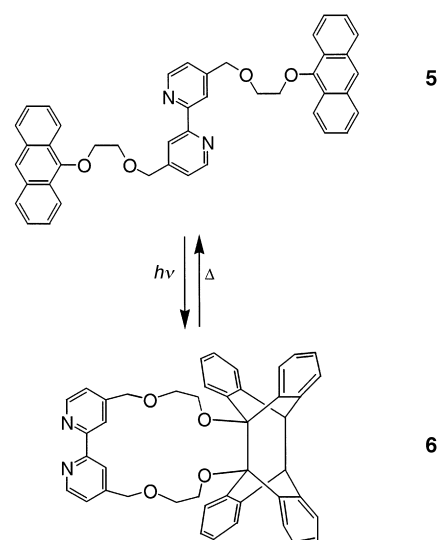
yields that the presence of either Hg^{II} or Na^+ induces a marked increase in photocyclization efficiency. Therefore, the transoid to cisoid conformational change in the bipy unit that is brought about by mercury complexation^[7] appears to preorganize the anthracene subunits for photocycloaddition (Scheme 2). Similarly, a weak interaction between Na^+ and the ether oxygen atoms would enable the two anthracene units to be kept in close proximity as the ring-closing process occurs.



Scheme 2. Schematic representations of the conformational changes in **1** and **2** brought about by metal interaction.

From a logic viewpoint^[2b-f] it may be argued that system **2** (and to some extent **1**) exhibits the OR logic operation if the two inputs are defined as chemical species (Na^+ and Hg^{II}) and the output is defined as the quantum yield of the photocycloaddition (Table 1).^[8] The output can be either low when no metal input is present, or high when either Na^+ , OR, Hg^{II} is bound to the receptors (Scheme 2). Quantum yields are also high when both Hg^{II} and Na^+ are present, as required for an OR gate. The values quoted here for the high output are amongst the largest known for the singlet-state cycloaddition of bis(anthracene) molecules.^[9] In contrast to **1** and **2**, the efficiency of the forward reaction to form the photoproduct **6** from **5** (Scheme 3)^[6] is extremely poor ($\Phi_R = 0.01$) and is unaffected by the presence of either Na^+ or Hg^{II} . Presumably the process is less favored because the *meta* substitution greatly enhances the strain in the crown ether photoproduct.

The rate of the thermal reverse reaction (k_{diss}) in acetonitrile for each system was measured in the presence and in the absence of Na^+ ions (Table 2). The complexation of HgCl_2 results in only a slight increase in the dissociation rate for systems **1** and **2**, presumably because binding at the distant



Scheme 3. Schematic representation of the formation of crown ether photocycloadduct **6** from **5**.

bipy unit results in no great change in the stability of each photoproduct. However, the rate of the thermal dissociation reaction is greatly reduced upon addition of Na^+ to the systems **1** and **2**. A similar effect has been observed in a related monotopic system^[3c] and is a result of the cation complexation within the crown ether cavity of the photoproduct greatly increasing its stability. This effect is most dramatic for system **2** where nearly a 100-fold decrease in the opening rate is observed upon the addition of Na^+ ions. The kinetic results for system **5** are consistent with the photoproduct being highly unstable for steric reasons.

To enable a comparison with the forward processes, the thermal reverse processes for **1** and **2** may also be described from a logic viewpoint if the output is defined as high when the kinetic stability of the photoproduct is high. It can be seen that in contrast to the forward processes, a high output only occurs if Na^+ ions are present; for a two input system, 4 out of the 16 possible logic functions depend on a single input only.^[2d]

To conclude, we have shown that the efficiency and the rate of interconversion between two photochromic states of a molecule can depend on a particular combination of two chemical inputs.

Experimental Section

1: A mixture of 3,3'-bis(hydroxymethyl)-2,2'-bipyridine^[5a] (0.5 g, 2.31 mmol), 9-(2-bromoethoxy)anthracene^[3a] (1.4 g, 4.62 mmol) and NaH (0.25 g, 10 mmol) in anhydrous THF (150 mL) was heated at reflux under nitrogen for 72 h. The reaction mixture was cooled and EtOH (15 mL) was added to decompose the excess NaH. The resulting solution was concentrated in vacuo, dissolved in CH_2Cl_2 (150 mL), and extracted

Table 2. Rate constants for the thermal dissociation reactions in degassed MeCN at 298 K.

System	$k_{\text{diss}} [10^{-6} \text{s}^{-1}]$	System	$k_{\text{diss}} [10^{-6} \text{s}^{-1}]$	System	$k_{\text{diss}} [10^{-6} \text{s}^{-1}]$
1	97	2	202	5	355
$[\text{Hg}(\text{1})\text{Cl}_2]$	104	$[\text{Hg}(\text{2})\text{Cl}_2]$	237	$[\text{Hg}(\text{5})\text{Cl}_2]$	490
1 + Na^+	29	2 + Na^+	2.9	5 + Na^+	370
$[\text{Hg}(\text{1})\text{Cl}_2]$ + Na^+	30	$[\text{Hg}(\text{2})\text{Cl}_2]$ + Na^+	2.4	$[\text{Hg}(\text{5})\text{Cl}_2]$ + Na^+	900

with H₂O (2 × 75 mL). The organic phase was dried (MgSO₄) and solvent was removed under reduced pressure. Purification by column chromatography (SiO₂, CH₂Cl₂, 2% MeOH) gave the desired product (0.41 g, 27%) as a pale green powder, m.p. 80 °C. ¹H NMR (400 MHz, CDCl₃): δ = 3.84 (t, 4H, OCH₂), 4.24 (t, 4H, OCH₂), 4.74 (s, 4H, CH₂), 7.36–7.41 (m, 10H, ArH), 7.95 (d, 4H, ArH), 8.17 (m, 4H, ArH), 8.28 (d, 4H, ArH), 8.63 (dd, 2H, ArH), FAB-MS calcd for C₄₄H₃₆N₂O₄: 657.2753 [M+H⁺]; found: 657.2781. Compounds **2** and **5** were prepared and characterized by similar methods.

Mercury complexes: The HgCl₂ complexes of **1**, **2**, and **5** were prepared by a slight adaptation of a literature method.^[5a] Complexation at the bipy unit was evidenced by shifts of the resonances corresponding to the bipy protons in the NMR spectra in CDCl₃ and CD₃CN.^[5a]

Photochemical studies: Photocycloaddition reactions were carried out using a Xenon lamp (see Table 1 for conditions). The ¹H NMR spectra of **3** and **4** in CD₃CN revealed no ethylenic proton resonances, and in the case of **3** a clear singlet peak at 4.51 ppm (2H) was evident, which indicated the formation of the 9,9'–10,10' photoproducts. The quantum yields of fluorescence of **1** and **2** in MeCN were largely unaffected by the presence of Group 1 cations and Hg^{II}, with only Na⁺ ions inducing any enhancement when bound to ligand **2** and its HgCl₂ complex (**1**, Φ_F = 0.04, **1**+Na⁺, Φ_F = 0.03, **2**, Φ_F = 0.05, **2**+Na⁺, Φ_F = 0.10).

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Evidence for Cooperativity in the Disproportionation of H₂O₂ Efficiently Catalyzed by a Tetranuclear Manganese Complex**

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Dedicated to Professor Stephen J. Lippard on the occasion of his 60th birthday

Intracellular hydrogen peroxide produced during O₂ metabolism in cells is an indiscriminate oxidizer of cellular components and its toxicity has been associated with oxidative stress,^[1] cancer,^[2] and aging.^[3,4] Disproportionation of intracellular hydrogen peroxide [Eq. (1)] by catalase provides a vital biological defense against this toxic oxygen metabolite.^[5]



In addition to intracellular sources, the photochemical generation of hydrogen peroxide is well established.^[6] Furthermore, in light of the possible health risks associated with halogenated byproducts produced by chlorination,^[7,8] the industrial consumption of hydrogen peroxide is expected to increase as it replaces chlorine as a bleaching and sterilizing agent.^[9] It has been proposed that the residual hydrogen peroxide used in industrial processes can be decomposed before disposal through the application of transition metal complexes or catalases.^[10]

Due to the toxicity of hydrogen peroxide and its pervasiveness in biological and industrial settings, the structure and function of catalases and catalase mimics is of considerable interest. While the majority of catalases contain a heme cofactor,^[11] a number of nonheme catalases require manganese as a cofactor.^[11–13] The synthesis, characterization, and reactivity studies of polynuclear manganese model systems have contributed significantly to our understanding of the structure and function of manganese catalases.^[14–27] We have an ongoing effort in the synthesis and isolation of well defined, oxo-bridged multinuclear manganese species and we report herein that the adamantane-shaped mixed-valence

- [1] a) *Photochromism—Molecules and Systems* (Eds.: H. Dürr, H. Bouas-Laurent), Elsevier, Amsterdam, **1990**; b) *Organic Photochromic and Thermochromic Compounds, Vol. 2* (Eds.: J. C. Crano, R. J. Gugliemetti), Kluwer Academic/Plenum, New York, **1999**.
- [2] a) J. M. Lehn, *Supramolecular Chemistry—Concepts and Perspectives*, VCH, Weinheim, **1995**; b) A. P. de Silva, I. M. Dixon, H. Q. N. Gunaratne, T. Gunnlaugsson, P. R. S. Maxwell, T. E. Rice, *J. Am. Chem. Soc.* **1999**, *121*, 1393, and references therein; c) M. Asakawa, P. R. Ashton, V. Balzani, A. Credi, G. Mattersteig, O. A. Matthews, M. Montalti, N. Spencer, J. F. Stoddart, M. Venturi, *Chem. Eur. J.* **1997**, *3*, 1992, and references therein; d) T. Gunnlaugsson, D. A. Mac Dónail, D. Parker, *Chem. Commun.* **2000**, 93, and references therein; e) F. Pina, M. Maestri, V. Balzani, *Chem. Commun.* **1999**, 107, and references therein; f) M. Inouye, K. Akamatsu, H. Nakazumi, *J. Am. Chem. Soc.* **1997**, *119*, 9160.
- [3] a) J. H. R. Tucker, H. Bouas-Laurent, P. Marsau, S. W. Riley, J. P. Desvergne, *Chem. Commun.* **1997**, 1165; b) D. Marquis, J. P. Desvergne, H. Bouas-Laurent, *J. Org. Chem.* **1995**, *60*, 7984; c) J. P. Desvergne, H. Bouas-Laurent, *J. Chem. Soc. Chem. Commun.* **1978**, 403.
- [4] a) G. Deng, T. Sakaki, K. Nakashima, S. Shinkai, *Chem. Lett.* **1992**, 1287; b) A. Beyeler, P. Belser, L. De Cola, *Angew. Chem.* **1997**, *109*, 2878; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2779.
- [5] a) J. Rebek Jr., J. E. Trend, R. V. Wattle, S. Chakravorti, *J. Am. Chem. Soc.* **1979**, *101*, 4333; b) P. D. Beer, A. S. Rothin, *J. Chem. Soc. Chem. Commun.* **1988**, 52.
- [6] For clarity, the photoproducts are depicted in a planar cisoid conformation.
- [7] K. T. Potts, K. A. Gheyson Raiford, M. Keshavarz-K, *J. Am. Chem. Soc.* **1993**, *115*, 2793.
- [8] For examples of photochromic logic devices in which one input is light and the output is absorbance or fluorescence, see references [2c] and [2f].
- [9] J. P. Desvergne, M. Gotta, J. C. Soullignac, H. Bouas-Laurent, *Tetrahedron Lett.* **1995**, *36*, 1259.

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Mn^{III}(Mn^{IV})₃ complex [Mn₄O₆(bpea)₄]³⁺ [28] (**1**; bpea = *N,N*-bis(2-pyridylmethyl)ethylamine) is an extremely efficient H₂O₂ disproportionation catalyst in the presence of the base Me₃(tacn) (Me₃(tacn) = 1,4,7-trimethyl-1,4,7-triazacyclononane; aqueous p*K*_a = 11.7), with the highest catalytic constant, *k*_{cat}, reported to date for any synthetic manganese complex. A base-mediated switch from Michaelis–Menten kinetics to cooperative kinetics also distinguishes the oxygen evolution promoted by **1**. The data presented herein for complex **1** reveal a novel biomimetic catalytic system exhibiting cooperative behavior.^[29–35]

The kinetics of oxygen evolution from the disproportionation of H₂O₂ was studied by a video recording the displacement of mineral oil in a gas buret. Treatment of an acetonitrile solution of **1**·3 ClO₄^[28] (bulk electrolysis, 0.228 mM)^[36, 37] with an excess of aqueous H₂O₂ (100 mM) immediately evolved O₂. The total volume of O₂ evolved (2.37 mL, 97.7 μmol) was consistent with disproportionation of H₂O₂ at an initial rate *v*₀ = −d[H₂O₂]/dt = 0.57 mM s^{−1}. Isotope-labeling studies (H₂¹⁸O₂^[38]) monitored by GC/MS showed peaks at *m/z* 32 and 36 in a 7:93 ratio and no *m/z* 34 (O₂) was observed. Together, these results indicate that both oxygen atoms of evolved O₂ originate from the same H₂O₂ molecule.

Initial rate data determined for a range of H₂O₂ concentrations (0.010–0.33 M) and **1**·3 ClO₄ (method B,^[36] Me₃(tacn) = 3.2 × 10^{−5}–3.2 × 10^{−3} M) show, at low concentrations of H₂O₂, that the rate of disproportionation is first order in both tetranuclear complex and H₂O₂ concentration; rate = *k*[**1**][H₂O₂], with an average rate constant *k* of 114 ± 14 M^{−1} s^{−1}. Complex **1** exhibits saturation kinetics (Figure 1).^[39]

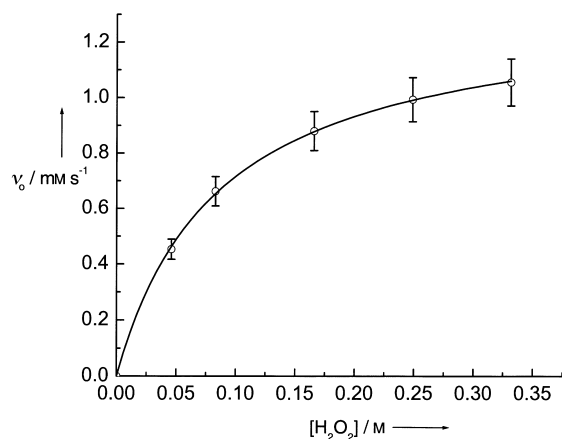


Figure 1. H₂O₂ saturation kinetics for **1**·3 ClO₄ (0.105 mM, Me₃(tacn) reduction) showing a fit of the Michaelis–Menten equation with *K*_m = 0.087 M and *V*_{max} = 1.34 ± 0.11 mM s^{−1}.

These data can be described by the Michaelis–Menten equation [Eq. (2)], where *S* is the substrate concentration, *K*_m is the substrate binding constant, and *V*_{max} is the maximum rate of the reaction.

$$\frac{v_0}{V_{\max}} = \frac{S}{K_m + S} \quad (2)$$

Nonlinear least-squares fitting of the data in Figure 1 to Equation (2) gave *K*_m = 0.087 M and *V*_{max} = 1.34 ± 0.11 mM s^{−1}

with a value of *k*_{cat} = 11.1 ± 0.9 s^{−1}.^[40] For comparison, kinetic measurements of the manganese catalase from *Lactobacillus plantarum*, modeled using Michaelis–Menten kinetics, gave *K*_m = 0.35 M and *k*_{cat} = 2.0 × 10⁵ s^{−1}.^[11]

In contrast to the activity of **1**, solutions of the (Mn^{IV})₄ complex **2**·4 ClO₄ were only slightly active. Addition of hydrogen peroxide (0.234 M) to an acetonitrile solution of **2**·4 ClO₄ (0.265 mM) exhibited an initial rate *v*₀ = 1.6 μM s^{−1}, in contrast to *v*₀ = 1.15 mM s^{−1} for **1** (Me₃(tacn) reduction)^[36] under the same conditions. On the other hand, we observed a substantially faster rate (83.7 mM s^{−1}) for **1**·3 ClO₄ prepared from **2**·4 ClO₄ using a large excess (4.8 equivalents) of Me₃(tacn).^[36] A number of manganese–oxo complexes were previously shown to exhibit significant rate enhancement for the disproportionation of hydrogen peroxide in the presence of exogenous base.^[17, 18, 26, 27, 41–44] It was proposed that at least a part of this effect was the base-facilitated deprotonation of H₂O₂ in the course of the coordination of hydroperoxide to the metal complex.

Substrate conversion rates were determined for several concentrations of **1**·3 ClO₄ and Me₃(tacn) and these results are shown in Figure 2. We propose that the sigmoidal shape of the O₂ evolution rate versus the number of base equivalents of

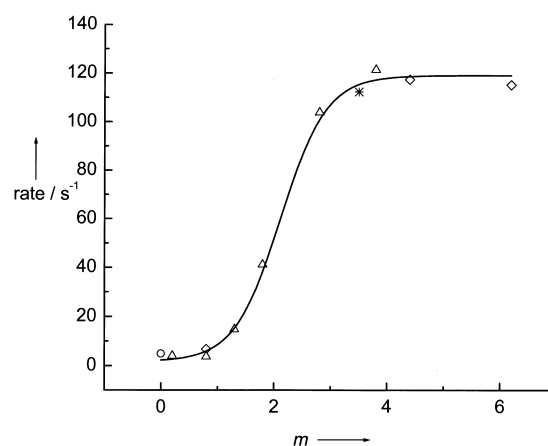


Figure 2. A composite of substrate conversion rate versus the number of base equivalents (*m*) of Me₃(tacn).^[45] Experimental conditions: 100 mM H₂O₂ and **1**·3 ClO₄ [○: **1** = 0.228 mM, bulk electrolysis; *: **1** = 0.096 mM, Me₃(tacn) reduction; ◇: **1** = 0.105 mM, Me₃(tacn) reduction; △: **1** = 0.245 mM, Me₃(tacn) reduction]. The curve is a nonlinear least-squares fit of a Boltzmann function to all of the data.

Me₃(tacn)^[45] is consistent with a base-activated switch in the mechanism of hydrogen peroxide disproportionation. Additional experimental evidence of such a switch is revealed by the saturation kinetics of **1** in the presence of excess base (Figure 3).^[39] These data reveal a dramatic deviation from the Michaelis–Menten kinetics observed for **1** with little or no excess base (Figure 1). The sigmoidal shape of the substrate conversion profile at this higher base concentration is indicative of cooperativity in the disproportionation reaction (vide infra).^[46–48] These data can be described with the Hill equation [Eq. (3)], where *n* is the Hill coefficient.^[49]

$$\frac{v_0}{V_{\max}} = \frac{S^n}{K_m^n + S^n} \quad (3)$$

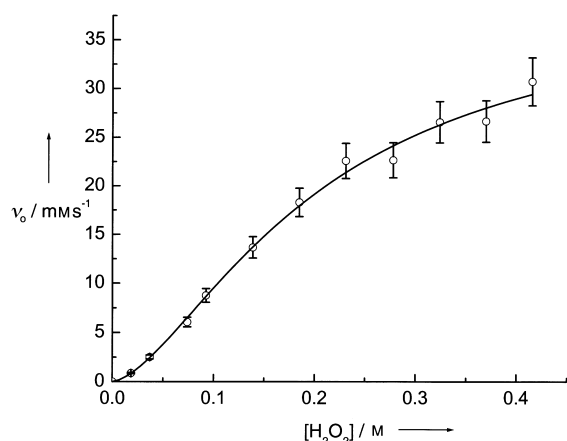
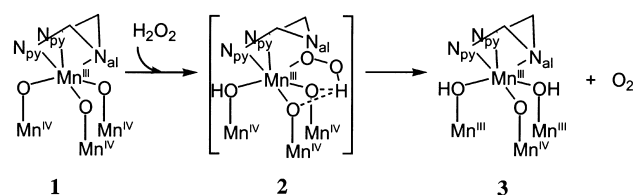


Figure 3. H_2O_2 saturation kinetics for **1**· 3ClO_4 (0.096 mM, $\text{Me}_3(\text{tacn})$ reduction, 4.2 equiv) showing the fit of the Hill equation [Eq. (3)].

A least-squares fit of the data in Figure 3 with Equation (3) gave $V_{\text{max}} = 38.9 \pm 3.1 \text{ mM s}^{-1}$, $K_m = 0.204 \text{ M}$, and $n = 1.58$, with a value of $k_{\text{cat}} = 405 \text{ s}^{-1}$. It is noteworthy that the crossover from an efficient, yet unremarkable, catalytic system exhibiting Michaelis–Menten kinetics to an extremely efficient one exhibiting cooperative kinetics renders a catalyst which has the highest k_{cat} reported for any synthetic manganese complex to date.^[14–22] Substitution of $n = 1$ in Equation (3) reduces the Hill equation to the Michaelis–Menten equation [Eq. (2)] and values of $n > 1$ indicates the extent of cooperativity.^[46–48] By comparison, data for hydrogen peroxide disproportionation with only a slight excess $\text{Me}_3(\text{tacn})$ (Figure 1) which were fit with the Michaelis–Menten equation fits equally well with the Hill equation with $n = 1.03$.

We propose that the observed cooperativity in the disproportionation of hydrogen peroxide by **1** at the higher base concentrations originates in cooperative binding or cooperative kinetics. A plausible initial reaction of **1** with H_2O_2 and one that is consistent with our previous studies (vide infra) is illustrated in Scheme 1.^[50] Reaction of **1** with H_2O_2 most likely



Scheme 1. Initial reaction of **1** with H_2O_2 . For the polydentate, Mn^{III} -coordinated bpea ligand, N_{py} and N_{al} represent the nitrogen atoms of the pyridyl and ethylamine moieties, respectively.

occurs via coordination of hydroperoxide to the more labile Mn^{III} site. The enhanced lability of **1** at the Mn^{III} site arises from elongation along the $\text{Mn}^{\text{III}}\text{--N}_{\text{al}}\text{--O}_{\text{oxo}}$ axis due to Jahn–Teller distortion of the d_5 Mn^{III} center.^[28] Lability of the amine nitrogen atom and flexibility of the bpea ligand at the Mn^{III} site of the dimeric complex $[\text{Mn}_2\text{O}_2(\text{OAc})(\text{bpea})_2](\text{ClO}_4)_2$ (**3**) has previously been proposed as a basis for its reactivity

with water or the hydroxide ion.^[51] Similar distortion of the Mn^{III} site of **1** could facilitate attack by H_2O_2 .

The oxo bridges of **1** are nucleophilic, as demonstrated by the enhanced oxo-bridge basicity of **1** by approximately 17 pK_a units following one-electron reduction of **2** and by the observation that **2** readily undergoes coupled e^-/H^+ and $e^-/2\text{H}^+$ processes.^[28] Hence, the reaction of **1** with H_2O_2 could lead to oxidation to yield O_2 and a $(\text{Mn}^{\text{III}})_3(\text{Mn}^{\text{IV}})$ tetramer (**3**; Scheme 1) in a net $2e^-/2\text{H}^+$ process. The $(\text{Mn}^{\text{III}})_3(\text{Mn}^{\text{IV}})$ tetramer could subsequently bind a second equivalent of hydroperoxide followed by reduction of H_2O_2 and restoration of **1** (Scheme 1).

The rate of H_2O_2 disproportionation in our studies was significantly affected by addition of the strong base $\text{Me}_3(\text{tacn})$. In control experiments, however, we observed that addition of $\text{Me}_3(\text{tacn})$ to H_2O_2 in acetonitrile in the absence of **1** resulted in only a very slow disproportionation of H_2O_2 . Slow base-catalyzed disproportionation of H_2O_2 in the absence of a transition metal complex is similar to that reported by others.^[52, 53] It is possible that a part of the effect of $\text{Me}_3(\text{tacn})$ in the present system could be base-facilitated deprotonation of H_2O_2 , similar to the effect noted above for other catalytic manganese systems. The striking difference in our system, however, is the sigmoidal behavior of rate with increasing base concentration, which leads to catalytic cooperativity.

The catalytic cooperativity observed in the present system could be due to either cooperative binding of hydroperoxide or cooperative kinetics of disproportionation. A two-electron reduction of the $[\text{Mn}_4\text{O}_6]^{3+}$ core of **1** leads to two additional Mn^{III} ions (**3**; Scheme 1). Reduction of Mn^{IV} to Mn^{III} leads to elongation of both the $\text{Mn}\text{--N}$ bonds^[54] as well as along the Jahn–Teller axis. In the absence of excess base, the proposed final step in H_2O_2 disproportionation involves H_2O_2 reduction and oxidation of the core to **1**. This step must be accompanied by contraction of the $\text{Mn}\text{--N}$ bonds as well as the Jahn–Teller axis. We propose that the higher $\text{Me}_3(\text{tacn})$ concentration could favor binding of two hydroperoxide ions in the $(\text{Mn}^{\text{III}})_3(\text{Mn}^{\text{IV}})$ oxidation states and the possibility of a subsequent concerted reduction and oxidation of hydrogen peroxide. In this proposal, the kinetically cooperative disproportionation cycle maintains the $(\text{Mn}^{\text{III}})_3(\text{Mn}^{\text{IV}})$ oxidation state throughout and thus avoids the energetic hurdles associated with expansion and contraction of the $[\text{Mn}_4\text{O}_6]$ core.

In conclusion, we have shown that one-electron reduction of the tetranuclear complex **2**, which exhibits very little reactivity toward H_2O_2 , leads to the more catalytically active complex **1**. The sigmoidal dependence of the disproportionation rate for **1** on $\text{Me}_3(\text{tacn})$ concentration is consistent with a base-mediated mechanism such as the one proposed. Addition of the $\text{Me}_3(\text{tacn})$ promotes crossover from a system exhibiting Michaelis–Menten kinetics to a catalytically cooperative kinetic system, to render a greatly more efficient catalyst. This proposal also incorporates the principal feature that we feel makes **1** catalytically active while **2** is relatively inert: a labile Mn^{III} site for the initial binding of hydroperoxo anion.

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- [1] H. Sies in *Oxidative Stress, Oxidants, and Antioxidants* (Ed.: H. Sies), Academic Press, London, **1991**, pp. xv–xxii.
- [2] B. Halliwell, J. M. C. Gutteridge in *Methods in Enzymology, Vol. 186* (Eds.: L. Packer, A. N. Glazer), Academic Press, San Diego, **1990**, pp. 1–85.
- [3] S. J. Stohs, *J. Basic Clin. Physiol. Pharmacol.* **1995**, *6*, 205–228.
- [4] V. Usmar-Darley, B. Halliwell, *Pharm. Res.* **1996**, *13*, 649–662.
- [5] W. F. Beyer, Jr., I. Fridovich in *Basic Life Sciences, Vol. 49* (Ed.: M. G. Simic), Plenum, New York, **1988**, pp. 651–661.
- [6] W. J. Cooper, E. S. Saltzman, R. G. Zika, *J. Geophys. Res.* **1987**, *92*, 2970–2980.
- [7] G. A. Boorman, V. Dellarco, J. K. Dunnick, R. E. Chapin, S. Hunter, F. Hauchman, H. Gardner, M. Cox, R. C. Sills, *Environ. Health Perspect.* **1999**, *107*, 207–217.
- [8] Chlorination agents include elemental chlorine, sodium hypochlorite, sodium chlorate, and sodium chlorite.
- [9] M. McCoy, *Chem. Eng. News* **1999**, *77*, 18–19.
- [10] P. Dhaese, *Chem. Today* **1996**, Jan./Feb., 19–21.
- [11] J. E. Penner-Hahn in *Manganese Redox Enzymes* (Ed.: V. L. Pecoraro), VCH, New York, **1992**, pp. 29–45.
- [12] J. E. Penner-Hahn in *Comprehensive Biological Catalysis, Vol. III* (Ed.: M. Sinnott), Academic Press, San Diego, **1998**, pp. 439–459.
- [13] G. C. Dismukes, *Chem. Rev.* **1996**, *96*, 2909–2926.
- [14] P. J. Pessiki, G. C. Dismukes, *J. Am. Chem. Soc.* **1994**, *116*, 898–903.
- [15] A. Gelasco, V. L. Pecoraro, *J. Am. Chem. Soc.* **1993**, *115*, 7928–7929.
- [16] T. Tanase, S. J. Lippard, *Inorg. Chem.* **1995**, *34*, 4682–4690.
- [17] M.-a. Sasayama, Y. Naruta, *Chem. Lett.* **1995**, 63–64.
- [18] Y. Naruta, K. Maruyama, *J. Am. Chem. Soc.* **1991**, *113*, 3595–3596.
- [19] M. Delroisse, A. Rabion, F. Chardac, D. Tétard, J.-B. Verlhac, L. Fraisse, J.-L. Séris, *J. Chem. Soc. Chem. Commun.* **1995**, 949–950.
- [20] P. Mathur, M. Crowder, G. C. Dismukes, *J. Am. Chem. Soc.* **1987**, *109*, 5227–5233.
- [21] R. T. Stibrany, S. M. Gorun, *Angew. Chem.* **1990**, *102*, 1195–1197; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1156–1158.
- [22] A. Gelasco, A. Askenas, V. L. Pecoraro, *Inorg. Chem.* **1996**, *35*, 1419–1420.
- [23] Y. Naruta, M.-a. Sasayama, K. Ichihara, *J. Mol. Catal. A* **1997**, *117*, 115–121.
- [24] T. C. Brunold, D. R. Gamelin, T. L. Stemmler, S. K. Mandal, W. H. Armstrong, J. E. Penner-Hahn, E. I. Solomon, *J. Am. Chem. Soc.* **1998**, *120*, 8724–8738.
- [25] A. Gelasco, S. Bensiek, V. L. Pecoraro, *Inorg. Chem.* **1998**, *37*, 3301–3309.
- [26] D. M. Hong, H. H. Wei, Y. Wang, *J. Chin. Chem. Soc.* **1998**, *45*, 701–706.
- [27] N. N. Gerasimchuk, A. Gerges, T. Clifford, A. Danby, K. Bowman-James, *Inorg. Chem.* **1999**, *38*, 5633–5636.
- [28] C. E. Dubé, D. W. Wright, S. Pal, P. J. Bonitatebus, Jr., W. H. Armstrong, *J. Am. Chem. Soc.* **1998**, *120*, 3704–3716.
- [29] K. D. Karlin, *Science* **1993**, *261*, 701–708.
- [30] D. H. Vance, A. W. Czarnik, *J. Am. Chem. Soc.* **1993**, *115*, 12165–12166.
- [31] D. Wahnnon, A.-M. Lebus, J. Chin, *Angew. Chem.* **1995**, *107*, 2594–2597; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2412–2414.
- [32] W. H. Chapman, Jr., R. Breslow, *J. Am. Chem. Soc.* **1995**, *117*, 5462–5469.
- [33] T. Koike, M. Inoue, E. Kimura, M. Shiro, *J. Am. Chem. Soc.* **1996**, *118*, 3091–3099.
- [34] K. G. Ragunathan, H.-J. Schneider, *Angew. Chem.* **1996**, *108*, 1314–1316; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1219–1221.
- [35] J. Suh, S. H. Hong, *J. Am. Chem. Soc.* **1998**, *120*, 12545–12552.
- [36] Complexes **1**·3ClO₄ and **2**·4ClO₄ were prepared as previously described.^[28] The Mn^{III}(Mn^{IV})₃ complex **1**·3ClO₄ was prepared by one-electron reduction of the (Mn^{IV})₃ complex **2**·4ClO₄ by either its bulk electrolysis in acetonitrile (method A) or by its reaction with 1.2 equiv of Me₃(tacn) (method B).^[28]
- [37] All reactions were run in 2 mL solutions of CH₃CN/H₂O at 22 °C. All chemicals were of reagent-grade quality and used as received. Aqueous H₂O₂ stock solution (50% w/w, Aldrich) was standardized with KMnO₄ and used directly or as diluted with acetonitrile.
- [38] 2% H₂¹⁸O₂ (91 ± 1% ¹⁸O, 9 ± 1% ¹⁶O) was purchased from ICON, Inc.
- [39] The error bars in Figures 1 and 3 are based on an estimated initial rate uncertainty of 8%.
- [40] The catalytic constant, or turnover number, is defined as $k_{\text{cat}} = V_{\text{max}}/[\text{cat}]$.
- [41] P. N. Balasubramanian, E. S. Schmidt, T. C. Bruice, *J. Am. Chem. Soc.* **1987**, *109*, 7865–7873.
- [42] E. J. Larson, V. L. Pecoraro, *J. Am. Chem. Soc.* **1991**, *113*, 3810–3818.
- [43] Y. Naruta, M.-a. Sasayama, *Chem. Lett.* **1994**, 2411–2414.
- [44] M. Kimura, T. Nishigaki, T. Koyama, K. Hanabusa, H. Shirai, *Macromol. Chem. Phys.* **1994**, *195*, 3499–3508.
- [45] One equivalent of Me₃(tacn) is required to reduce **2**·4ClO₄ to **1**·3ClO₄ (ref. ^[28]), hence the effective number of base equivalents of Me₃(tacn) (*m*) is one less than the total number of equivalents added to the reaction mixture.
- [46] K. E. Neet, *Methods Enzymol.* **1995**, *249*, 519–567.
- [47] K. E. Neet, *Methods Enzymol.* **1980**, *64*, 139–192.
- [48] E. Zeffren, P. L. Hall, *The Study of Enzyme Mechanisms*, Wiley, New York, **1973**.
- [49] A. V. Hill, *J. Physiol.* **1910**, *40*, iv–vii.
- [50] We cannot rule out an alternative Scheme in which a N_{pyr}, rather than N_{al}, dissociates at the Mn^{III} site.
- [51] S. Pal, M. M. Olmstead, W. H. Armstrong, *Inorg. Chem.* **1995**, *34*, 4708–4715.
- [52] J. L. Roberts, Jr., H. Sugimoto, W. C. Barrette, D. T. Sawyer, *J. Am. Chem. Soc.* **1985**, *107*, 4556–4557.
- [53] G. Rábai, *J. Chem. Soc. Chem. Commun.* **1991**, 1083–1084.
- [54] H. H. Thorp, G. W. Brudvig, *New J. Chem.* **1991**, *15*, 479–490.

A Practical Asymmetric Synthesis of Enantiomerically Pure 3-Substituted Pyroglutamic Acids and Related Compounds**

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In recent years, tailor-made χ -constrained amino acids have emerged as critically important key structural units in the de novo design of peptide-based drugs with enhanced receptor selectivity and stability to metabolic degradation.^[1, 2] Incorporation of these amino acids in strategic positions of a peptide chain allows rational design of the three-dimensional topographical structure of the peptide and, thus, opens a new avenue for exploration of the fundamental chemical–physical basis for peptide-mediated biological information transfer.^[2] In particular, to explore the topographical requirements of the recently discovered human melanocortin receptors,^[3] we need a series of hitherto unknown conformationally con-

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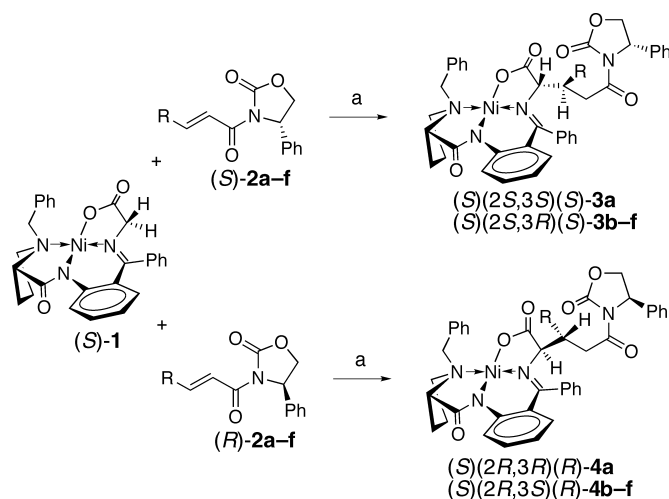
strained glutamic acid and proline derivatives. Since pyroglutamic acids (PGAs) can be readily transformed into glutamic acids, glutamines, and prolines, we reasoned that by developing a convenient asymmetric method for preparation of the corresponding PGAs, we would have access to the whole family of these extraordinarily valuable compounds.^[4]

For construction of the carbon skeleton of C-substituted glutamic/pyroglutamic acids, Michael addition reactions between chiral equivalents of a nucleophilic glycine and the correspondingly substituted acrylic acid derivatives represent the most methodologically straightforward solution. These types of reactions have been extensively studied by many groups using various chiral equivalents of nucleophilic glycine.^[5, 6] With the exception of a few cases which feature diastereoselectivity of over 98 % *de* in the addition reactions,^[7] synthetic efficiency of the reported methods is compromised by the lack of generality and poor stereochemical outcome. Accordingly, our ultimate objectives were to develop a synthetic protocol for asymmetric Michael addition reactions which fulfilled all requirements for efficacy, including complete chemical (> 98 % yield) and stereochemical (> 98 % *ee*) control, and broad substrate generality. In this communication we report a highly successful solution to the aforementioned synthetic objectives.

Recently we have discovered that addition reactions between *N*-(*E*-enoyl)oxazolidin-2-ones and nickel(II)-complexes of Schiff bases of glycine with *o*-[*N*- α -pycolylamine]-benzophenone or -acetophenone readily occurred in DMF at room temperature, in the presence of 10–15 mol % DBU (1,8-diazabicyclo[5.4.0]undec-7-ene); the corresponding products were afforded in quantitative yield and with virtually complete diastereoselectivity.^[8] Our first attempt to realize the asymmetric version by employing a nickel(II) complex of the chiral Schiff base of glycine with (*S*)-*o*-[*N*-(*N*-benzylpropyl)amino]benzophenone **1** for reaction with the *N*-(*E*-enoyl)oxazolidin-2-ones, though successful, did not give the desired result.^[9] Specifically, the problem we met was poor *si/re* face stereocontrol of the complex (*S*)-**1** derived enolate, while the face selectivity of the *N*-(*E*-enoyl)oxazolidin-2-ones was perfect, giving rise only to the products of *like*^[10] relative topology.

Reasoning how to improve the stereochemical outcome, we decided to try the additions of complex (*S*)-**1** with chiral 4-substituted *N*-(*E*-enoyl)oxazolidin-2-ones (**2**, Scheme 1). Assuming that the reactions between the chiral equivalent of glycine and the Michael acceptors might have two distinct cases (matched and mismatched stereochemistry), we anticipated that the matched case would afford the target addition products with the desired stereochemical outcome. Regarding the chiral oxazolidin-2-ones (**2**), we decided to use the derivatives developed in our group, that is the 4-phenyl-oxazolidin-2-ones,^[11] as they have been shown to be superior in controlling the stereochemistry of the conjugate addition reactions^[12] compared to the more commonly used 4-benzyl and 4-isopropyl analogues.

First we investigated the addition between complex (*S*)-**1** and (4*S*)-*N*-crotonyl-4-phenyloxazolidin-2-one (**2a**, Scheme 1). To our satisfaction, the reaction, conducted in DMF at ambient temperature (22 °C) in the presence of



Scheme 1. a) DMF, DBU (15 mol %). R = Me (**a**), iPr (**b**), Ph (**c**), 2-naphthyl (**d**), 4-MeO-C₆H₄ (**e**), or 4-CF₃-C₆H₄ (**f**). DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

15 mol % DBU, was complete in 5 min to afford **3a** as the only product in quantitative yield (Table 1, entry 1). The absolute stereochemistry of the glutamic acid residue in **3a** was found to be (2*S*,3*S*),^[13] in agreement with the previously established diastereoselectivity in the reactions between the achiral

Table 1. Addition reactions of (*S*)-**1** with (*S*)- or (*R*)-**2a-f**.^[a]

Entry	Compound	Time	Yield [%] ^[b]	Product ^[c]
1	(<i>S</i>)- 2a	5 min	99	(2 <i>S</i> ,3 <i>S</i>)- 3a
2	(<i>R</i>)- 2a	10 min	97	(2 <i>R</i> ,3 <i>R</i>)- 4a
3	(<i>S</i>)- 2b	30 min	97	(2 <i>S</i> ,3 <i>R</i>)- 3b
4	(<i>R</i>)- 2b	4 hrs	67 ^[d]	(2 <i>R</i> ,3 <i>S</i>)- 4b
5	(<i>S</i>)- 2c	8 min	97	(2 <i>S</i> ,3 <i>S</i>)- 3c
6	(<i>R</i>)- 2c	30 min	97	(2 <i>R</i> ,3 <i>S</i>)- 4c
7	(<i>R</i>)- 2d	2 hrs	97	(2 <i>R</i> ,3 <i>S</i>)- 4d
8	(<i>S</i>)- 2e	5 min	97	(2 <i>S</i> ,3 <i>R</i>)- 3e
9	(<i>S</i>)- 2f	30 min	97	(2 <i>S</i> ,3 <i>R</i>)- 3f

[a] All reactions were performed in DMF in the presence of DBU (15 mol %) at ambient temperatures. Ratio of (*S*)-**1**:(4*S*)- or (4*R*)-**2** was 1:1.05–1.1. [b] Yield of diastereo- and enantiomerically pure products. [c] In all cases only one stereochemical product (*de* > 98 %) was detected by NMR analysis of the crude reaction mixtures. The absolute configuration of the products was determined on the basis of their optical properties, as well as by comparison of the optical rotations of the corresponding pyroglutamic acids later formed from the products with the literature data. [d] Incomplete (70 %) conversion of the starting materials.

substrates.^[8] Assuming that the reaction of (*S*)-**1** and (*S*)-**2a** represents the case of matching stereochemical requirements of the chiral auxiliaries, we expected that the addition between (*S*)-**1** and (*R*)-**2a** might be the mismatch case, and, thus, less stereoselective. To our surprise, under the same reaction conditions, the addition of complex (*S*)-**1** with (*R*)-**2a** occurred with a similar reaction rate giving rise to **4a** as the sole product in quantitative yield (entry 2). Determination of the stereochemistry of compound **4a** gave a totally unexpected result. The absolute configuration of the two newly created stereogenic centers in **4a** was found to be 2*R*,3*R*. This stereochemical outcome was not anticipated based on previous knowledge of the stereocontrolling properties of com-

plex (*S*)-**1**, which showed strong preference for the α -*S* absolute configuration in alkylation, Michael, and aldol addition products.^[14, 15]

Whatever the origin of this unexpected stereochemical outcome, we next decided to study the generality of these almost diastereoselective addition reactions. The addition of complex (*S*)-**1** with oxazolidin-2-one (*S*)-**2b**, bearing a bulky isopropyl group, occurred relatively easily (30 min) giving (2*S*,3*R*)-**3b** as the sole product, which was obtained in quantitative yield (entry 3). In contrast, the reaction of (*S*)-**1** with the 4*R*-configured enantiomer **2b**, proceeded at a substantially slower rate to give (2*R*,3*S*)-**4b** as the only diastereomeric product (entry 4). These results suggest that the reaction between complex (*S*)-**1** and (4*S*)-**2b** represents a case of matched stereochemical preferences of the chiral starting compounds, while the addition of (*S*)-**1** with (4*R*)-**2b** is a mismatched case. In contrast to our expectations, both cases featured virtually complete diastereoselectivity. The only difference was the reaction rate, a difference which was more pronounced in the additions of the sterically demanding isopropyl derivatives (4*R*)- and (4*S*)-**2b**.

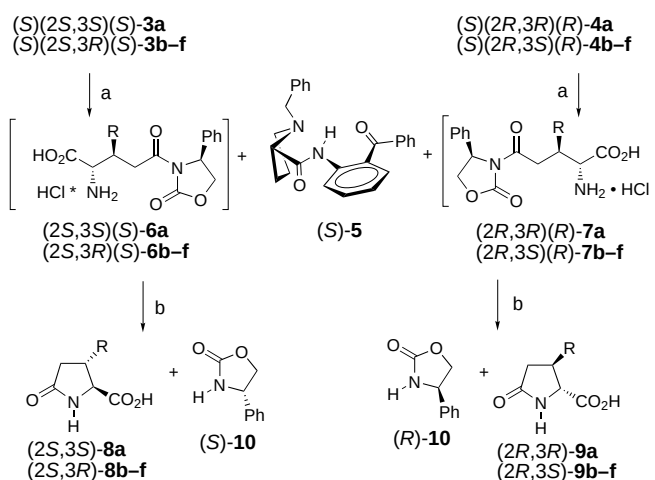
Similar patterns of reactivity and stereochemical outcome were observed in the reactions between complex (*S*)-**1** with cinnamic acid derived (4*S*)- and (4*R*)-oxazolidin-2-ones **2c**. Thus, the reactions afforded as the sole products (2*S*,3*R*)-**3c** and (2*R*,3*S*)-**4c**, respectively, in quantitative chemical yields (Table 1, entries 5 and 6). The reaction rate of the former was substantially higher than that of the latter. Considering the difference in the stereochemical requirements for methyl and phenyl groups, it is interesting to note that the reactions of (*S*)-**1** with (4*S*)-**2a** and (4*S*)-**2c** occurred at virtually the same rate.

To further demonstrate the generality of our method, we investigated additions of complex (*S*)-**1** with several aromatic oxazolidin-2-ones bearing the more sterically demanding β -naphthyl group (**2d**) or derivatives of cinnamic acid with electron-withdrawing (CF₃, **2e**) or electron-donating (OMe, **2f**) substituents on the phenyl ring. All three reactions were conducted under same reaction conditions and featured virtually quantitative yields and diastereoselectivities. The sole products **4d**, **3e**, and **3f** were observed in the crude reaction mixtures by NMR analysis (entries 7–9). Regarding the reaction rates, the additions of β -naphthyl- and *p*-methoxy-containing derivatives with complex (*S*)-**1** proceeded markedly slower than the reactions of (4*R*)-**2c** and (4*S*)-**2c**, respectively (entries 6 relative to 7, and 5 relative to 9), while the reaction of trifluoromethyl derivative (4*S*)-**2e**, which possesses a more electrophilic C–C double bond, was as fast as the addition of crotonyl derivative (*S*)-**2a** (entry 1 relative to 8).

Considering the results obtained, it is reasonable to suggest that the steric and/or electronic natures of the substituents do not influence the stereochemical outcome but only affect the reaction rates; this indicates that the substituent on the C–C double bond is not directly involved in the stereoselective step of the addition reaction. On the other hand, the observed stereochemical outcome clearly suggests that the steric preferences of the chiral oxazolidin-2-ones overwhelm those of the chiral complex (*S*)-**1**. One can assume that application of achiral nickel complexes or other derivatives of glycine, instead of (*S*)-**1**, would be a feasible option to enhance the

preparative value of the method, provided, of course, that the achiral derivatives feature the same high reactivity and virtually complete diastereoselective outcome of the corresponding addition reactions.^[17]

With these promising results in hand, we next sought to find a simple way to prepare the target pyroglutamic acids from the addition products, along with complete recycling of both chiral auxiliaries. Following our standard procedure for preparing the pyroglutamic acids from the corresponding complexes obtained by additions of (*S*)-**1** with α,β -unsaturated carboxylic esters,^[6a,b] we decomposed compounds **3a–f** and **4a–f** with 2 N hydrochloric acid in MeOH to afford NiCl₂, the hydrochloride of the chiral ligand (*S*)-**5**, and the corresponding glutamic acid ω -amide **6** or **7** containing the residue of the oxazolidinone (Scheme 2). We were pleased to



Scheme 2. a) MeOH/HCl; (b) 1. NH₄OH; 2. Dowex. R substituents as in Scheme 1.

find that treatment of this mixture with concentrated ammonia cleanly resulted in the intramolecular cyclization of compounds **6** and **7** to give the target pyroglutamic acids **8** and **9**, respectively, and the free (4*S*)- or (4*R*)-oxazolidinone **10**. The treatment of the reaction mixture with ammonia also converted the hydrochloride of ligand (*S*)-**5** to afford the free base (*S*)-**5**, allowing a simple and complete extraction of both chiral auxiliaries (4*S*)- and (4*R*)-**10**, and (*S*)-**5** from the aqueous ammonia solution with chloroform. Taking advantage of the basic nature of (*S*)-**5**, its further separation from (4*S*)- and (4*R*)-**10** was effectively achieved by precipitating the hydrochloric salt of (*S*)-**5** with gaseous HCl, while (4*S*)- or (4*R*)-**10** remained in the chloroform solution. By this simple procedure both chiral auxiliaries (4*S*)- or (4*R*)-**10** and (*S*)-**5** were recycled in 87–95% yield, and were used for the preparation of fresh starting Ni(II)-complex (*S*)-**1**, and (4*S*)- or (4*R*)-*N*-(*E*-enoyl)-4-phenyloxazolidin-2-ones (**2**). The enantiomerically pure pyroglutamic acids **8** and **9** were isolated effectively (93–95% yield) from the solution using a cation-exchange resin and could be recrystallized from hexane/THF for analytical purposes.

This is a highly practical asymmetric synthesis of chemically and biomedically important enantiomerically pure β -substituted pyroglutamic acids and, with the possibility of further

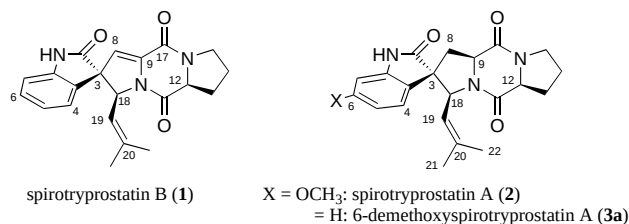
transformations of the products,^[4] a wide range of synthetic applications can be readily envisaged. The simplicity of the procedures, the low cost of the reagents, and the good chemical and optical yields of the products, make this a useful alternative to existing methods.^[5, 6]

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A Rapid Total Synthesis of Spirotryprostatin B: Proof of Its Relative and Absolute Stereochemistry**

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A variety of prenyl-containing alkaloids, clearly derived from the amino acids tryptophan and proline and linked by a diketopiperazine arrangement, have been isolated from natural sources.^[1, 2] One such alkaloid which engaged our interest is spirotryprostatin A (**2**),^[3] wherein a “prenylidene” group serves to meld N₆ of the tryptophan to the β -carbon atom of the oxidized indolo sector. Recently, our laboratory



disclosed the total synthesis of spirotryprostatin A (**2**).^[4] Coproduced with the A compound in *Aspergillus fumigatus*, is spirotryprostatin B (**1**),^[3] which lacks the C6-methoxyl substituent of the A congener **2**. Clearly, the additional site of unsaturation in the B system adds incremental complexity to the prospect of its total synthesis.

The relative stereochemistry of the A compound **2**, assigned on the basis of NMR “through space” connectivities between carbons 3, 18, 9, and 12 was fully corroborated by our total synthesis,^[4] which also served to establish the absolute configuration. In the case of spirotryprostatin B (**1**), the “prolyl” center (C12) is spectroscopically isolated from the prenylated tryptophan domain (see Scheme 5). Thus, while the relative configurational arrangements at the C3 and C18 centers of the A and B alkaloids (**2** and **1**) are the same, the relationship between these diads and the proline-derived C12 atom could not be asserted with rigor in the latter compound. As we shall show, the surmise of the original discoverers of spirotryprostatin B (**1**) turns out to be correct.

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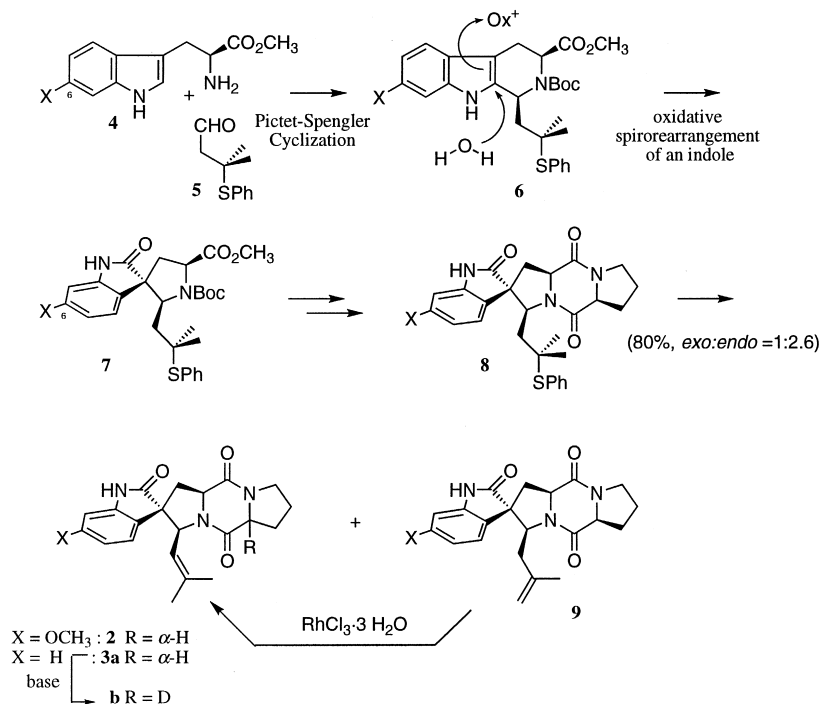
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- [1] For reviews, see: a) M. Goodman, S. Ro in *Burger's Medicinal Chemistry and Drug Discovery*, Vol. 1, 5th ed. (Ed.: M. E. Wolff), John Wiley and Sons, Inc., New York, **1995**, pp. 803–861; b) J. Gante, *Angew. Chem.* **1994**, *106*, 1780; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699; c) A. Giannis, T. Kolter, *Angew. Chem.* **1993**, *105*, 1303; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244; d) V. J. Hruby, F. Al-Obeidi, W. Kazmierski, *Biochem. J.* **1990**, *268*, 249; e) V. J. Hruby, *Med. Res. Rev.* **1989**, *9*, 343; f) C. Cativiela, M. D. Díaz-de-Villegas, *Tetrahedron: Asymmetry* **1998**, *9*, 3517.
- [2] a) V. J. Hruby, X. Qian in *Methods in Molecular Biology*, Vol. 35, *Peptide Synthesis and Purification Protocols* (Eds.: M. W. Pennington, B. M. Dunn), Humana Press, Clifton, NJ, **1994**, pp. 249–286; b) V. J. Hruby, G. Li, C. Haskell-Luevano, M. Shenderovich, *Biopolymers (Pept. Sci.)* **1997**, *43*, 219–266.
- [3] W. Fan, B. A. Boston, R. A. Kesterson, V. J. Hruby, R. D. Cone, *Nature* **1997**, *385*, 165, and references therein.
- [4] For a recent review, see: *Tetrahedron: Asymmetry* **1999**, *10*, 2245.
- [5] For reviews, see: a) R. O. Duthaler, *Tetrahedron* **1994**, *50*, 1539; b) R. M. Williams, *Synthesis of Optically Active α -Amino Acids*, Pergamon Press, Oxford, **1989**.
- [6] For recent papers, see: a) V. A. Soloshonok, C. Cai, V. J. Hruby, L. V. Meervelt, N. Mischenko, *Tetrahedron* **1999**, *55*, 12031; b) V. A. Soloshonok, C. Cai, V. J. Hruby, L. V. Meervelt, *Tetrahedron* **1999**, *55*, 12045; c) J. Ezquerro, C. Pedregal, I. Merino, J. Florez, J. Barluenga, S. Garcia-Granda, M.-A. Llorca, *J. Org. Chem.* **1999**, *64*, 6554, and references therein.
- [7] D. Seebach, M. Hoffman, *Eur. J. Org. Chem.* **1998**, 1337.
- [8] V. A. Soloshonok, C. Cai, V. J. Hruby, *Tetrahedron Lett.* **2000**, *41*, 135.
- [9] V. A. Soloshonok, C. Cai, V. J. Hruby, *Tetrahedron: Asymmetry* **1999**, *10*, 4265.
- [10] D. Seebach, V. Prelog, *Angew. Chem.* **1982**, *94*, 696; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 654.
- [11] a) E. Nicolas, K. C. Russell, V. J. Hruby, *J. Org. Chem.* **1993**, *58*, 766; b) G. Li, M. A. Jarosinski, V. J. Hruby, *Tetrahedron Lett.* **1993**, *34*, 2561; c) S. Liao, Y. Han, W. Qui, M. Bruck, V. J. Hruby, *Tetrahedron Lett.* **1996**, *37*, 7917.
- [12] D. R. Williams, W. S. Kissel, J. J. Li, *Tetrahedron Lett.* **1998**, *39*, 8593.
- [13] The corresponding pyroglutamic acids were isolated from the diastereomerically pure nickel(II) complexes (as depicted in Scheme 2) and their spectroscopic and optical properties compared with the literature data (ref. [6]).
- [14] Exceptions were cases where α -(R) absolute configuration is a consequence of the Cahn-Ingold-Prelog priority (see ref. [16]) and also some aldol addition reactions where the α -(R) stereochemistry is a result of the rearrangement of the corresponding aldol products forming the β -hydroxy-coordinated complexes. For general patterns of reactivity and stereocontrol properties of complex (S)-**1**, see ref. [15].
- [15] a) Yu. N. Belokon, *Pure Appl. Chem.* **1992**, *64*, 1917; b) V. A. Soloshonok in *Biomedical Frontiers of Fluorine Chemistry* (Eds.: I. Ojima, J. R. McCarthy, J. T. Welch), American Chemical Society, Washington, DC, **1996**, Chap. 2; c) V. A. Soloshonok in *Enantiocontrolled Synthesis of Fluoro-Organic Compounds: Stereochemical Challenges and Biomedical Targets* (Ed.: V. A. Soloshonok), Wiley, Chichester, **1999**.
- [16] R. S. Cahn, C. Ingold, V. Prelog, *Angew. Chem.* **1966**, *78*, 413; *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 385.
- [17] For instance: the reactions, recently introduced by us (see ref. [7]), of achiral nickel complexes with the chiral oxazolidin-2-ones, proceeded with high stereochemical outcome but at substantially reduced reaction rates (unpublished results).

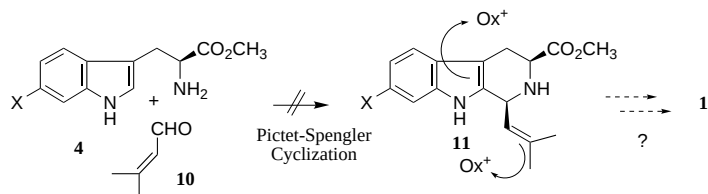
In addition to learning how to overcome the significant challenge posed by the presence of the C8–C9 double bond of the B system, total synthesis^[5] emerged as the only way for our laboratory to gain access to appropriate quantities of this compound for assessment of its potential utility as a cancer chemotherapeutic agent (reportedly operating by cell cycle inhibition at the G2/M phase).

We naturally gave thought to reaching our goal by retracing and modifying our route to the A system **2**, which also had led to a total synthesis of 6-demethoxyspirotryprostatin A (**3a**). However, the routes we had used to reach the A compound **3a** (see Scheme 1), while reasonably concise (13 steps), were plagued by several difficult specificity issues necessitating



Scheme 1. Synthesis of spirotryprostatin A (**2**). Ox⁺ = oxidant, Boc = *tert*-butoxycarbonyl.

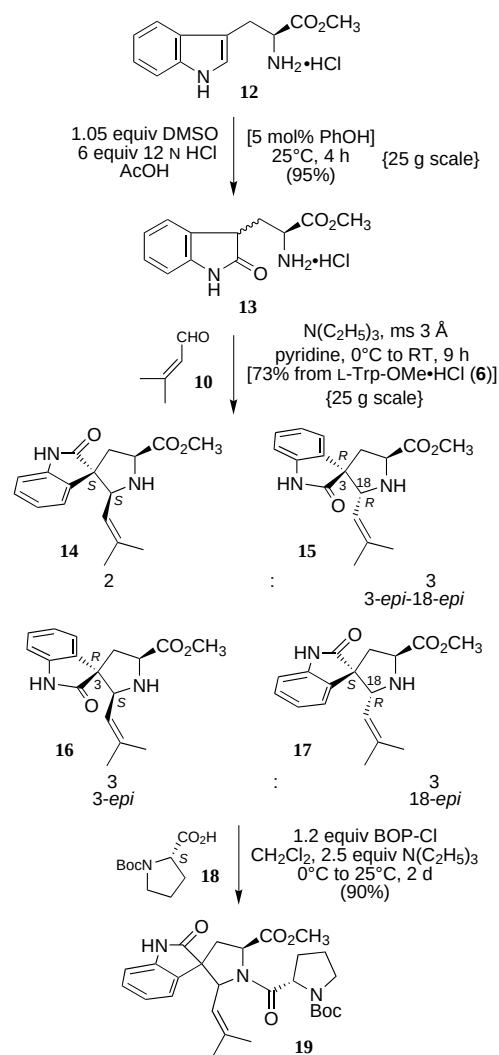
awkward management of functional groups. Moreover, attempted metal exchange at C9 in **3a**, bearing the intact diketopiperazine had resulted in metal hydrogen exchange at C12 (cf. **3b**), thus further complicating prospects for late stage introduction of the 8–9 double bond from a previously synthesized dihydro precursor.^[6] Another serious factor complicating our synthesis of **2** was our failure to install the prenyl group by direct Pictet–Spengler incorporation of senecialdehyde (**10**, Scheme 2). We and others^[6] have not been able to reduce this apparently simple concept to practice



Scheme 2. Attempted Pictet–Spengler cyclizations with prenyl aldehyde (**10**).

in the face of the lability of **10**. We started by asking whether a Mannich reaction^[7] on an oxindole would enable direct incorporation of the required prenyl function.

The readily available *L*-tryptophan methyl ester (**12**) was converted (95 %) into the oxindole derivative hydrochloride **13** (Scheme 3).^[8] Treatment of **13** with **10**, under the conditions shown, afforded a 73 % yield of prenyl “insertion” products. Detailed NMR studies revealed that, at this stage, a four component mixture (**14**–**17**) had been generated.

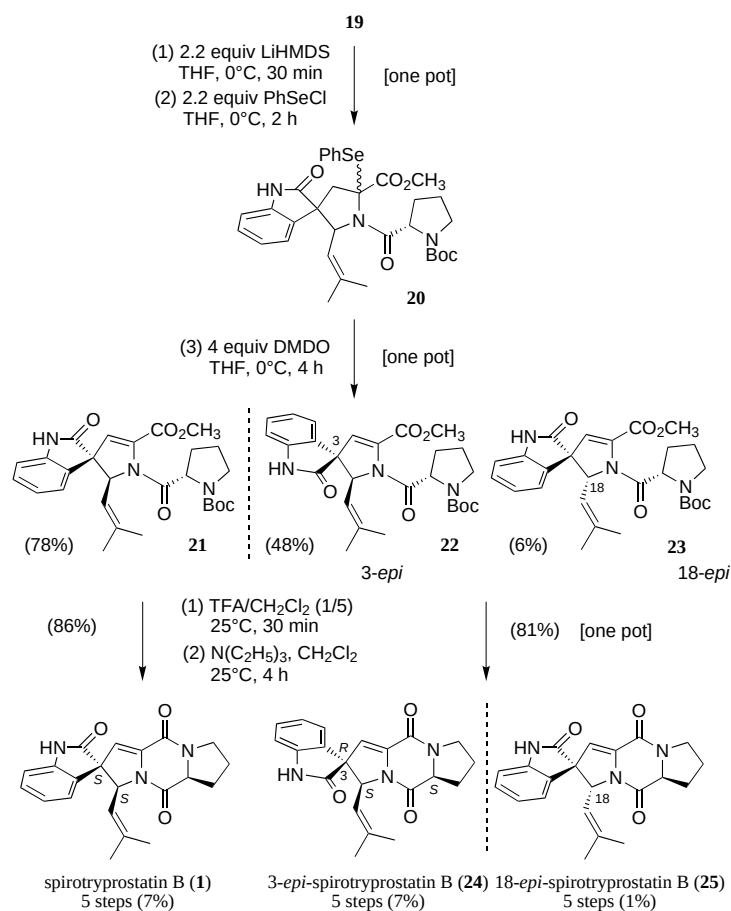


Scheme 3. The Mannich route to the spirooxindole system. DMSO = dimethyl sulfoxide, ms = molecular sieves, RT = room temperature, BOP = Bis-(2-oxo-3-oxazolidinyl)phosphoryl.

Following chromatography, compound **15**, with the 3,18-bis-*epi* configuration (3*R*,18*R*), could be obtained in a homogeneous state. The other three isomers coeluted. There was no advantage to be gained from independent forward processing of **15** since, under our acylation conditions with *N*-Boc-*L*-proline (**18**), extensive equilibration had occurred at C3 and C18. Indeed, attempted acylation of **15** with **18** afforded a product **19** whose NMR characteristics were the same as those of the mixture arising from conducting the prolinoylation on the **14**–**17** mixture. Identification of the components of the acylation mixture **19** (no stereoassignments intended) was no

small matter. Given the serious complications in NMR spectral analysis of the prolinoylation products, further aggravated by the presence of the amidic and carbamate rotamers, we relied solely on mass spectroscopy to establish that coupling had actually occurred.

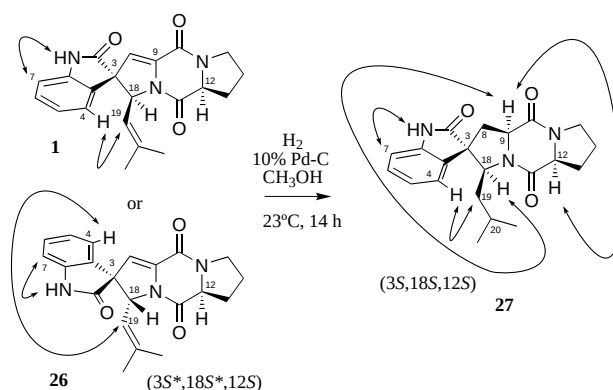
At this stage, we hoped to exploit the fact that in these acylation products, the enolizable tryptophyl α -center (C9) appears in the context of an ester (flanked by an *N*-acyl group). By contrast, the corresponding α -center in the proline sector (C12) is presented in an amide-like setting (this time flanked by a carbamoyl type nitrogen). In the event, treatment of **19**, with lithium 1,1,1,3,3,3-hexamethyldisilazane, followed by selenylation, led to a crude product presumed to be **20** by ESI-MS (Scheme 4). Upon exposure of the selenide **20** to the action of dimethyldioxirane, oxidative elimination occurred readily, yielding a mixture of the products **21–23**. Compound **21** could be separated from the latter two (**22** and **23**) by silica gel chromatography. The other fraction eluted as a mixture of **22** and **23**, substantially favoring the former according to NMR analysis. Fortunately, there had been a major simplification in converting the **14–17** mixture into the dehydro intermediates **21–23**. Thus, the pre-**2** stereoisomer **14** was the least prevalent isomer at the nonacylated stage (18% of a 4-component mixture). In the penultimate dehydro series it is approximately 40% (and readily purified) of a three component mixture, one member of which (**23**) is quite minor.



Scheme 4. Synthesis of spirotryprostatin B (**1**). HMDS = 1,1,1,3,3,3-hexamethyldisilazane, DMDO = dimethyldioxirane.

Deprotection of the prolyl nitrogen of **21** followed by triethylamine induced cyclization, afforded spirotryprostatin B (**1**), identical by the criteria of ^1H , COSY, HMBC, and ^{13}C NMR spectroscopy methods with the natural product.^[9] Moreover, the CD spectrum and the optical rotation ($[\alpha]_D^{25}$ of synthetic material -165.4 ; $[\alpha]_D$ reported -162.1) established both materials to have the same absolute stereochemistry.

In order to confirm that spirotryprostatin B is **1**, rather than **26**, the fully synthetic compound was subjected to catalytic hydrogenation (Scheme 5). In this way, the proton at C9 would serve as a linkage site at the level of NMR analysis.



Scheme 5. Introduction of the 9-H NMR spectroscopy linker for nuclear Overhauser experiments.

Indeed, with greater than 90% *de*, catalytic hydrogenation of the fully synthetic compound afforded **27**. This compound, corresponding to tetrahydrospirotryprostatin B, exhibited all of the expected nuclear Overhauser enhancements (Scheme 5) between the α -protons (9-H, 12-H, and 18-H). Compound **27** could also be obtained by hydrogenation of 6-demethoxy-spirotryprostatin A (**3a**) synthesized by the old route (Scheme 1). These findings rigorously establish spirotryprostatin B to have the relative and absolute stereochemistry implied in expression **1**.

Processing of the mixture of **22** and **23** in the same fashion, followed by chromatographic separation afforded 3-*epi*-spirotryprostatin B (**24**)^[10] and 18-*epi*-spirotryprostatin B (**25**)^[11] in a ratio of 7:1. Following careful chromatography, these compounds were obtained as homogenous entities. Missing at the end of the four step processing of mixture **14–17** was the 3,18-bis-*epi* compound which would have been derived from **15**. Its nonappearance in the sequence might reflect a failure of its precursor to undergo the oxidative dehydrogenation sequence or could be due to unfavorable stereoequilibration at an undetermined stage.

We note that while our total synthesis, as it is now conducted, lacks stereocontrol at carbons 3 and 18, it does produce copious quantities of **1**. (Currently we deliver approximately 500 mg per batch of this highly active compound, in five easily executed steps, starting with 5.5 g of trivially available **12** and resorting to only

two chromatographic purifications.) We expect to explore fresh possibilities for dealing with the last remaining stereochemical issues. However, chemical synthesis is already the preferred way to amass formidable quantities of **1**, and biological investigations reflecting its now ready availability are beginning in earnest.

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- [1] For the tryptostatin, see: C.-B. Cui, H. Kakeya, G. Okada, R. Onose, M. Ubukata, I. Takahashi, K. Isono, H. Osada, *J. Antibiot.* **1995**, *48*, 1382–1384.
- [2] For the fumitremorgins, see: M. Yamazaki, H. Fujimoto, T. Kawasaki, *Chem. Pharm. Bull.* **1980**, *28*, 245–254.
- [3] a) C.-B. Cui, H. Kakeya, H. Osada, *Tetrahedron* **1996**, *52*, 12651–12666; b) H. Osada, S. L. Choi, H. Kakeya, K. Isono, F. Yamashita, *Chem. Abstr.* **1997**, *127*, 261790.
- [4] a) S. D. Edmondson, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1190–1193; *Angew. Chem. Int. Ed.* **1998**, *37*, 1138–1140; b) S. Edmondson, S. J. Danishefsky, L. Sepp-Lorenzino, N. Rosen, *J. Am. Chem. Soc.* **1999**, *121*, 2147–2155.
- [5] Two other total syntheses of **1** have been accomplished, by groups headed by Prof. Robert Williams at Colorado State University (*J. Am. Chem. Soc.* **2000**, in press) and Prof. Lawrence Overman at the University of California, Irvine (personal communication). The work described herein was disclosed by S.D. at the H. C. Brown Award Symposium at the 219th National Meeting of the American Chemical Society, San Francisco, CA, on March 26, 2000. We thank Professor Williams and Professor Overman for sharing their results with us.
- [6] a) D. M. Harrison, R. B. Sharma, *Tetrahedron* **1993**, *49*, 3165–3184; b) for a successful *N*-acyliminium Pictet–Spengler condensation, see: H. Wang, A. Ganesan, *Org. Lett.* **1999**, *1*, 1647–1649; H. Wang, A. Ganesan, *Tetrahedron Lett.* **1997**, *38*, 4327–4328.
- [7] For a Mannich reaction with oxo-tryptamine and benzaldehyde, see: J. Harley-Mason, R. F. Ingleby, *J. Chem. Soc.* **1958**, 3639–3642; for a Mannich reaction with acetone, see: M. Incze, G. Dörnyei, M. Kajtár-Peredy, C. Szántay, *Collect. Czech. Chem. Commun.* **1999**, *64*, 408–416.
- [8] W. E. Savage, A. Fontana, *Int. J. Pept. Protein Res.* **1980**, *15*, 285–297.
- [9] Spirotryprostatin B (**1**): $[\alpha]_D^{25} = -165.4$ ($c = 0.92$, CHCl_3); ^1H NMR (500 MHz, CDCl_3): $\delta = 1.27$ (s, 3H, 22-H), 1.56 (s, 3H, 21-H), 1.92–2.03 (m, 2H, 13 β -H, 14 α -H), 2.01–2.15 (m, 1H, 14 β -H), 2.48 (m, 1H, 13 α -H), 3.57 (ddd, $J = 12.3, 9.3, 3.0$ Hz, 1H, 15 α -H), 3.84 (dt, $J = 12.3, 8.1$ Hz, 1H, 15 β -H), 4.34 (dd, $J = 10.0, 5.8$ Hz, 1H, 12-H), 5.21 (dm, $J = 8.9$ Hz, 1H, 19-H), 5.42 (d, $J = 8.9$ Hz, 1H, 18-H), 5.77 (s, 1H, 8-H), 6.85 (d, $J = 7.8$ Hz, 1H, 7-H), 6.99 (t, $J = 7.6$ Hz, 1H, 5-H), 7.06 (d, $J = 7.4$ Hz, 1H, 4-H), 7.23 (t, $J = 7.8$ Hz, 1H, 6-H), 7.69 (s, 1H, 1-H); ^{13}C NMR (75.5 MHz, CDCl_3): $\delta = 18.22$ (C-22), 22.07 (C-14), 25.27 (C-21), 29.23 (C-13), 44.82 (C-15), 61.55 (C-12), 61.86 (C-3), 64.11 (C-18), 110.05 (C-7), 116.47 (C-8), 120.43 (C-19), 122.18 (C-5), 127.21 (C-3a), 127.71 (C-4), 129.04 (C-6), 138.16 (C-9), 138.28 (C-20), 140.70 (C-7a), 155.07 (C-17), 162.56 (C-11), 178.52 (C-2); HRMS (FAB): calcd for $\text{C}_{21}\text{H}_{22}\text{N}_3\text{O}_3$ $[M + \text{H}]^+$: 364.1661, found: 364.1650.
- [10] 3-*epi*-Spirotryprostatin B (**24**): $[\alpha]_D^{25} = -250.6$ ($c = 0.87$, CHCl_3); ^1H NMR (500 MHz, CDCl_3): $\delta = 1.35$ (s, 3H, 22-H), 1.69 (s, 3H, 21-H), 1.93–2.03 (m, 2H, 13 β -H, 14 α -H), 2.11 (m, 1H, 14 β -H), 2.41 (m, 1H, 13 α -H), 3.53 (ddd, $J = 12.7, 9.7, 3.6$ Hz, 1H, 15 α -H), 3.84 (dt, $J = 12.6, 7.8$ Hz, 1H, 15 β -H), 4.23 (dd, $J = 10.4, 5.7$ Hz, 1H, 12-H), 5.28 (d, $J = 9.3$ Hz, 1H, 18-H), 5.56 (dm, $J = 9.2$ Hz, 1H, 19-H), 5.86 (s, 1H, 8-H), 6.82 (d, $J = 7.7$ Hz, 1H, 7-H), 7.07 (t, $J = 7.6$ Hz, 1H, 5-H), 7.20 (d, $J = 7.3$ Hz, 1H, 4-H), 7.25 (t, $J = 7.3$ Hz, 1H, 6-H), 7.40 (s, 1H, 1-H); ^{13}C NMR (75.5 MHz, CDCl_3): $\delta = 18.19$ (C-22), 22.00 (C-14), 25.83 (C-21), 29.54 (C-13), 44.71 (C-15), 61.63 (C-12), 62.30 (C-3), 66.59 (C-18), 109.81 (C-7), 115.84 (C-8), 119.78 (C-19), 123.36 (C-5), 124.07 (C-4), 129.44 (C-6), 131.32 (C-3a), 136.84 (C-20), 138.36 (C-9), 140.15 (C-7a), 154.65 (C-17), 163.38 (C-11), 175.56 (C-2); HRMS (FAB): calcd for $\text{C}_{21}\text{H}_{22}\text{N}_3\text{O}_3$ $[M + \text{H}]^+$: 364.1661, found: 364.1675.
- [11] 18-*epi*-Spirotryprostatin B (**25**): $[\alpha]_D^{25} = -24$ ($c = 0.14$, CHCl_3); ^1H NMR (500 MHz, CDCl_3): $\delta = 1.23$ (s, 3H, 22-H), 1.66 (s, 3H, 21-H), 1.96–2.05 (m, 2H, 13 β -H, 14 α -H), 2.13 (m, 1H, 14 β -H), 2.45 (m, 1H, 13 α -H), 3.55 (ddd, $J = 12.4, 9.3, 3.3$ Hz, 1H, 15 α -H), 3.84 (dt, $J = 12.7, 8.5$ Hz, 1H, 15 β -H), 4.38 (dd, $J = 10.0, 5.8$ Hz, 1H, 12-H), 5.27 (d, $J = 9.3$ Hz, 1H, 18-H), 5.49 (dm, $J = 9.3$ Hz, 1H, 19-H), 5.83 (s, 1H, 8-H), 6.81 (d, $J = 7.7$ Hz, 1H, 7-H), 7.07 (t, $J = 7.6$ Hz, 1H, 5-H), 7.25 (m, 2H, 6-H, 4-H), 7.47 (s, 1H, 1-H); ^{13}C NMR (75.5 MHz, CDCl_3): $\delta = 18.11$ (C-22), 22.01 (C-14), 25.87 (C-21), 28.79 (C-13), 45.12 (C-15), 61.66 (C-12), 62.39 (C-3), 66.74 (C-18), 109.69 (C-7), 114.88 (C-8), 118.18 (C-19), 123.38 (C-5), 124.51 (C-4), 129.43 (C-6), 129.04 (C-6), 130.60 (C-3a), 137.92 (C-20), 140.59 (C-7a), 154.61 (C-17), 162.59 (C-11), 175.27 (C-2); HRMS (FAB): calcd for $\text{C}_{21}\text{H}_{22}\text{N}_3\text{O}_3$ $[M + \text{H}]^+$: 364.1661, found: 364.1650.