

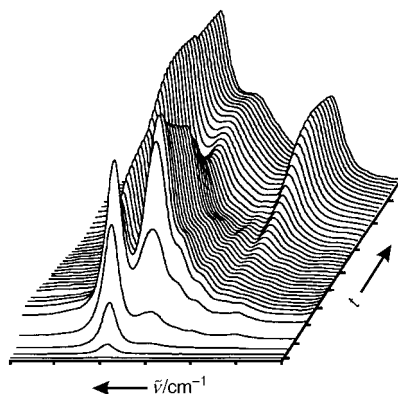
COVER PICTURE

The cover picture shows two new catalytic asymmetric reactions in front of an enantiomeric pair of lurs—one of the national symbols of Denmark. Chiral bisoxazoline copper complexes catalyze the reactions. The reaction on the left is the direct enantioselective Mannich reaction of carbonyl compounds with imines to give highly functionalized 4-oxo-glutamic esters, while the reaction on the right is the enantioselective nitro-Mannich reaction of nitro alkanes with imines to give optically active β -nitro- α -amino esters. Why the lur in relation to the present chemistry? Lurs exist as pairs of enantiomers and they probably belong to some of the first man-made pair of enantiomers, as they are from the Bronze Age (1000–500 BC). The lurs are the oldest musical instruments that can still be played, and are used as enantiomers. Side by side stood two lur-players, symmetric in every detail, the soft curves of the instruments swaying upwards and outwards at each side. The sonorous notes emphasized the solemn nature of the rituals. It is conceivable that the sound of the lur also accompanied the crackle of the funeral pyre when one of the tribe was cremated and laid to rest in the burial mound of his forefathers. The lurs are made of bronze (copper) and were discovered in lakes; less than 50 are known. The most famous pair of lurs was found in Brudevælde and is the pair shown on the cover. Further information about lurs can be obtained from kaj@chem.au.dk. The picture of the lurs and the accompanying music (click here to listen to the music: www.angewandte.com) are used with permission from The National Museum. For more details about the two new catalytic asymmetric reactions, see Jørgensen et al. on p. 2992 ff. and p. 2995 ff.



Spectroscopy at the working catalyst: Modern procedures of in situ spectroscopy are being increasingly applied to the preparation and selection of suitable reaction networks, the determination of the stoichiometry of individual intermediate steps of a network, and the identification and characterization of active centers, intermediates, the activation processes of the reactants, and the surface reactions. The picture exemplifies the changes in an IR spectrum during the course of a reaction.

Angew. Chem. **2001**, *113*, 3040–3059



M. Hunger,* J. Weitkamp* .. 2954–2971

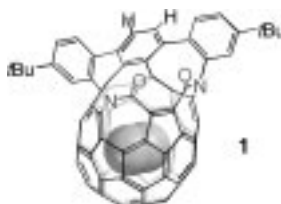
In situ IR, NMR, EPR, and UV/Vis Spectroscopy: Tools for New Insight into the Mechanisms of Heterogeneous Catalysis

Keywords: active centers • heterogeneous catalysis • in situ spectroscopy • reaction mechanisms • solid catalysts

HIGHLIGHTS

A window of opportunity: With the formation of a large orifice in the C₆₀ core (**1**) and the successful insertion of He and H₂, two critical steps have been realized towards the development of an efficient synthetic approach for the preparation of endohedral fullerene complexes.

Angew. Chem. **2001**, *113*, 3061–3062



J.-F. Nierengarten* 2973–2974

Ring-Opened Fullerenes: An Unprecedented Class of Ligands for Supramolecular Chemistry

Keywords: fullerenes • host–guest systems • inclusion compounds • supramolecular chemistry

VIPs

The following communications are “Very Important Papers” in the opinion of two referees. They will be published shortly (those marked with a diamond will be published in the next issue). Short summaries of these articles can be found on the *Angewandte Chemie* homepage at the address <http://www.angewandte.com>

Synthesis of the C1–C13 Fragment of Kendomycin: Atropisomerism around a C-Aryl Glycosidic Bond

H. J. Martin, M. Drescher,
H. Kählig, S. Schneider,
J. Mulzer*



[NdI₂(thf)₅]: The First Crystallographically Authenticated Neodymium Complex

M. N. Bochkarev*,
I. L. Fedushkin, S. Dechert,
A. A. Fagin, H. Schumann*



From Split-Pool Libraries to Spatially Addressable Microarrays and Its Application to Functional Proteomic Profiling

N. Winssinger, J. L. Harris,
B. J. Backes, P. G. Schultz*



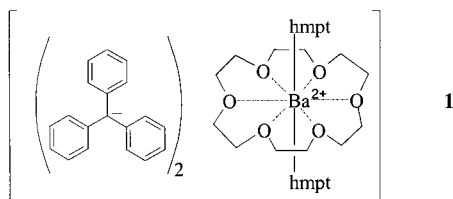
High Turnover Numbers for Catalytic Selective Epoxidation of Alkenes with 1 atm Molecular Oxygen

Y. Nishiyama, Y. Nakagawa,
N. Mizuno*

Zero-Strain Intercalation Cathode for Rechargeable Li-Ion Cell

J. Cho, Y. J. Kim, T.-J. Kim,
B. Park*

Even a century after the discovery of the Grignard reagents, similar compounds of the heavy alkaline earth metals are still lacking. Owing to the industrial need for such compounds (in particular as anionic polymerization catalysts), the last few years have been marked by a rapid, extensive development which has culminated in the first structurally characterized alkylbarium derivatives such as the crown ether complex of bis(triphenylmethyl)barium (**1**). hmpt = hexamethylphosphoric acid triamide.



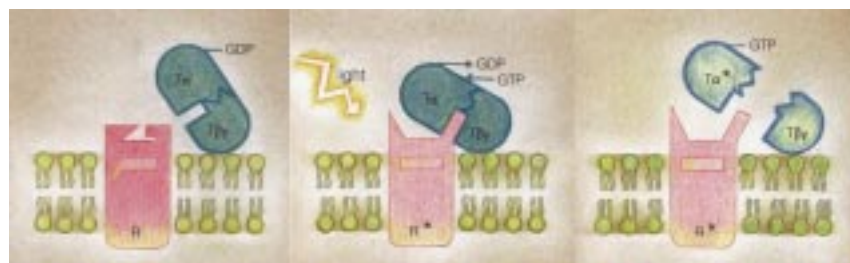
Angew. Chem. **2001**, *113*, 3063–3065

M. Westerhausen* 2975–2977

100 Years after Grignard: Where Does the Organometallic Chemistry of the Heavy Alkaline Earth Metals Stand Today?

Keywords: alkaline earth metals • barium • calcium • carbanions • Grignard reactions

The visual pigment rhodopsin constitutes the interface between the physical event of light absorption and the biochemical process of visual transduction within the photoreceptor cells. The signal transduction is initiated by an 11-*cis*→all-*trans* photoisomerization of the retinal chromophore of rhodopsin which causes a series of thermally driven conformational changes of the chromophore and the protein moiety. A rhodopsin conformation is generated which allows interaction with a heterotrimeric G-protein (see picture). Two recent publications follow the chromophore motions after light absorption by cross-linking experiments and by solid-state NMR spectroscopy.



Angew. Chem. **2001**, *113*, 3065–3069

W. Gärtner* 2977–2981

The Light Shall Show the Way—Or: The Conformational Changes of the Retinal Chromophore in Rhodopsin upon Light Activation

Keywords: NMR spectroscopy • photochemistry • retinal • rhodopsin • signal transduction

COMMUNICATIONS

Simply decomposing InCp at room temperature in the presence of hexadecylamine leads to the formation of indium nanowires with a crystalline bct structure, a mean diameter of about 200 nm, and lengths in the micrometer range. The method can be extended to the preparation of In₃Sn nanowires of about 100 nm in diameter (a transmission electron micrograph is shown in the picture). Cp = C₅H₅[−].



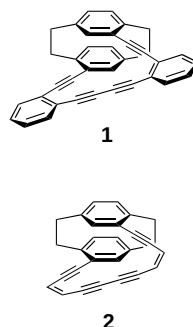
Angew. Chem. **2001**, *113*, 3072–3074

K. Soulantica, A. Maisonnat, F. Senocq, M.-C. Fromen, M.-J. Casanove, B. Chaudret* 2984–2986

Selective Synthesis of Novel In and In₃Sn Nanowires by an Organometallic Route at Room Temperature

Keywords: electron microscopy • indium • nanostructures • nanowires • tin

Enhanced global delocalization throughout the “stepped” π-electron systems of the [2.2]paracyclophane/dehydrobenzoannulene (PC/DBA) hybrids **1** and **2** is strongly suggested by a comparison of their electronic absorption spectra with those of model compounds with complete and interrupted classical aromatic delocalization. A distinct bathochromic shift (for **1**) and greater absorption intensity at higher wavelengths (for **1** and **2**) is observed versus the corresponding model hydrocarbons.



Angew. Chem. **2001**, *113*, 3074–3077

A. J. Boydston, L. Bondarenko, I. Dix, T. J. R. Weakley, H. Hopf,* M. M. Haley* 2986–2989

[2.2]Paracyclophane/Dehydrobenzoannulene Hybrids: Transannular Delocalization in Open-Circuited Conjugated Macrocycles

Keywords: alkynes • annulenes • aromaticity • cyclophanes • macrocycles

It is both an [18]trannulene and a [60]fullerene. It is aromatic and a hexa-substituted benzene. It is formed by the first proven example of an S_N2' reaction in a fullerene. It is intensely colored and stable. It is $C_{60}F_{15}[CBr(CO_2Et)_2]_3$, the first example of a new class of fullerene derivatives (see Schlegel diagram).



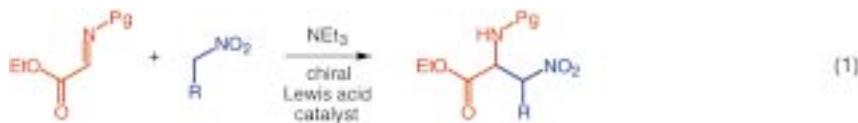
X.-W. Wei, A. D. Darwish,
O. V. Boltalina, P. B. Hitchcock,
J. M. Street, R. Taylor* 2989–2992

The Remarkable Stable Emerald Green
 $C_{60}F_{15}[CBr(CO_2Et)_2]_3$: The First
[60]Fullerene That Is also the First
[18]Trannulene

Keywords: annulenes • aromaticity •
fluorine • fullerenes • nucleophilic
substitution

Angew. Chem. **2001**, *113*, 3077–3080

 **The catalytic and asymmetric nitro-Mannich reaction** of nitro compounds to α -imino esters catalyzed by chiral bisoxazoline–copper complexes [Eq. (1); Pg = protecting group] gave β -nitro- α -amino esters with excellent diastereo- and enantioselectivities. The reactions can be performed under ambient conditions.



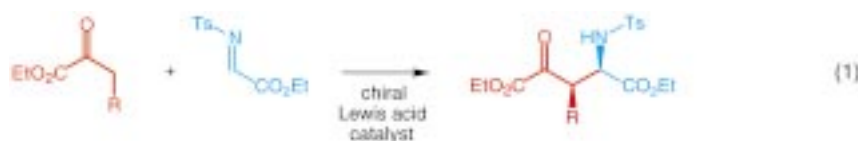
N. Nishiwaki, K. Rahbek Knudsen,
K. V. Gothelf,
K. A. Jørgensen* 2992–2995

Catalytic Enantioselective Addition of
Nitro Compounds to Imines—A Simple
Approach for the Synthesis of Optically
Active β -Nitro- α -Amino Esters

Keywords: amino acids • asymmetric
catalysis • imines • Lewis acids • nitro
compounds

Angew. Chem. **2001**, *113*, 3080–3083

 **The development of a direct catalytic asymmetric Mannich reaction** is shown and its potential demonstrated by the synthesis of optically active lactones via 4-hydroxyglutamic acid ester derivatives. The catalytic asymmetric Mannich reaction of carbonyl compounds with α -imino esters [Eq. (1); Ts = *p*-toluenesulfonyl] gives good yields and diastereoselectivities, as well as excellent enantioselectivities.



K. Juhl, N. Gathergood,
K. A. Jørgensen* 2995–2997

Catalytic Asymmetric Direct Mannich
Reactions of Carbonyl Compounds with
 α -Imino Esters

Keywords: amino acids • asymmetric
catalysis • imines • Lewis acids •
Mannich reactions

Angew. Chem. **2001**, *113*, 3083–3085

A double shell of Platonic solids: A trajectory that connects the six-membered rings of I_h-C_{80} is followed by the two La atoms of $La_2@C_{80}$ according to maximum entropy/Rietveld analysis of the synchrotron powder-diffraction data. The result is a pentagonal-dodecahedral charge-density distribution for La_2 (brown in the picture) inside the icosahedral C_{80} cage (green).



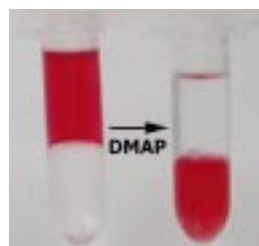
E. Nishibori, M. Takata,* M. Sakata,
A. Taninaka, H. Shinohara . . 2998–2999

Pentagonal-Dodecahedral La_2 Charge
Density in $[80-I_h]$ Fullerene: $La_2@C_{80}$

Keywords: fullerenes • lanthanum •
structure elucidation • synchrotron
radiation • X-ray diffraction

Angew. Chem. **2001**, *113*, 3086–3087

 **4-dimethylaminopyridine (DMAP) is the answer** to the quest for an efficient transfer of metallic nanoparticles from organic to aqueous solutions. The picture shows the transfer of gold nanoparticles from toluene to water by the addition of DMAP (0.1M, pH 10.5). This method enables the generation of high concentrations of nanoparticles with better monodispersity than those commonly prepared in water.



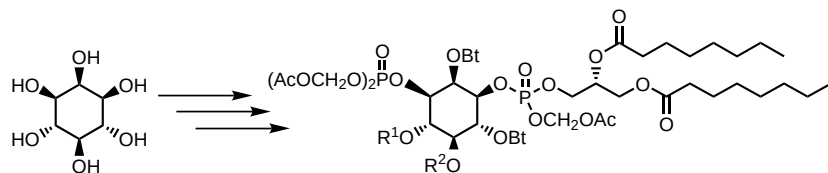
D. I. Gittins, F. Caruso* 3001–3004

Spontaneous Phase Transfer of
Nanoparticulate Metals from Organic to
Aqueous Media

Keywords: colloids • gold •
nanostructures • phase-transfer catalysis •
transition metals

Angew. Chem. **2001**, *113*, 3089–3092

A crucial role in the regulation of epithelial chloride secretion is played by the phosphoinositide $\text{PtdIns}(3,4,5)\text{P}_3$. Membrane-permeant derivatives of this and other naturally occurring phosphoinositides have been synthesized. These derivatives, which can be bioactivated, were used in investigations on nasal epithelia of patients suffering from cystic fibrosis (see general scheme).



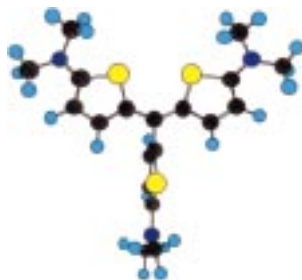
Angew. Chem. **2001**, *113*, 3093–3096

C. Dinkel, M. Moody,
A. Traynor-Kaplan,
C. Schultz * 3004–3008

Membrane-Permeant 3-OH-
Phosphorylated Phosphoinositide
Derivatives

Keywords: cyclitols • cystic fibrosis •
phosphoinositides • secretion • signal
transduction

Deep purple: By the transformation of readily available *N,N*-disubstituted 2-aminothiophenes and 2-aminothiazoles into heterocyclic analogues of Crystal Violet, such as, tris(2-dimethylamino-5-thienyl)methinium perchlorate (cation shown), the series of dyes derived from these heterocyclic structural units as the important chromophoric component is significantly extended and a gap in the class of heteroanalogous triphenylmethane dyes has been closed.



Angew. Chem. **2001**, *113*, 3097–3100

A. Noack, A. Schröder,
H. Hartmann * 3008–3011

Synthesis and Spectral Characterization
of a New Class of Heterocyclic Analogues
of Crystal Violet Dyes

Keywords: chromophores •
Crystal Violet • dyes/pigments •
sulfur heterocycles

The stabilizing effect of a dG:dC base-pair can also be imparted to a DNA duplex by a non-hydrogen-bonding, non-shape-complementary nucleoside analogue when inter-strand stacking interactions come into play. This is the case, for example, with dBP, which has a bipyridyl (BP) residue as a nucleobase surrogate (the picture shows a dBP:dBP pair).



Angew. Chem. **2001**, *113*, 3101–3103

C. Brotschi, A. Häberli,
C. J. Leumann * 3012–3014

A Stable DNA Duplex Containing a Non-
Hydrogen-Bonding and Non-Shape-
Complementary Base Couple: Interstrand
Stacking as the Stability Determining
Factor

Keywords: DNA recognition • DNA
structures • N ligands • nucleobases •
oligonucleotides

Visible light can greatly enhance the oxidative degradation of organic compounds such as rhodamine B and salicylic acid, which are test compounds for organic pollutants. The degradations are significantly accelerated relative to reactions carried out in the dark. The mechanism involves the photoassisted generation of $\text{HO}^\bullet$ radicals, which react with the pollutants to form the degraded products.

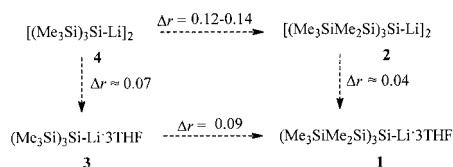
X. Tao, W. Ma, T. Zhang,
J. Zhao * 3014–3016

Efficient Photooxidative Degradation of
Organic Compounds in the Presence of
Iron Tetrasulfophthalocyanine under
Visible Light Irradiation

Keywords: EPR spectroscopy • iron •
photochemistry • photodegradation •
pollutants

Angew. Chem. **2001**, *113*, 3103–3105

It was fortunate that the first extended branched polysilyl anion $(\text{Me}_3\text{SiMe}_2\text{Si})_3\text{Si}^-$ could be crystallographically characterized in two forms—as the THF complex **1** and as the dimer **2**. Comparison with the analogous $(\text{Me}_3\text{Si})_3\text{SiLi}$ systems **3** and **4** shows that β - Me_3Si substitution and complexation by THF elongate the Si–Li distance by 0.09–0.14 Å and 0.04–0.07 Å, respectively.



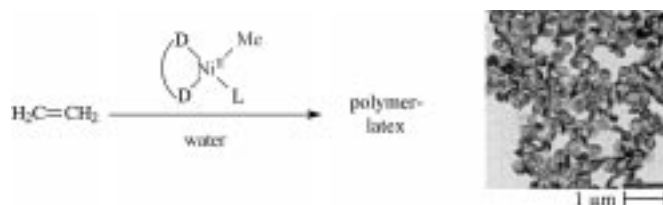
Angew. Chem. **2001**, *113*, 3106–3109

Y. Apeloig,* M. Yuzefovich,
M. Bendikov, D. Bravo-Zhivotovskii,*
D. Bläser, R. Boese 3016–3020

Branched Star-Type Polysilyllithium
Compounds: The Effects of β -Silyl
Substitution and of Complexation on
Their Molecular Structure

Keywords: anions • density functional
calculations • lithium • silicon

Semicrystalline microparticles of high molecular mass polyethylene can be prepared as aqueous dispersions (see picture) by catalytic emulsion polymerization of ethylene with a mini-emulsified catalyst precursor ($\text{D} = \text{salicylaldimine}$, $\text{L} = \text{pyridine}$). Stable polymer latexes are formed.



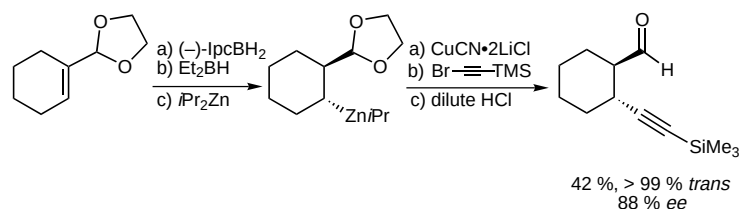
Angew. Chem. **2001**, *113*, 3112–3115

F. M. Bauers, S. Mecking* . 3020–3022

High Molecular Mass Polyethylene
Aqueous Latexes by Catalytic
Polymerization

Keywords: catalysis • colloids •
dispersions • polyethylene •
polymerization

By a sequence comprising asymmetric hydroboration, boron–zinc exchange, and transmetalation with copper, allylations, alkynylations, and allenylations of unsaturated ketals or acetals can be performed with good enantioselectivities (see scheme, $(-)\text{-IpcBH}_2 = (-)\text{-isopinocampheylborane}$, $\text{TMS} = \text{Me}_3\text{Si}$). The products correspond to a formal Michael addition with inversion of polarity.



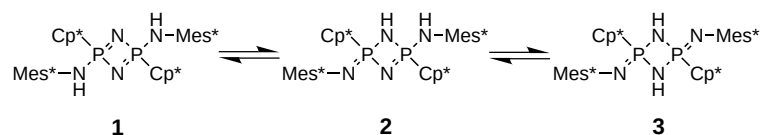
Angew. Chem. **2001**, *113*, 3109–3112

E. Hupe, P. Knochel* 3022–3025

Formal Enantioselective Michael
Addition with Umpolung of Reactivity

Keywords: asymmetric synthesis •
hydroboration • Michael addition •
umpolung • zinc

The tautomeric four-membered-ring P–N heterocycles 1, 2, and 3 can be interconverted; the formation of the cyclodiphosph(v)azene **1** is enthalpically and entropically favored. The crystal structures of the tautomers **1** and **3** have been determined and indications are given regarding the mechanism of the interconversion.



Angew. Chem. **2001**, *113*, 3115–3117

J. Tirré, D. Gudat, M. Nieger,
E. Niecke* 3025–3028

Reversible Tautomeric Transformation
between a Bis(amino)cyclodi-
phosph(v)azene and a
Bis(imino)cyclodiphosph(v)azene

Keywords: NMR spectroscopy •
phosphorus • phosphorus heterocycles •
tautomerism

Rapid-scan FT-IR spectral imaging allows high-throughput screening of the activities of components of a catalyst library in a truly parallel fashion. The gas-phase reaction products from 16 supported catalysts can be investigated simultaneously with the reactor system described.

Angew. Chem. **2001**, *113*, 3117–3120

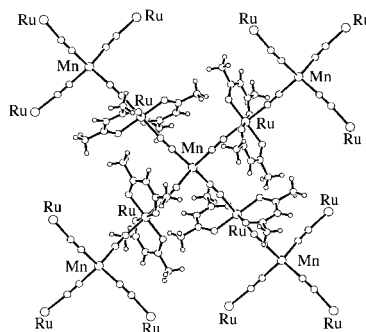
C. M. Snively, G. Oskarsdottir,
J. Lauterbach* 3028–3030

Parallel Analysis of the Reaction
Products from Combinatorial Catalyst
Libraries

Keywords: catalysts • combinatorial
chemistry • heterogeneous catalysis •
high-throughput screening • vibrational
spectroscopy



Cyano-mediated interaction of the alternating high-spin Mn^{II} centers and low-spin Ru^{III} centers in $[\text{Mn}[\text{Ru}(\text{acac})_2(\text{CN})_2]_n]$ (Hacac = acetylacetone) results in long-range ferromagnetic ordering below 3.6 K. This novel $\text{Mn}^{\text{II}}\text{Ru}^{\text{III}}$ coordination polymer, which has a diamond-like structure (see picture), was constructed from $[\text{Ru}(\text{acac})_2(\text{CN})_2]^-$ and Mn^{2+} ions.



W.-F. Yeung, W.-L. Man, W.-T. Wong, T.-C. Lau,* S. Gao* 3031–3033

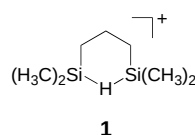
Ferromagnetic Ordering in a Diamond-Like Cyano-Bridged $\text{Mn}^{\text{II}}\text{Ru}^{\text{III}}$ Bimetallic Coordination Polymer

Keywords: coordination polymers • cyanides • magnetic properties • manganese • ruthenium

Angew. Chem. **2001**, *113*, 3121–3123



The unusually high kinetic stability of the silylation **1** is due to the presence of a three-center, two-electron Si-H-Si bond. NMR data (^1H , ^2H , ^{13}C , ^{29}Si) are consistent with the static, symmetrical structure shown, which was further corroborated by the results of density functional calculations.



T. Müller* 3033–3036

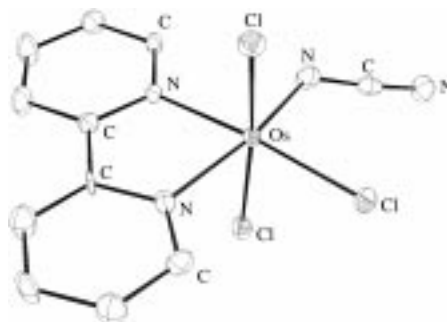
A Silyl Cation with a Three-Center Si-H-Si Bond

Keywords: cations • density functional calculations • isotope effects • NMR spectroscopy • silicon

Angew. Chem. **2001**, *113*, 3123–3126



Oxo-like reactivity exists for a new series of osmium complexes such as $[\text{Os}^{\text{IV}}(\text{bpy})(\text{Cl})_3(\text{NCN})]^-$ (bpy = 2,2'-bipyridine, see structure) containing the cyanoimido ligand. This ligand is formed directly at the metal center by the reaction of Os^{VI} -nitrido precursors with tetraethylammonium cyanide. In the cyanoimido complexes there is an extensive electron-transfer chemistry at the metal center and an extensive functional-group chemistry based on the ligand.



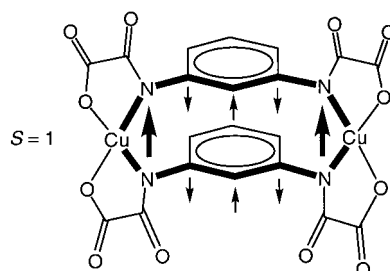
M. H. V. Huynh,* P. S. White, C. A. Carter, T. J. Meyer* ... 3037–3039

Formation and Reactivity of the Osmium(IV)–Cyanoimido Complex $[\text{Os}^{\text{IV}}(\text{bpy})(\text{Cl})_3(\text{NCN})]^-$

Keywords: acylation • alkylation • cyanoimido ligands • osmium • protonation

Angew. Chem. **2001**, *113*, 3127–3129

First organic radicals, now metal complexes: A successful extension to metal complexes of a well-known organic radical approach to ferromagnetism is exemplified by the triplet ground-state molecule containing two Cu^{II} centers connected by a double *m*-phenylenediamide skeleton of the cyclophane type shown in the scheme.



I. Fernández, R. Ruiz,* J. Faus, M. Julve, F. Lloret,* J. Cano, X. Ottenwaelder, Y. Journaux,* M. C. Muñoz . 3039–3042

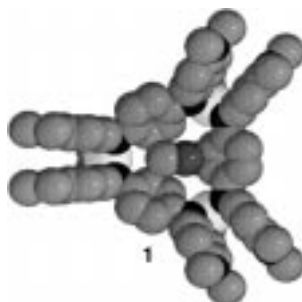
Ferromagnetic Coupling through Spin Polarization in a Dinuclear Copper(II) Metallacyclophane

Keywords: amide ligands • copper • density functional calculations • magnetic properties • metallacycles

Angew. Chem. **2001**, *113*, 3129–3132



A triangle with a calixarene-like cone configuration: the trimetallamacrocycle $[\{\text{Pd}(\text{en})(\text{L})\}_3]^{6+}$ (**1**; $\text{L} = N,N'$ -bis(4-dimethylaminophenyl)-1,4-benzoquinonediimine, en = ethylenediamine; see picture for a space-filling view). The coordination array of the redox-active π -conjugated bridging quinonediimine spacer of **L** and the palladium unit determines whether conjugated **1** or a conjugated polymeric complex is formed.



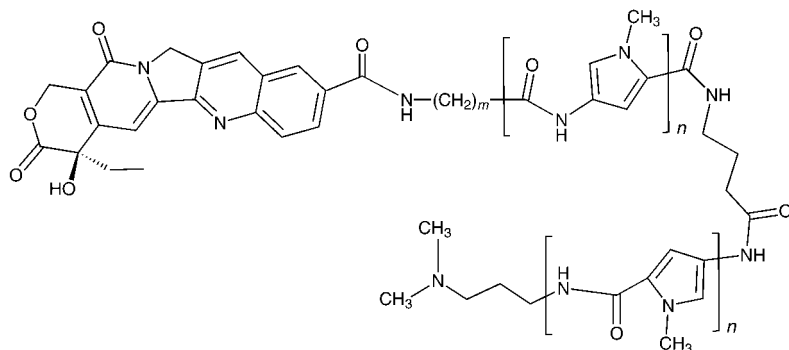
T. Moriuchi, M. Miyaishi, T. Hirao* 3042–3045

Conjugated Complexes Composed of Quinonediimine and Palladium: Controlled Formation of a Conjugated Trimetallic Macrocycle

Keywords: bridging ligands • coordination chemistry • macrocycles • palladium • stacking interactions

Angew. Chem. **2001**, *113*, 3132–3135

The covalent linkage of a hairpin polyamide, which binds in the minor groove, to camptothecin (see picture) provides an efficient system to direct topoisomerase I mediated DNA cleavage to specific sites. These conjugates are equally as potent at targeting the enzyme to a single site in a DNA fragment as camptothecin conjugates of ligands that bind in the major groove (triplex-forming oligonucleotides).



Angew. Chem. **2001**, *113*, 3135–3138

P. B. Arimondo, C. Bailly,
A. S. Boutorine, V. A. Ryabinin,
A. N. Syniakov, J.-S. Sun, T. Garestier,
C. Hélène* 3045–3048

Directing Topoisomerase I Mediated
DNA Cleavage to Specific Sites by
Camptothecin Tethered to Minor- and
Major-Groove Ligands

Keywords: antitumor agents • DNA
cleavage • DNA recognition • drug
research • topoisomerase

Caged but not trapped! Caged reagents are molecules from which biological effectors are released by photolysis. Here, *p*-hydroxyphenacyl bromide is used to derivatize a model peptide containing a thiophosphotyrosine residue in order to mimic phosphorylation at tyrosine. Upon photolysis, a biologically active peptide is released. Peptides and proteins caged on thiophosphotyrosine will be useful in signal transduction research.

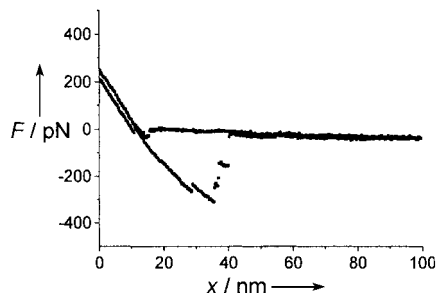
Angew. Chem. **2001**, *113*, 3139–3141

K. Zou, W. T. Miller, R. S. Givens,
H. Bayley* 3049–3051

Caged Thiophosphotyrosine Peptides

Keywords: bioorganic chemistry •
peptides • photolysis • thiophosphate

The adhesion forces between individual molecules of Lewis^x trisaccharide antigen (Le^x) have been measured in water and in calcium solution by using atomic force microscopy (AFM, see graph). These results demonstrate the self-recognition capability of this antigen, and reinforce the hypothesis that carbohydrate–carbohydrate interaction could be considered as the first step in the cell-adhesion process in nature.



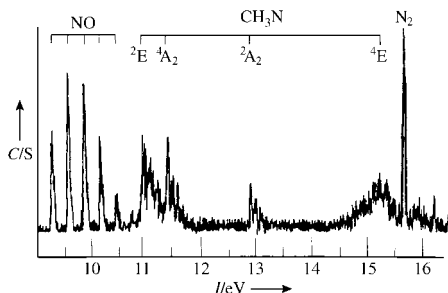
Angew. Chem. **2001**, *113*, 3142–3145

C. Tormas, J. Rojo, J. M. de la Fuente,
A. G. Barrientos, R. García,
S. Penadés* 3052–3055

Adhesion Forces between Lewis^x
Determinant Antigens as Measured by
Atomic Force Microscopy

Keywords: antigens • atomic force
microscopy • carbohydrates • gold •
noncovalent interactions

Pyrolysis of CH₃N₃ under the protection of NO generates a continuous methylnitrene CH₃N diradical beam that enables the ionization energies of different ionic states of the CH₃N diradical to be determined by HeI photoelectron spectroscopy (PES; see spectrum) and both ab initio and density functional theory (DFT) calculations. The ab initio and DFT results are in excellent agreement with the PES experiment and show that the CH₃N diradical has C_{3v} symmetry and the ground state of the CH₃N radical cation is the ²E state.



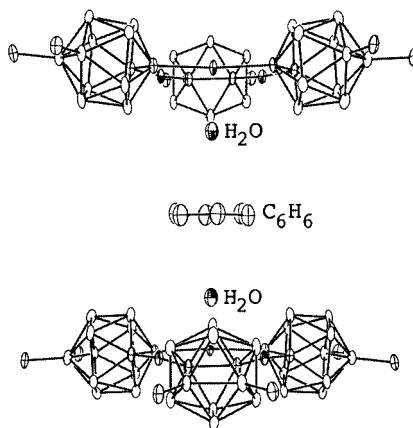
Angew. Chem. **2001**, *113*, 3145–3147

W. Jing, S. Zheng, Z. Xinjiang,
Y. Xiaojun, G. Maofa,
W. Dianxun* 3055–3057

The CH₃N Diradical: Experimental and
Theoretical Determinations of the
Ionization Energies

Keywords: ab initio calculations • density
functional calculations • ionization
potentials • nitrenes • photoelectron
spectroscopy • radicals

Two cyclic Lewis acidic receptors, namely *B*-hexamethyl-9-mecuracarborand-3, [9,12-(CH₃)₂-C₂B₁₀H₈Hg]₃, each host a single water molecule. A benzene molecule is situated between the two complexed water molecules in a sandwich fashion that suggests there is O–H⋯π hydrogen bonding (see structure).



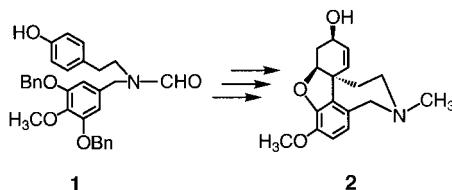
Angew. Chem. **2001**, *113*, 3148–3150

H. Lee, C. B. Knobler,
M. F. Hawthorne * 3058–3060

A Hydrogen-Bonded
[(Mercuracarborand–Water)₂–Benzene]
π-Sandwich Complex

Keywords: carboranes • host–guest
systems • hydrogen bonds •
pi interactions • sandwich complexes

Old problems, new ideas! The biomimetic phenol coupling of norbelladine derivatives such as **1** (Bn = benzyl) to form galanthamine (**2**), a drug used in the treatment of Alzheimer's disease, has been greatly improved by the use of the hypervalent-iodine oxidation reagent phenyliodine(III) bis(trifluoroacetate) (PIFA).



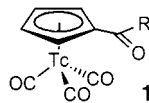
Angew. Chem. **2001**, *113*, 3150–3152

M. Node,* S. Kodama, Y. Hamashima,
T. Baba, N. Hamamichi,
K. Nishide 3060–3062

An Efficient Synthesis of (±)-Narwedine
and (±)-Galanthamine by an Improved
Phenolic Oxidative Coupling

Keywords: biomimetic synthesis • drug
research • enzyme inhibitors • iodine
reagents • oxidation

Instant radiopharmaceuticals—just add [^{99m}TcO₄][−] in water! Half-sandwich complexes of the type [(RCOCp)^{99m}Tc(CO)₃] (e.g. **1**) were synthesized in a single step in water from [^{99m}TcO₄][−]. The acidification of the Cp ring by a carbonyl substituent and the use of K₂[H₃BCO₂] as a reducing agent and in situ source of CO are instrumental in the synthesis.



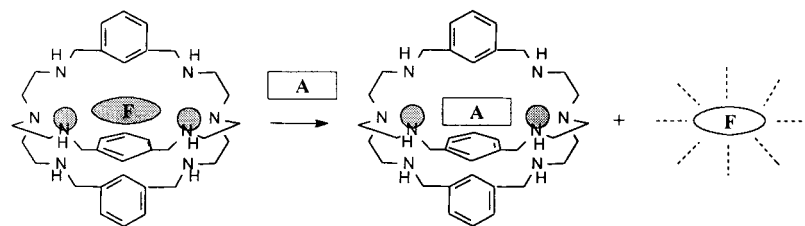
Angew. Chem. **2001**, *113*, 3152–3156

J. Wald, R. Alberto,* K. Ortner,
L. Candrea 3062–3066

Aqueous One-Pot Synthesis of
Derivatized Cyclopentadienyl–
Tricarbonyl Complexes of ^{99m}Tc with an
In Situ CO Source: Application to a
Serotonergic Receptor Ligand

Keywords: carbonylation •
cyclopentadienyl ligands •
radiopharmaceuticals • rhenium •
technetium

Displacement of the loosely encapsulated fluorescent indicator coumarine 343 (F) from a dicopper(II) cryptate enables the selective fluorimetric detection of the HCO₃[−] ion (A) in water (see scheme, ● = Cu²⁺). The fluorophore is quenched when encapsulated, but displays its full emission when released to the solution.



Angew. Chem. **2001**, *113*, 3156–3159

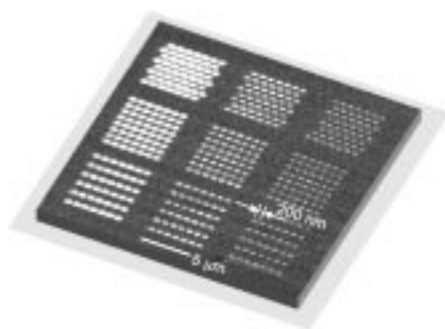
L. Fabbrizzi,* A. Leone,
A. Taglietti 3066–3069

A Chemosensing Ensemble for Selective
Carbonate Detection in Water Based on
Metal–Ligand Interactions

Keywords: anions • cryptates •
fluorescence spectroscopy • molecular
recognition • sensors

Lattices of single polystyrene particles were constructed by using a combinatorial approach to analyze particle pattern recognition properties. Dip-pen nanolithography was used to generate chemical templates of 16-thiohexadecanoic acid on a gold surface to study the two-dimensional assembly of amine- and amide-modified particles (see picture).

Angew. Chem. **2001**, *113*, 3159–3161



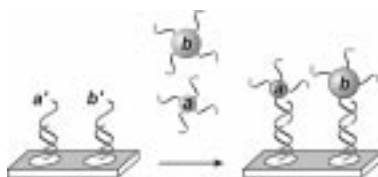
L. M. Demers, C. A. Mirkin* 3069–3071

Combinatorial Templates Generated by Dip-Pen Nanolithography for the Formation of Two-Dimensional Particle Arrays

Keywords: colloids • electrostatic interactions • immobilization • monolayers • nanolithography

Nanoscale construction work with DNA: Dip-pen nanolithography (DPN) is used to generate nanostructures which can be subsequently functionalized with oligonucleotides **a'** and **b'** and used to assemble, in an orthogonal manner, gold nanoparticles (**a**, **b** in scheme) functionalized with sequences complementary to the DPN-generated structures.

Angew. Chem. **2001**, *113*, 3161–3163



L. M. Demers, S.-J. Park, T. A. Taton, Z. Li, C. A. Mirkin* 3071–3073

Orthogonal Assembly of Nanoparticle Building Blocks on Dip-Pen Nanolithographically Generated Templates of DNA

Keywords: DNA recognition • gold • monolayers • nanolithography • nanostructures • self-assembly



Supporting information on the WWW (see article for access details).

* Author to whom correspondence should be addressed



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Issue 15, 2001 was published online on August 3, 2001.

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<http://www.angewandte.com>

CORRIGENDA

The assignment of the ^{15}N NMR signals of N_5^+ in the Communication by **K. O. Christe et al.** in Issue 13/14, 1999, pp. 2004–2010 were inadvertently reversed. The signal at $\delta = -237.3$ (calculated: -235) corresponds to the central N atom N3, and that at $\delta = -100.4$ (calculated: -95) to N1 (see for example Table 1 in this contribution).

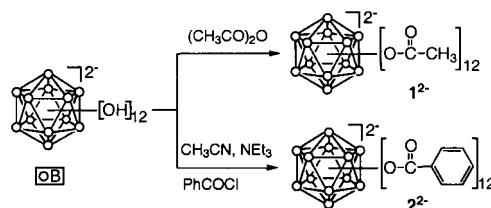
The ^{13}C NMR data given in the Communication by **R. Langer et al.** in Issue 4, 2001, pp. 726–728 for dinitro[2.2]paracyclophane **2b** were for a mixture of isomers. Data for the pseudo-*para* isomer as well as revised data for the nitro[2.2]paracyclophane **2a** and the amino[2.2]paracyclophane **3a** are given here.

2a: $\delta = 34.36, 34.69, 34.91, 35.93, 129.45, 129.86, 132.31, 133.07, 133.12, 136.37, 137.27, 137.73, 139.26, 139.68, 142.02, 149.17$.

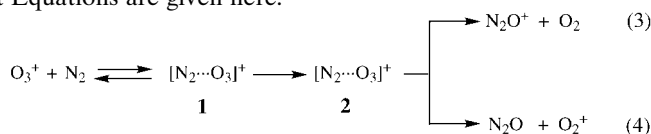
2b: $\delta = 34.17, 34.60, 129.13, 133.87, 136.46, 137.36, 142.00, 149.71$.

3a: $\delta = 32.23, 33.03, 34.95, 35.37, 122.36, 122.99, 124.60, 126.82, 131.48, 132.43, 133.23, 135.44, 138.91, 138.97, 141.06, 144.72$.

In the Communication by **M. F. Hawthorne et al.** in Issue 9, 2001, pp. 1661–1664 there was an error in the equation at the top of the frontispiece: it should read R_2O not $(\text{R}^2\text{CO})_2\text{O}$, and the text at the bottom the page should read “Globular molecules with an icosahedral core—12(12)-closomers—are obtained by total esterification and etherification of $[\text{B}_{12}(\text{OH})_{12}]^{2-}$. For details as well as for the transformation of an anionic closomer ether into the neutral *hypercloso* analogue see the following two communications”. An error in Scheme 1 was also inadvertently introduced; the correct scheme is given here.



In the Communication by **G. de Petris et al.** in Issue 10, 2001, pp. 1938–1941 complexes **1** and **2** in Equations (3) and (4) were erroneously written. The correct Equations are given here.



In the minireview by **P. Braunstein** and **N. M. Boag** in Issue 13, 2001, pp. 2427–2433 the isolobal signs were inadvertently missing from the second sentence of the abstract, the second sentence should read:

The C, Si, and P donor atoms of these ligands are sp^3 -hybridized and the ligands are related to each other by the isolobal analogy: $(\text{CR}_3)^- \rightarrow (\text{SiR}_3)^- \rightarrow \text{PR}_3$.

We would like to apologize for this error.

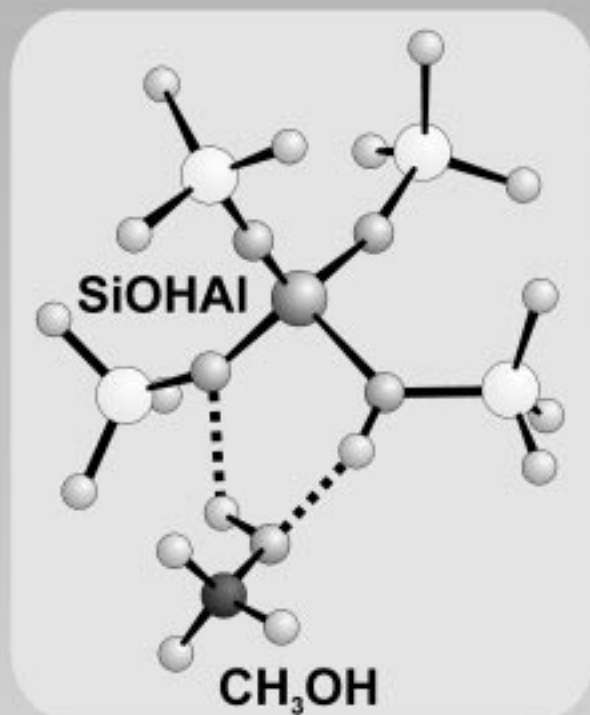
In the communication by **Y. Gu et al.** in Issue 12, 2001, pp. 2320–2322 the protein surface coverages listed in Table 1 were incorrect. Since that publication, the authors identified and corrected a systematic error in the method used to determine surface coverages. The corrected Table is given here. The corrected data show that a spacer arm of three ethylene glycol units (in Gal-3) is sufficient for rgp120 recognition of a galatosyl receptor presented at the surface of a planar DOPC membrane. Longer spacer arm lengths (in Gal-4 and -5) result in less efficient recognition. The authors apologize for this error.

Table 1. Summary of rgp120 binding behavior at planar DOPC membranes, either pure or doped with 5 % (mol mol⁻¹) of Gal-3, -4, or -5 in the outer leaflet.^[a]

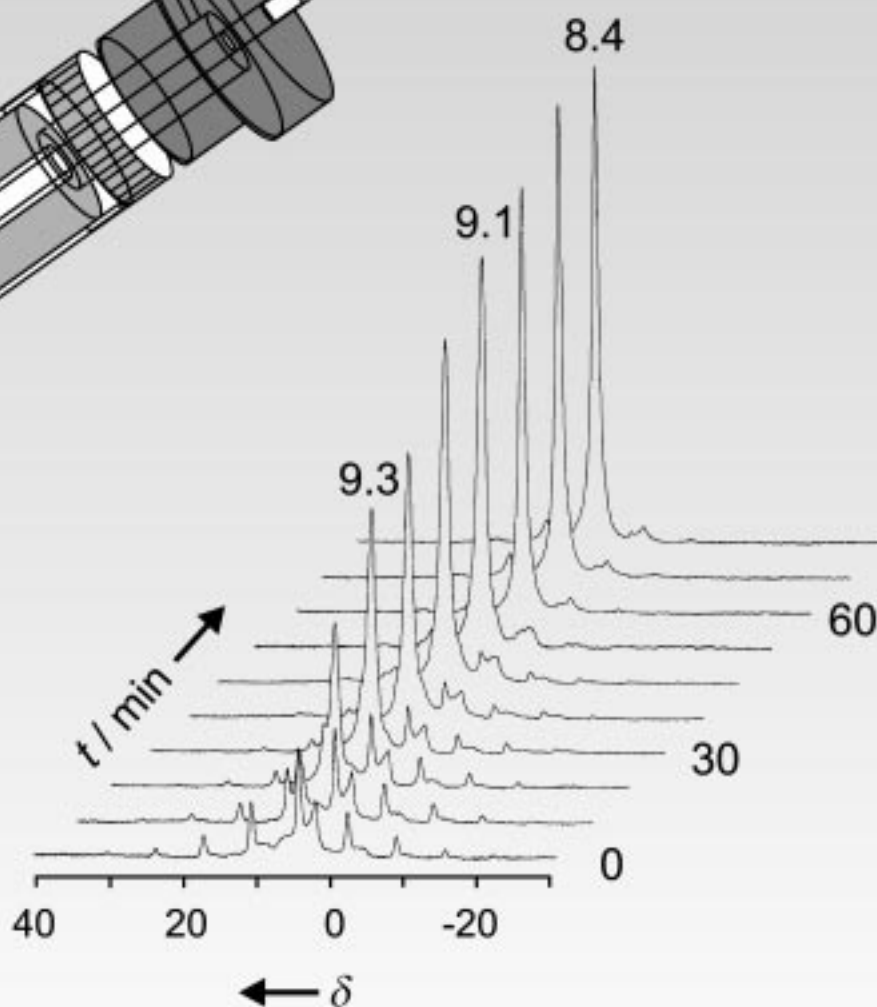
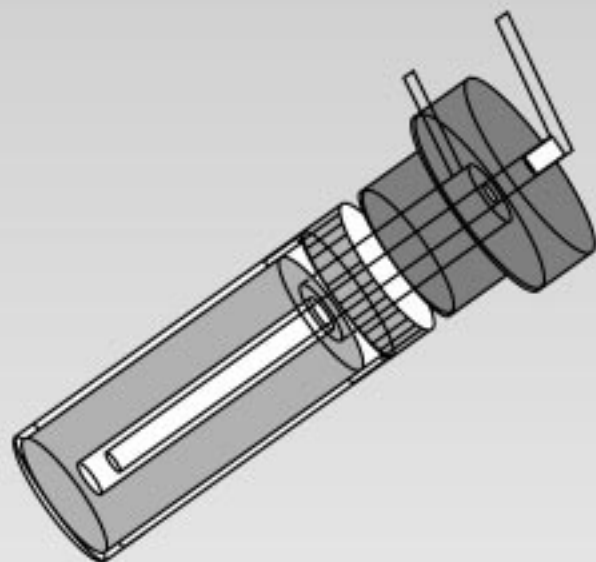
	DOPC	Gal-3	Gal-4	Gal-5
K_a ($\times 10^6$)	–	5.4 ± 1.8	3.8 ± 0.57	2.5 ± 0.57
cooperativity coefficient (ω)	–	1.44 ± 0.20	1.50 ± 0.28	1.59 ± 0.11
surface coverage [$\text{mol cm}^{-2} \times 10^{-13}$] ^[b]	1.6 ± 0.23	3.6 ± 0.57	2.2 ± 0.19	2.2 ± 0.13

[a] All values listed are the mean and standard deviation of two experimental trials. [b] Surface coverages were determined at a dissolved rgp120 concentration of 208 nM, using a modification of the method described by Haldy et al.^[26]

In situ Spectroscopy



B_0



In situ IR, NMR, EPR, and UV/Vis Spectroscopy: Tools for New Insight into the Mechanisms of Heterogeneous Catalysis

Michael Hunger* and Jens Weitkamp*

The development of new solid catalysts for use in industrial chemistry has hitherto been based to a large extent upon the empirical testing of a wide range of different materials. In only a few exceptional cases has success been achieved in understanding the overall, usually very complex mechanism of the chemical reaction through the elucidation of individual intermediate aspects of a heterogeneously catalyzed reaction. With the modern approach of combinatorial catalysis it is now possi-

ble to prepare and test much more rapidly a wide range of different materials within a short time and thus find suitable catalysts or optimize their chemical composition. Our understanding of the mechanisms of reactions catalyzed by these materials must be developed, however, by spectroscopic investigations on working catalysts under conditions that are as close as possible to practice (temperature, partial pressures of the reactants, space velocity). This demands the develop-

ment and the application of new techniques of in situ spectroscopy. This review will show how this objective is being achieved. By the term in situ (Lat.: in the original position) is meant the investigation of the chemical reactions which are taking place as well as the changes in the working catalysts directly in the spectrometer.

Keywords: active centers • heterogeneous catalysis • in situ spectroscopy • reaction mechanisms • solid catalysts

1. Introduction

The elucidation of reaction mechanisms in heterogeneous catalysis is a process that involves the design and selection of a suitable reaction network, the determination of the stoichiometry of each individual intermediate step of this network, and the identification and characterization of the active centers, the intermediate products, the activation processes of the reactants, and the surface reactions. In addition to the methods of reaction kinetics increasing use is being made of the modern procedures of in situ spectroscopy for the elucidation of these reaction networks. In this review an overview of the state of development and the applications of the most important techniques of IR, NMR, EPR, and UV/Vis spectroscopy in heterogeneous catalysis will be presented. IR, NMR, and EPR spectroscopy are universally used methods for the investigation of catalytically active sites in solid catalysts and the reactants interacting with these sites. UV/Vis spectroscopy is particularly suitable for the observa-

tion of chemically excited states of hydrocarbons. An important advantage of all four methods lies in the possibility of being able to investigate working catalysts in contact with the reactants under near-practice conditions, that is under reasonably high partial pressures of these reactants. This requirement is also met by X-ray diffraction (XRD), X-ray absorption spectroscopy (EXAFS, XANES), and Raman spectroscopy.^[1–4] These methods, not described in this work, are finding increasing application in the characterization of active sites in solid catalysts under in situ conditions. Similarly, spectroscopic methods that require the use of high vacuum, such as X-ray and UV photoemission spectroscopy (XPS and UPS), electron energy loss spectroscopy (EELS), and Auger electron spectroscopy (AES) will not be dealt with.^[1–4] In these methods, which have convincingly established their strength in numerous investigations of ideal solid-state surfaces, the practical partial pressures of the reactants lie several orders of magnitude below those of industrial processes (*pressure gap*). Special attention will be devoted to zeolites, not only due to their ideal crystalline structures, but also in view of their widespread use in numerous processes of industrial chemistry. In addition, a large number of amorphous and crystalline solid catalysts of importance to industrial chemistry will be discussed.

Much of the spectroscopic work on heterogeneously catalyzed reactions was performed under batch conditions,

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that is after a single loading of the activated catalyst with reactants in a sealed sample or spectroscopic cell. In industrial chemistry, however, processes are frequently operated under flow conditions, that is, with a continuous feed stream of reactants and removal of products. For this reason, work on the development and application of new in situ techniques for spectroscopic investigations under flow conditions is being pursued by a growing number of groups. Catalyst formation and deactivation as well as the formation of surface complexes and intermediates in the steady state of a reaction can be observed spectroscopically with these techniques. An overview of new applications of the most important of these spectroscopic techniques will also be presented. Owing to the extensive nature of this topic, no claim to completeness is made. An introduction to the basic principles and classical uses of spectroscopic methods in heterogeneous catalysis may be found in the literature.^[2–4]

2. Infrared Spectroscopy

Infrared spectroscopy is based upon the interaction of electromagnetic radiation with species that possess a permanent or induced dipole moment and the excitation of different vibrational states. An IR spectrometer usually records the energy of the electromagnetic radiation which is transmitted through a sample as a function of the wavenumber or frequency. Today, nondispersive spectrometers are almost exclusively used when the total spectrum is analyzed by an interference process and transformed into the frequency or wavenumber range by means of a Fourier transform (FTIR

spectroscopy). In recent years diffuse reflectance spectroscopy (DRS) in the region of infrared radiation has been used increasingly for the characterization of solid catalysts. The so-called DRIFT technique (*diffuse reflectance infrared Fourier transform*) is used in those cases where the sample does not have adequate transparency. A quantitative evaluation of band intensities requires the knowledge of extinction coefficients which are a function of the wavenumber. In some cases the extinction coefficients can be taken from the literature, but they are often limited in their accuracy. The high sensitivity of IR spectroscopy is of considerable advantage for in situ investigations in the area of heterogeneous catalysis. With its help it is possible to carry out investigations under both batch conditions and flow conditions.

Figure 1 shows the scheme of an IR flow cell developed by Karge and Nießen for transmission operation which is

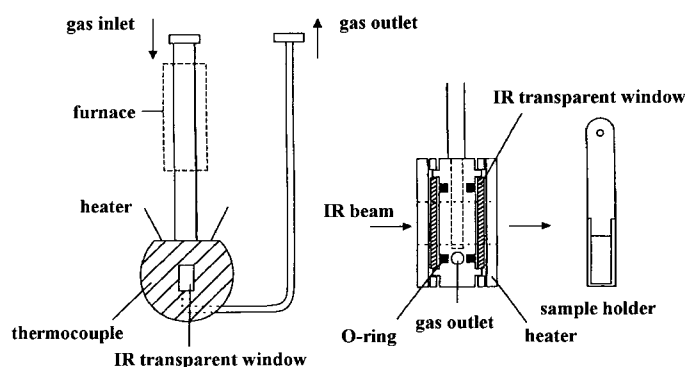


Figure 1. Schematic representation of an IR flow cell for in situ investigations of heterogeneously catalyzed reactions.^[5]

Michael Hunger studied physics at the University of Leipzig and gained his doctorate in 1984 at the Department of Experimentalphysik. At that time he was engaged on the spectroscopic investigation of solid acids, in particular zeolites. In 1992, after his Habilitation and a number of periods abroad, he moved to the Institute of Chemical Technology of the University of Stuttgart. Two years later he was appointed Lecturer in Chemical Technology. In 1999 he was appointed Professor at the Faculty of Chemistry of the University of Stuttgart. In the same year he was appointed member of the management board of the DECHEMA Working Group Zeolites. His interests are centered on the development of new techniques of solid-state NMR and IR spectroscopy for the characterization of micro- and mesoporous solid catalysts and in situ spectroscopy of heterogeneous catalytic reactions.



M. Hunger



J. Weitkamp

Jens Weitkamp studied chemistry at the University of Karlsruhe and the Free University of Berlin. He obtained his doctorate and Habilitation at the Faculty of Chemical Engineering of the University of Karlsruhe. From 1986 to 1988 he held the post of C3 professor for Chemical Technology at the University of Oldenburg, and since 1988 he has been Head of the Institute of Chemical Technology of the University of Stuttgart. His main areas of research are heterogeneous catalysis, separation and purification technology by adsorption, and materials science with an emphasis on zeolites. He is currently President of the International Zeolite Association, Editor-in-Chief of the journal Microporous and Mesoporous Materials and a member of the board of directors of DECHEMA e.V., Frankfurt am Main, as well as of the Deutsche Wissenschaftliche Gesellschaft für Erdöl, Erdgas und Kohle e.V., Hamburg.

characterized by a particularly short optical path and thus a low gas-phase excitation.^[5] The cell allows in situ calcination of a self-supporting sample, compressed to a very thin wafer ($\rho_{\text{surface}} \approx 10 \text{ mg cm}^{-2}$), in an oven located in the upper section. After calcination the sample is moved into the optical path with a magnet where it is measured in a vacuum or under the influence of an adsorbate or reactant. The most important areas of application of in situ spectroscopy in heterogeneous catalysis are the characterization of the framework of solid catalysts after their preparation and during the process of catalyst modification, the investigation of the species acting as adsorption centers or catalytically active sites, and the characterization of adsorbate complexes and intermediates on the surfaces of solid catalysts.^[6]

2.1. IR Investigations of the Preparation of Solid Catalysts and the Interaction of Catalytically Active Sites with Reactants

Classical areas of application of IR spectroscopy in the characterization of solid catalysts are the investigation of framework vibrations of silicates and aluminosilicates and the OH stretching region of acidic solid-state catalysts.^[7–12] The region of OH stretching of calcined, crystalline aluminosilicates is made up of bands of the terminal and defect SiOH groups at 3740 cm^{-1} , the high-frequency (HF) bands of the accessible bridging OH groups in zeolites at 3610 to 3650 cm^{-1} , and the low-frequency (LF) bands of bridging OH groups in the small cages of zeolites at about 3540 cm^{-1} .^[10] IR spectroscopy is frequently used for the characterization of bridging OH groups which are acting as Brønsted centers in zeolites. Different probe molecules (e.g. pyridine, benzene, CO) are used for the determination of the accessibility and acid strength of these Brønsted centers,^[13, 14] when the decay or displacement of the stretching bands of the OH groups are investigated. Lewis acid sites of these materials and the framework oxygen atoms acting as bases are characterized, for example, with pyridine or with chloroform and methylacetylene (see ref. [13, 14] and references therein).

An interesting technique for the investigation of the preparation and modification of solid catalysts is the ATR/FTIR spectroscopy (ATR: *attenuated total reflectance*). This technique, developed by Moser and co-workers, is based upon a multiple reflection of the infrared radiation within a cylindrical crystal rod (CIR: *cylindrical internal reflectance*) which is led through the reaction space of the synthesis autoclave or reactor when it comes into contact with the fluid or solid-phase material under investigation.^[15, 16] Calabro et al. used ATR-FTIR spectroscopy for in situ investigations of the synthesis of silicon-rich, mesoporous MCM-41 materials.^[17] In the spectral window restricted by the use of ZnS as crystal rod, bands of template deformation vibrations occur at 1500 to 1350 cm^{-1} and those of internal asymmetric Si-O-Si stretch vibrations at 1250 to 1000 cm^{-1} . At room temperature, a synthesis experiment with cetyltrimethylammonium hydroxide as template showed two dominant bands at 1104 cm^{-1} and 997 cm^{-1} , an indication that this template promotes the formation of double 4-ring units (D4R), that are cubic

octamers ($\text{Si}_8\text{O}_{20}^{8-}$). These D4R units disintegrate rapidly on increasing the temperature with the formation of oligomers with very different band positions. The resulting MCM-41 material shows two bands at 1045 to 1060 cm^{-1} and 1210 to 1221 cm^{-1} , the intensity and wavenumbers of which are dependent upon the texture. Holmes et al. undertook analogous investigations and made a quantitative evaluation of the intensities of these bands. They found that the rate of synthesis of the MCM-41 material is determined by the dissolution process of the silicon source.^[18]

In the investigation of the reduction of V_2O_5 catalysts by NH_3 and H_2 Sullivan et al. used infrared emission spectroscopy (IRES: *infrared emission spectroscopy*).^[19] Here the sample was situated on a heated sample table within the IR cell. The radiation emitted from the heated sample is collected by a parabolic mirror and deflected into the FTIR spectrometer whose IR source is switched off.^[19, 20] By means of IRES, the near-surface species can be investigated preferentially. With this technique Sullivan et al. observed the stepwise reduction of V^{5+} and V^{4+} in V_2O_5 and found that the rate of reduction is limited by the solid-state diffusion of O atoms.^[19]

2.2. H/D Exchange on Solid Catalysts

H/D exchange between surface OH groups and adsorbate molecules is a valuable method for the investigation of acid strength of OH groups and the mechanism of acid-catalyzed reactions. Armendariz et al. investigated the H/D exchange between deuterated benzene and the OH groups on sulfated ZrO_2 catalysts.^[21] The authors observed that OH groups whose stretching bands appear at 3630 cm^{-1} form hydrogen bonds to adsorbed benzene molecules, whereas H/D exchange occurs between the OH groups at 3300 cm^{-1} and adsorbed C_6D_6 molecules. On the basis of this finding the OH groups absorbing at 3300 cm^{-1} were assigned a greater acid strength than those at 3630 cm^{-1} . Lee et al. investigated the H/D exchange between CH_4 , C_2H_6 , C_3H_8 , and C_6H_{14} with the acidic OH groups of deuterated ferrierite.^[22] By comparison of the activation energies of the H/D exchange reaction determined experimentally (130 to 143 kJ mol^{-1}) with data calculated by quantum-mechanical methods, the authors derived a reaction mechanism which passed through a neutral transition state.

Kondo et al. have investigated the mechanism of the double-bond isomerization of 1-butene on acid forms of mordenite and ZSM-5 zeolite.^[23–25] With deuterated zeolites the authors were able to establish that starting from the π complex of 1-butene at the SiODAl groups, a reaction to *cis*- and *trans*-2-butene occurred even at 230 K without an H/D exchange of the acidic OD groups. H/D exchange, which causes a reduction in the intensity of the OD stretching band and an increase in the OH stretching band of the π complex, was observed only after a further increase in temperature. At temperatures below 230 K , the double-bond isomerization of 1-butene thus occurs without formation of a protonated intermediate species. On D-ZSM-5 zeolite a temperature above 250 K leads to dimerization of the 2-butenes, whereas

on D-mordenite 2-butenes form paraffinic alkoxy groups even at temperatures as low as 230 K.^[26]

In the reaction of acetone and ammonia on the acidic zeolites H-Y, H-Beta, H-ZSM-5, and H-mordenite, Kotrla et al. observed the formation of dimethylketimine ((CH₃)₂C=NH, DMKI).^[27] A band at 1707 cm⁻¹ was identified as the C=N- stretching band of the >C=NH₂⁺ group of the protonated DMKIH⁺, which forms ion-pair complexes with framework oxygen anions. This assignment was confirmed by a H/D exchange between the catalyst and the reactants, which shifts the C=N- stretching band to 1683 cm⁻¹ (>C=NHD⁺).

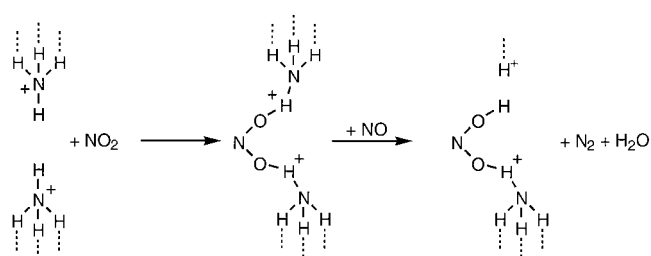
2.3. IR Investigations of the Decomposition of NO on Metal-Containing Solid Catalysts

The reactive surface species which are formed during the decomposition of NO and the selective catalytic reduction (SCR) of NO_x on zeolites exchanged with copper have already been reviewed.^[9] The characteristic wavenumbers of these surface complexes can be obtained from the literature.^[28–39] A frequently used catalyst for the decomposition of NO is Cu-ZSM-5 zeolite (see ref. [40, 41] and references therein). After loading this material with NO, Ganemi et al. observed bands at 1631, 1589 to 1575, and 2130 cm⁻¹ which the authors ascribed to nitrate groups bridged to Cu²⁺-O-Cu²⁺ dimers, monodentate nitrate species bonded to isolated copper ions, and NO⁺ ions on the zeolite framework.^[40] The authors postulated N₂O₃ species bound to Cu²⁺-O-Cu²⁺ dimers as the reactive intermediates which are not detectable spectroscopically because of their short life span.

Konduru et al. used different transient species techniques to investigate the dynamic behavior of the adsorbates during the decomposition of NO on Cu-ZSM-5 zeolites.^[41] With these techniques the authors were able to demonstrate that NO decomposition on Cu-ZSM-5 zeolites leads to a very rapid establishment of the equilibrium between gaseous NO and Cu⁺(NO) and Cu²⁺(NO₃)⁻¹ species on the catalyst surface. The formation of N₂ immediately after the appearance of the band of Cu⁺(NO) species suggests that the NO decomposition is initiated by Cu⁺ ions.

2.4. IR Investigations of the Selective Catalytic Reduction of NO_x

The mechanism of the selective catalytic reduction of NO with NH₃ on H-ZSM-5 zeolites and H-mordenite has been investigated by Eng and Bartholomew.^[42] Bands for NH₃ and NO₂ species appeared in the in situ FTIR spectra of the most reactive samples. Based on their experimental data, the authors suggested a reaction mechanism in which a NO₂ species forms an active surface complex with pairwise aligned NH₃ species which reacts with NO to form N₂ (Scheme 1). Centeno et al. used the DRIFT technique for the investigation of the SCR of NO with NH₃/O₂ on an V₂O₅/Al₂O₃ catalyst.^[43] They interpreted their results on the basis of an Eley–Rideal mechanism the essence of which is that an adsorbed reactant, that is here the ammonium species, reacts



Scheme 1. Proposed mechanism for the catalytic reduction of NO with NH₃ on H-ZSM-5 zeolites and H-mordenites.

with other, nonadsorbed reactants, that is in this case gaseous NO. The authors rejected a coordinately bound ammonia since mainly NH₄⁺ ions were observed at the reaction temperature with the highest NO turnover.

Acke et al. investigated the SCR of NO with HNCO and O₂ on a Pt/γ-Al₂O₃ catalyst at temperatures of 373 to 723 K.^[44] During the reaction the authors observed the formation of isocyanate species at 2263 and 2240 cm⁻¹, surface-coordinated ammonia at 1587 and 1270 cm⁻¹, and ammonium ions at 1656, 1500, and 1477 cm⁻¹ (Figure 2). The authors proposed a

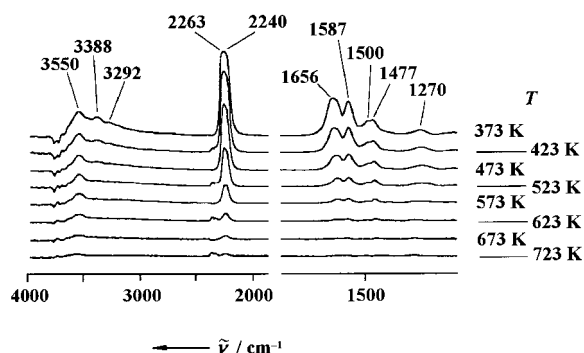


Figure 2. IR spectra of γ-Al₂O₃ recorded after the reaction of a mixture of HNCO, NO, and O₂ at temperatures in the range 373 to 723 K.^[44]

mechanism in which adsorbed ammonia and ammonium species are important species in the formation of N₂ and N₂O. Similarly, Captain and Amiridis investigated the SCR of NO with propene on a Pt/γ-Al₂O₃ catalyst.^[45] The in situ FTIR spectra of Pt/γ-Al₂O₃ and γ-Al₂O₃ measured during the introduction of NO into the measurement cell indicated the formation of nitrate species on the γ-Al₂O₃ support and the adsorption of NO onto the platinum. After the introduction of C₃H₆, the authors observed the formation of carboxyl species and the adsorption of CO onto the platinum. In contrast, when a mixture of NO, C₃H₆, and O₂ was used, two bands of cyanide and isocyanate species appeared. The occurrence of the band of the isocyanate species under optimal reaction conditions indicates that these surface complexes are reactive intermediates of the SCR. In the reaction of NO and NO₂ on H-mordenite, Gerlach et al. observed a band which they attributed to chemisorbed nitrosyl cations (NO⁺).^[46] The authors were able to demonstrate that these nitrosyl cations are very reactive and react with C₃H₆ to form acrylonitrile. The authors suggested a reaction mechanism with 3-nitrosopropene and propenal oxime as intermediates. Zeolites exchanged with cobalt proved to be particularly active in the

SCR of NO with methane. NO is adsorbed mainly as highly stable dinitrosyl species on Co zeolites, whereas mononitrosyl species are very unstable.

2.5. The Conversion of Hydrocarbons on Metal-Containing Solid Catalysts

De La Cruz and Sheppard have investigated the dehydrogenation ethane on a Pt/SiO₂ catalyst.^[47] Even at a reaction temperature of 90 K the authors observed a band at 2920 cm⁻¹, which is indicative of the formation of a monolayer of di-σ-C₂H₄ or di-σ/di-π-C₂H₄ species on platinum particles. Whereas the IR spectrum of the Pt/SiO₂ catalyst loaded with ethene contained bands of π-C₂H₄ complexes, the authors found no indications of the formation of such species after the dehydrogenation of ethane at 294 K. Weng et al. have carried out high-temperature in situ FTIR investigations (1023 to 1073 K) on the oxidative coupling of methane on LaOF and BaF₂/LaOF catalysts.^[48] They established that gas-phase oxygen is activated to superoxide (O₂⁻) on the catalyst surface. This O₂⁻ species reacts with CH₄ with the formation of C₂H₄ and CO₂.

The synthesis of methanol from H₂/CO₂ and H₂/CO on Cu/SiO₂ and Cu/ZrO₂/SiO₂ catalysts has been investigated by Fisher and Bell.^[49, 50] Cu/ZrO₂/SiO₂ proved to be the more effective catalyst on which both CO₂ and CO interact preferentially with ZrO₂ leading to the formation of monodentate and bidentate carbonate and bidentate bicarbonate. During the course of the surface reaction, these species are finally hydrogenated to solely bidentate formate species and methoxy species, where the presence of copper has an accelerating effect. Copper also promotes the reductive elimination of the methoxy species as methanol. A further increase in the activity of Cu/SiO₂ was observed by modification of the catalyst with a mixture of TiO₂ and ZrO₂.^[51] Wokaun and co-workers used the DRIFT technique for the investigation of the synthesis of methanol on Cu/ZrO₂.^[52] In a multistep experiment, paraformaldehyde and formic acid (¹³C labeled and in the natural abundance) were adsorbed and then hydrogenated. The authors established that methanol is synthesized solely via η²-H₂CO (η²-H₂¹³CO: 1130 cm⁻¹) as intermediate, not via formate species, whose asymmetric and symmetric ¹³C–O stretching vibrations appear at 1525 and 1335 cm⁻¹, respectively.

Voskoboinikov et al. used the DRIFT technique for the investigation of the Diels–Alder cyclodimerization of 1,3-butadiene to vinylcyclohexene on H-Y, Cu-Y, Cu-Beta, and Cu-EMT zeolites.^[53] During the conversion of butadiene on H-Y zeolites, the authors observed broad bands of alkenyl cations, which are formed by the cationic oligomerization of the reactant. In contrast, the formation of polycyclic naphthenes with a low ratio of CH₃ to CH₂ groups occurred on zeolites exchanged with copper.

2.6 Acid-Catalyzed Reactions of Hydrocarbons

Dardas et al. and Süer et al. have used a CIR cell (see Section 2.1) in their investigation of the cleavage of heptane

on H-Y zeolite under subcritical and supercritical conditions.^[54, 55] At a pressure of 4 MPa the authors observed an increased concentration of heptane in the pores of the cracking catalyst and a change in the stretching vibrations of the C–H bonds as a result of increased generation of intermolecular hydrogen bonds. Supercritical conditions led to a partial regeneration of the catalysts deactivated in the subcritical range.

In their work Bordiga et al. turned their attention to the polymerization of acetylene, methylacetylene, and ethylacetylene on H-ZSM-5 catalysts.^[56] The authors identified as catalytically active sites bridging OH groups whose OH stretching bands appear at 3610 cm⁻¹. The bands at 3260 and 1950 cm⁻¹ occurring immediately after the adsorption of acetylene were explained by OH and C=C stretching vibrations of the adsorbed complexes bonded through hydrogen bonds. In contrast, ethylacetylene does not form hydrogen-bonded complexes. These molecules were protonated immediately after adsorption. After an increase in the reaction temperature to 420 K, the start and progress of the polymerization of the acetylene could be monitored by the appearance of bands at 1702, 1640, 1580, and about 1500 cm⁻¹. These bands correspond to C=C stretching vibrations of monomeric C₂H₃⁺, dimeric C₄H₅⁺, trimeric C₆H₇⁺, and polymeric C_{2n}H_{2n+1}⁺ species, respectively. Geobaldo et al. investigated the reaction of propene and the formation of oligomers on H-mordenite at 100 to 300 K (Figure 3).^[57] The weakening of

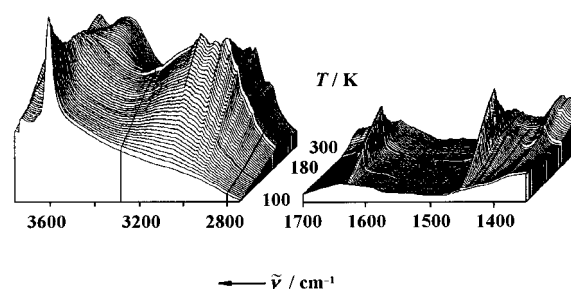


Figure 3. FTIR spectra of H-mordenite recorded during the reaction of propene in a temperature range of 100 to 300 K.^[57]

the bands of the bridging OH groups at 3609 cm⁻¹ observed with increasing temperature is caused by hydrogen bond formation of these hydroxy protons with propene molecules, which at the same time leads to a shift of the OH band to 3100 cm⁻¹ and the appearance of a C=C stretching band of the adsorbate molecule at 1632 cm⁻¹. A band at 1380 to 1366 cm⁻¹ is an indication of the existence of CH(CH₃)₂ groups in protonated species. The protonation of the adsorbed propene molecules and their oligomerization commenced at temperatures above 180 K and led to characteristic stretching vibration bands of the CH₃ and CH₂ groups in oligomers.

Trombetta et al. investigated the skeletal isomerization of *n*-butene to isobutene on γ-Al₂O₃ and H-ZSM-5 zeolite and the interaction of 1-butene, *cis*-2-butene, *trans*-2-butene, *sec*-butanol, and *tert*-butyl alcohol on these catalysts.^[58, 59] γ-Al₂O₃ proved to be the more suitable catalyst for the isomerization of *n*-butene to isobutene at 753 K. At 733 K the H-ZSM-5 zeolite exhibited a higher turnover of *n*-butene but with a low

selectivity for isobutene. At 250 K the reaction of *n*-butene on H-ZSM-5 zeolite led to the formation of *tert*-butoxy species which are considered to be intermediates in the polymerization of isobutene.^[59] In contrast, no IR bands of *tert*-butoxy species were detected on γ -Al₂O₃. In the opinion of the authors at the temperatures which are necessary to generate alkoxy species on γ -Al₂O₃ (>473 K) the decomposition of these surface species occurs faster than their generation.^[58] The adsorption and reaction of isobutene on deuterated D-mordenite has been investigated by Ishikawa et al.^[60] Below 160 K the authors observed the formation of adsorbate complexes with strong π bonding character to the acidic OD groups of the D-mordenite. The increase in the band intensity of the interaction-free SiODAl groups occurring at 170 K was explained by a dimerization of the π complexes of the isobutene molecules. By adsorption of 2,4,4-trimethyl-1-pentanol on ZrO₂ and comparison of the spectra it was possible to show that isobutene dimerization on the D-mordenite leads to the formation of 2,4,4-trimethyl-1-pentoxo species. During investigations on the skeletal isomerization of *n*-butene to isobutene on H-ferrierite, Wichterlova et al. found a relationship between the isobutene yield and the concentration of bridging OH groups in the 10-ring channels.^[61] According to the authors, Lewis acid sites in the neighborhood of bridging OH groups promote the dimerization and oligomerization of the butene and the subsequent cleavage of the reaction products into C₃, C₄, and C₅ alkanes.

Flego et al. investigated the hydrocarbon deposition arising during the alkylation of isobutane with 1-butene on a LaH-Y zeolite (coke).^[62] They observed a decrease in oligomerization of 1-butene with an increase in the fraction of isobutane in the reaction mixture. Attempts at desorption of the coke deposition at temperatures above 523 K led to the formation of a band attributable to aromatic compounds at 3050 cm⁻¹.

The disproportionation of ethylbenzene on H-Y zeolite has been investigated by Karge and co-workers under flow conditions.^[63] The authors found that from the start of the reaction to the establishment of the steady state the composition of the sorbates on the catalyst changes continuously, with a constant decrease in the ethylbenzene concentration. After an induction period of about one hour the steady state was reached simultaneously on the adsorbent and in the gas phase. This experimental finding was used to suggest that the induction period in the disproportionation of ethylbenzene is caused by a delayed establishment of the equilibrium between the gas phase and the adsorbate phase consisting of benzene, ethylbenzene, and the different diethylbenzenes.

Veefkind and Lercher studied the amination of methanol on H-mordenite.^[64] For this purpose methoxy groups were prepared on the catalyst and their reaction with ammonia was observed. The authors found that the reaction rate of the methylammonium ions formed from methoxy groups was comparable to the reaction rate of the direct alkylation of ammonium ions. In view of the low number of free Brønsted sites which are available for the formation of methoxy groups under the reaction conditions, the amination of alkoxy species as a reaction pathway should be, however, of low significance.^[64]

2.7 Base-Catalyzed Reactions of Hydrocarbons

In recent years increased efforts have been devoted to the development of solid catalysts for base-catalyzed reactions. FTIR spectroscopy has proved a valuable method for the characterization of the base strength of these materials (see ref. [13] and references therein). One reaction investigated by a number of groups is the alkylation of toluene with methanol which is directed towards side chain alkylation by basic solid catalysis. An important intermediate step, which was investigated with the help of IR spectroscopy, was the conversion of methanol on the solid base.^[65, 66] King and Garces investigated the side chain alkylation of toluene with methanol on Cs-X, Rb-X, K-X, Na-X, and Li-X zeolites.^[67] In the reaction of methanol on Cs-X, Rb-X, and K-X zeolites, bands of monodentate and bidentate surface formate species were observed. IR spectroscopic experiments suggest that only the monodentate surface formate species contribute to the side chain alkylation of toluene. In their investigations on the side chain alkylation of toluene on hydrotalcite, MgO and Cs-X, Rb-X, K-X, and Na-X zeolites, Lercher and co-workers found a preferred adsorption of toluene on strongly basic zeolites, whereas less basic zeolites adsorbed methanol preferentially.^[68, 69] Based on these spectroscopic investigations three criteria for an active catalyst for side chain alkylation of toluene with methanol could be formulated: 1) adequate base strength for the dehydrogenation of methanol to formaldehyde, 2) sites for the stabilization of toluene and for polarization of the methyl group of this molecule, and 3) establishment of a suitable sorption equilibrium of the reactants toluene and methanol.

3. NMR Spectroscopy

Modern NMR spectrometers (NMR: *nuclear magnetic resonance*) record the decay of the magnetic induction of nuclear spin produced by irradiation with a radio frequency pulse which after a Fourier transform produces the spectrum of resonance signals of nuclear spins as a function of frequency. The most important parameter of solid-state NMR spectroscopy is the chemical shift, δ , which is caused by the shielding effect of the electron shell and which gives information on the chemical nature of the atoms under study. Information on the near structure of the atoms can be obtained with help of the dipole–dipole interaction between spatially neighboring nuclear spins and the quadrupolar interaction between the electrical nuclear quadrupole moment (in nuclei with spin $I \geq 1$) and the electrical field gradient at the site of the nucleus. The dipolar interaction gives data on distances between nuclei, whereas the quadrupole interaction reflects the charge distribution in the environment of the nucleus and thus the atom coordination. The broad solid-state NMR signals produced by solid-state interactions require the use of the technique of rapid sample rotation around an axis in the magic angle (MAS: *magic angle spinning*) with whose help the anisotropic solid-state interactions can be averaged out or at least reduced. Air-bearing turbines with sample rotation frequencies of up to 30 kHz are

used almost exclusively for rapid sample rotation. The use of the MAS technique causes significantly higher demands on the preparation of samples and the procedures of in situ experiments than is the case for IR and EPR spectroscopy. For in situ measurements under batch conditions, catalyst samples can be fused in symmetrical glass ampoules which fit in MAS rotors (see ref. [70–72]). Another possibility exists in the use of special loading apparatus such as, for example, CAVERN equipment (CAVERN: cryogenic adsorption vessel enabling rotor nestling), which allows the preparation of samples in gas-tight MAS rotors (Figure 4).^[73]

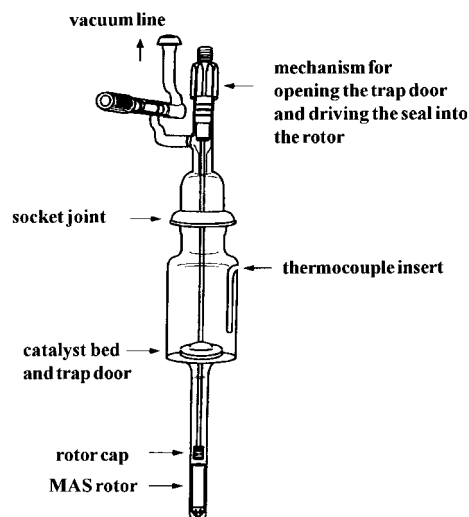


Figure 4. Schematic representation of a CAVERN apparatus for the preparation of catalyst samples for in situ MAS NMR spectroscopy.^[73]

With the CAVERN equipment connected to a vacuum line catalyst samples can be calcined and repeatedly loaded with reactants before the MAS rotor is sealed with a gas-tight cap. In a technique used by many groups for in situ MAS NMR investigations of heterogeneously catalyzed reactions under flow conditions, MAS turbines with an axially arranged injection tube are used through which a continuous flow of reactants is led into the MAS NMR rotor during the NMR experiment.^[74] After the conversion of the reactants in the catalyst bed shaped as a hollow cylinder the reaction products leave the MAS NMR rotor through an annular gap in the rotor cap or are instead bled off at this point through a pump. In the latter case it is possible to couple the in situ MAS NMR probe directly with an on-line gas chromatograph (Figure 5).^[75–77] In another in situ NMR/GC technique, the heterogeneously catalyzed reaction is carried out in a micro-pulse reactor and stopped within a few hundred milliseconds by cooling with liquid nitrogen (pulse-quench reactor). The catalyst loaded with reactants is then transferred to a MAS NMR rotor at low temperatures and measured.^[78] Investigations on reaction kinetics are carried out with MAS NMR probes with Laser pulse heating.^[79–81] In this technique the course of a reaction as a function of time is monitored by the use of short heating pulses. Further methods for in situ MAS NMR spectroscopy may be found in the literature.^[82–85]

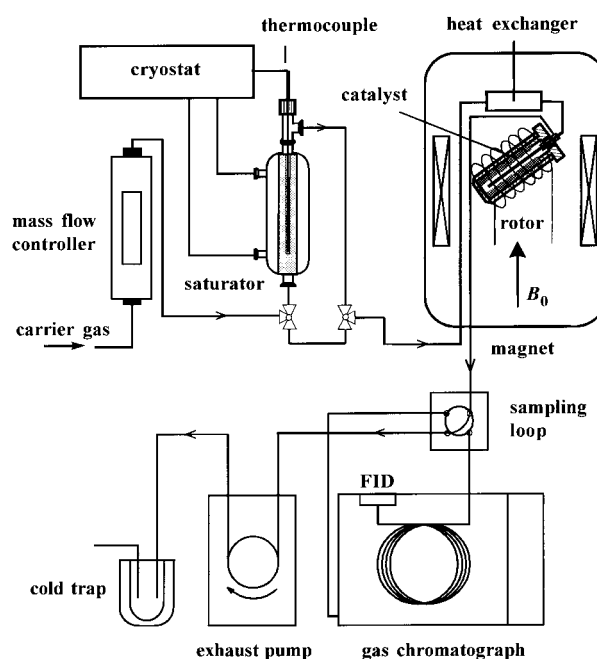


Figure 5. Schematic representation of the equipment applied for in situ MAS NMR experiments under flow conditions coupled with on-line gas chromatography.^[77]

The wide range of the chemical shifts of the reactants and their characteristic resonance positions are an advantage for the use of ^{13}C MAS NMR spectroscopy in in situ investigations of heterogeneously catalyzed reactions. With direct excitation of the ^{13}C nuclei, the intensities of the NMR signal appearing in the spectra can be correlated directly with the concentration of the spins. Disadvantages for NMR spectroscopy are its low sensitivity in comparison to IR and EPR spectroscopy and the observation times of up to a few milliseconds, which impedes the investigation of short-lived intermediates. Currently, a number of groups is working on the development of a technique to improve the sensitivity of NMR spectroscopy by the injection of Laser-polarized xenon in the sample volume. With this technique, the polarization of the spin system necessary for the NMR experiment is amplified by contact of the resonant nuclei with optically excited xenon.^[86–88] This is linked with the possibility of a selective excitation of the nuclei of atoms and molecules on the outer surface of a solid catalyst.^[89] Although many of the atoms which serve as building blocks for the catalyst framework are accessible to NMR spectroscopy by means of isotopes with a nuclear spin, further discussion will be concentrated on investigations of surface OH groups and the reactants of heterogeneously catalyzed reactions

3.1. The Characterization of Acid Sites and Their Interaction with Probe Molecules and Reactants

Calcined solid catalysts provide ^1H MAS NMR signals of metal OH groups at chemical shifts of $\delta = -0.5$ to $+0.5$, SiOH groups at $\delta = 1.2$ to 2.2 , and bridging OH groups in zeolites at $\delta = 3.6$ to 4.3 .^[6, 72] Hydrogen bonds of these OH groups to

neighboring oxygen atoms or probe molecules shift these resonance positions to greater values by 20 ppm. A considerable advantage of ^1H MAS NMR spectroscopy compared to IR spectroscopy lies in the quantitative assessment of the signal intensities, which permits a direct determination of the OH concentrations. Different probe molecules are used for characterization of the acid strength of solid-state OH groups. Pyridine forms a hydrogen bond with weakly acidic hydroxy protons (low field shift by ca. 8 ppm) and is protonated by strongly acidic bridging OH groups (signals at $\delta = 15$ to 20).^[6, 72] A likewise frequently used probe molecule is trimethylphosphane (TMP), which gives a ^{31}P MAS NMR signal at $\delta \approx -3$ through interaction with Brønsted acid centers, whereas in an interaction with Lewis acid centers a ^{31}P MAS NMR signal is produced at $\delta = -32$ to -58 .^[90, 91]

A more precise differentiation of the acid strength of strongly acidic OH groups is possible with the aid of the ^{13}C NMR shift of the carbonyl atoms of adsorbed acetone molecules. The resonance shift relative to the resonance position of the carbonyl carbon in pure acetone arising from adsorption onto acidic OH groups of zeolites lies between 10.1 ppm for H-SAPO-5 and 18.7 ppm for H-ZSM-22.^[92] Haw et al. have reported a scale of ^{13}C NMR shifts of the carbonyl atoms of acetone adsorbed on different acidic catalysts and they compare these values with the resonance positions of the molecules in H_2SO_4 solutions of different concentration.^[73] According to Haw et al., the acid strength of the bridging OH groups in the zeolite H-ZSM corresponds to an 80 vol% H_2SO_4 solution.

3.2. H/D Exchange on Acidic Catalysts

In analogy to IR spectroscopy, ^1H MAS NMR spectroscopy also offers the possibility to investigate H/D exchange between surface OH groups and adsorbed molecules over a large temperature range. With this technique Beck et al. calculated activation energies of 107, 85, and 60 kJ mol^{-1} , respectively, for H/D exchange between deuterated benzene and acidic HNa-Y, USY, and H-ZSM-5 zeolites.^[93] Freude and co-workers were able to confirm these values applying a Laser MAS NMR probe.^[94, 95] They calculated activation energies of 102 and 93 kJ mol^{-1} for H-Y zeolites which were exchanged by 85 and 92 %, respectively. According to Beck et al. the activation energies for benzene complexes determined by quantum mechanical probability density calculations, in which the benzene is attached to the acidic bridging OH groups of the zeolites by van der Waals bonds agreed well with the experimental values.^[93] The authors concluded from this that H/D exchange between the OH groups and benzene does not take place through the formation of a carbocation intermediate of protonated benzene. This assumption was confirmed by ^{13}C MAS NMR investigations of benzene adsorbed on H-Y zeolite. No signal for a carbocation appeared, only a signal for adsorbed benzene.

Stepanov et al. investigated the H/D exchange between deuterated propane and the acidic OH groups of H-ZSM-5 zeolite by ^1H MAS NMR spectroscopy over a temperature range of 457 to 543 K.^[96] The authors determined activation

energies of 108 kJ mol^{-1} for an exchange of the propane methyl groups, and 117 kJ mol^{-1} for an exchange of the propane methylene group. These values correspond to the activation energies which were calculated by quantum-chemical methods for a hydrogen exchange of methane (122 kJ mol^{-1})^[97] and ethane (118 kJ mol^{-1}).^[98] The calculations were based upon a pentacoordinate transition state of the carbon atoms for the hydrogen exchange. A further argument against the assumption of a propyl cation as transition state was that the exchange was not regiospecific.^[96] In contrast, Sommer et al. observed a regiospecific exchange for the H/D exchange between deuterated isobutane and the acidic OH groups of H-ZSM-5 zeolite, with an activation energy of 50 kJ mol^{-1} .^[99] The authors explained the differences between the activation energies for H/D exchange derived experimentally and those mentioned previously by differences in the reactivities of linear and branched alkanes. The regioselectivity, on the other hand, is an indication of the formation of *tert*-butyl cations as intermediates in the reaction.^[99]

3.3 The Reaction of Hydrocarbons on Acidic Catalysts

In recent years a number of reviews on in situ ^{13}C MAS NMR spectroscopy of adsorbed complexes on solid catalysts and the investigation of heterogeneously catalyzed reactions has been published.^[70, 71, 73, 100–103] After the adsorption of olefines and alcohols onto acidic H-ZSM-5 and H-Y zeolites, various groups have observed the formation of alkoxy species with the ^{13}C MAS NMR shifts given in parenthesis for the carbon atoms attached to oxygen: methoxy groups ($\delta = 58$),^[104] ethoxy groups ($\delta = 68$),^[98] isopropoxy groups, ($\delta = 87$),^[105] and *tert*-butoxy groups ($\delta = 77$ to 86).^[106–108] Because of the low activation energy for the transition of the alkoxy species into the alkylcarbenium ion state, Aronson et al. called these species surface complexes with carbenium-like properties.^[106] During investigations of the synthesis of methyl *tert*-butyl ether (MTBE) from isobutene and methanol on a H-Beta zeolite under flow conditions, ^{13}C MAS NMR signals of secondary and tertiary carbon atoms in alkoxy species were observed at chemical shifts of $\delta = 77$ to 90.^[109] A simultaneous analysis of the products leaving the MAS NMR rotor reactor by on-line gas chromatography showed that the intensities of the signals correlate with the yields of MTBE.^[77] With different zeolite catalysts weaker signals for the alkoxy species were found at $\delta = 77$ to 90 for the less active materials (H-Y, H-ZSM-5).^[110] These observations suggest that alkoxy species can act as reactive components and have an influence upon the course of the reaction.

Today it is assumed that reactive carbenium ions on acidic solids only exist as transition states.^[111] Consequently these species cannot be observed directly by NMR spectroscopy. An indirect proof of carbenium ions adsorbed on acidic catalysts is possible, however, with reactions whose mechanisms are based upon the trapping of these species by other reactants. Stepanov and Luzgin have investigated the reaction of acetonitrile with 1-octene and *tert*-butyl alcohol on acid H-ZSM-5 zeolite at 296 K under batch conditions, and

observed a ^{13}C MAS NMR signal at $\delta = 108$ which they attributed to *N*-alkylnitrilium cations.^[112] The authors explained the formation of these alkylnitrilium cations by the "trapping" of chemically unstable alkylcarbenium ions which themselves are formed by adsorption of 1-octene on the acidic OH groups of the H-ZSM-5 zeolite. This experiment was confirmed by Barich et al. with a pulse-quench reactor (see Section 3).^[113] Parameters of the ^{13}C shielding of the alkylnitrilium cation calculated by probability density calculations agree very well with the values determined experimentally.

Carbenium ions present on the surface of acidic catalysts which are detectable directly by NMR spectroscopy usually possess an alkyl-substituted, cyclic structure and are formed, for example by the reaction of the respective olefine or diene with the acidic zeolite. For a H-Y zeolite which was loaded with ^{13}C -enriched propene, Haw et al. observed ^{13}C MAS NMR signals at $\delta = 249$, 145, and 47 which they assigned to alkyl-substituted cyclopentadienyl cations.^[114] A signal of the trivalent carbon atom of the carbenium ion of propene, the propyl-2 cation, which occurred in superacid solutions at $\delta \approx 320$ was not observed.^[114] The formation of alkyl-substituted cyclopentadienyl cations was also observed in the reaction of methanol and acetone on H-ZSM-5 zeolite with a pulse-quench reactor.^[78, 115, 116] It was assumed, however, that these very stable carbenium ions are the final step of a number of reactions and at the best play only a secondary role in the course of the reaction. Reviews on carbenium ions in acidic zeolites observed with in situ ^{13}C MAS NMR spectroscopy are found in the literature.^[73, 100]

With isotopically labeled reactants under controlled conditions in fused glass ampoules it is possible to use ^{13}C MAS NMR spectroscopy for the clarification of reaction pathways. With this technique, Derouane et al. and Ivanova et al. have investigated the isopropylation of benzene on H-ZSM-11 zeolite,^[117] the transformation of *n*-hexane on aluminum/magnesium oxides and K-L zeolites modified with Pt and Pd,^[118, 119] the dehydrogenating cyclodimerization of propane and the alkylation of benzene with propane on gallium-modified H-ZSM-5 zeolites,^[120, 121] and the activation of propane on H-ZSM-5 zeolite.^[122] The mechanism of the formation of *n*-propylbenzene from cumene in the presence of benzene as side reaction of cumol synthesis on H-ZSM-11 zeolite has been investigated by Ivanova et al. by the reaction of two different benzene/cumene mixtures under identical reaction conditions.^[117] In reaction 1 the carbon atoms in the aromatic ring of cumenes were enriched with ^{13}C , in experiment 2 those of benzene. The ^{13}C -enriched aromatic carbon atom of cumene to which the isopropyl group is bonded has a chemical shift of $\delta = 149$. The disappearance of this signal in experiment 1 and its appearance in experiment 2 is caused by the transfer of propyl groups from the ^{13}C -enriched cumene molecules to the nonenriched benzene molecule and vice versa. Thus this experiment provides the evidence for an intermolecular reaction mechanism for the formation of *n*-propylbenzene on acid zeolites.

A large number of ^{13}C MAS NMR investigations is concerned with the conversion of methanol into hydrocarbons (MTG: methanol to gasoline, MTO: methanol to olefins) on acid zeolites under batch conditions, that is in sealed glass

ampoules. The zeolites H-ZSM-5,^[123–129] H-SAPO-5,^[127, 130] H-SAPO-11,^[130] H-SAPO-34,^[128, 131–133] H-Y,^[127] H-EMT,^[127] H-ZSM-12,^[134] H-ZSM-23,^[130] H-erionite,^[135] H-mordenite,^[127] and H-offretite,^[135, 136] as well as montmorillonite,^[137] and saponite^[137] have been used as catalysts. Even at reaction temperatures of 373 to 423 K, dehydration of methanol ($\delta = 50$) adsorbed on acidic OH groups to dimethyl ether ($\delta = 61$) occurs. This intermediate is converted into C_2 hydrocarbons such as ethene ($\delta = 123$) after a further increase in temperature. In a series of the work described above methoxy groups, whose signal appears at $\delta \approx 58$, have been debated as reactive species. In in situ ^{13}C MAS NMR experiments on the conversion of methanol on a H-ZSM-5 zeolite under flow conditions signals occurring at chemical shifts of $\delta = 12$ to 25 and 125 to 131 were explained by the formation of a hydrocarbon pool consisting of C_4 to C_6 olefins (Figure 6).^[138]

Simultaneous with the measurement of the ^{13}C MAS NMR spectra, the reaction products leaving the MAS NMR rotor reactor were analyzed by gas chromatography with the technique illustrated in Figure 5 (numerical values on the left side of Figure 6). The presence of C_4 to C_6 olefins on a

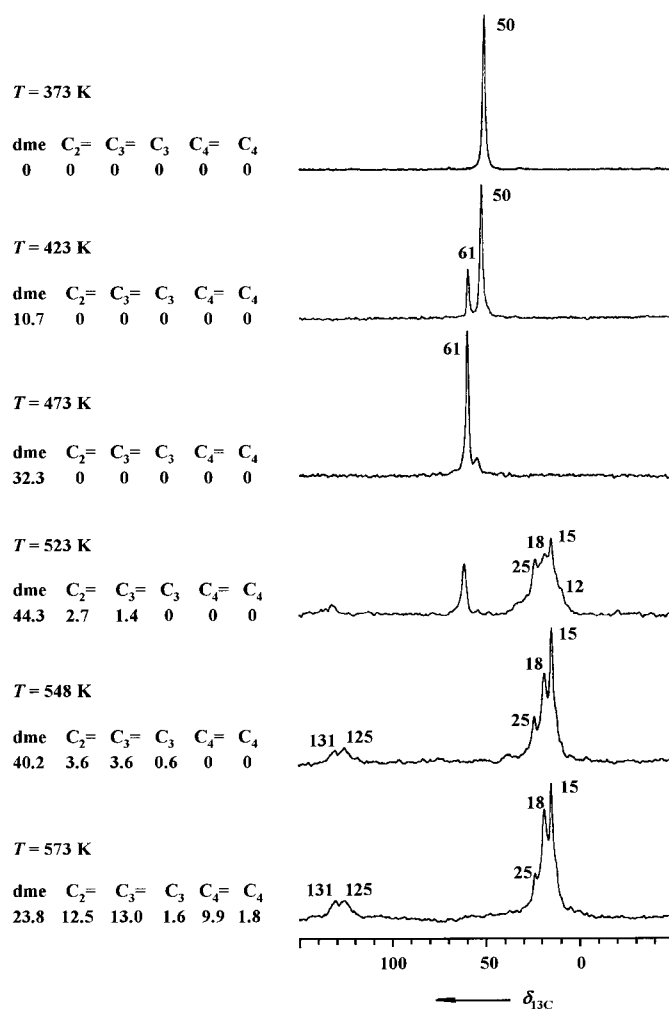


Figure 6. ^{13}C MAS NMR spectra recorded during the reaction of $^{13}\text{CH}_3\text{OH}$ on H-ZSM-5 zeolite under flow conditions with a modified residence time of $W/F = 25 \text{ g h mol}^{-1}$ and at reaction temperatures of 373 to 573 K. On the left side the yields (%) of dimethyl ether (dme), ethene ($\text{C}_2=$), propene ($\text{C}_3=$), propane (C_3), butenes ($\text{C}_4=$), and butanes (C_4) measured simultaneously by on-line gas chromatography are given.^[138]

working zeolite catalyst during the conversion of methanol provides experimental confirmation of the hydrocarbon pool mechanism previously suggested by von Haag in 1984.^[139] According to this mechanism, a pool of higher hydrocarbons, predominantly olefins, to which on the one hand methanol and/or dimethyl ether couple and which otherwise undergo cationic β -cleavage, is present in the pores of acid zeolites during the conversion of methanol.

3.4. The Base-Catalyzed Conversion of Hydrocarbons

Unlike the Brønsted acid sites of solid catalysts, which are directly accessible to FTIR and ^1H MAS NMR spectroscopy, the characterization of oxygen atoms of the catalyst framework acting as base sites requires the use of molecular probes. Because of the necessary isotopical enrichment and the strong quadrupolar interactions of these nuclei (spin $I = 5/2$), the use of ^{17}O MAS NMR spectroscopy has hitherto been of limited use.^[140–143] Bosacek has suggested the use of the ^{13}C NMR shift of methoxy groups which are bound to the Si-O-Al bridges of the zeolite framework for the determination of the base strength of these materials.^[144, 145] After the preparation of these methoxy groups by loading the catalysts with methyl iodide, Bosacek found a correlation between the chemical shifts of the methoxy groups and the mean Sanderson electronegativity, S^m ,^[146] of the zeolite framework. According to this correlation, a low ^{13}C NMR shift of the methoxy groups ($\delta = 54$ for CsNa-X zeolite)^[145] corresponds to a low mean electronegativity or a high base strength of the zeolite framework and vice versa. Methoxy groups on highly basic guest components, for example clusters of alkali metal acetates and oxides on Y and X zeolites, give signals in the range $\delta = 48.7$ to 50.0 .^[147–149]

^{13}C MAS NMR investigations on the side chain alkylation of toluene with methanol on X and Y zeolites exchanged with alkali metal cations have been carried out under both batch and flow conditions.^[148, 150, 151] In agreement with Lazo et al. and Murray et al., signals observed at $\delta = 166$ to 168 were attributed to formate species which arise from the conversion of methanol on the basic zeolite framework (see also Section 2.7).^[152, 153] Formaldehyde, which would be formed by the dehydration of methanol, could not be detected. It was observed, however, that during the side chain alkylation of toluene on an impregnated X zeolite exchanged and impregnated with cesium ions, the formation of ethylbenzene was first initiated at temperatures at which methanol was already fully converted into formate species.^[151] The formate species formed from methanol could thus act as reactive surface species. This assumption was supported by in situ experiments under flow conditions where an increase in the toluene content in the reactant flow caused a reduction in the ^{13}C MAS NMR signal of the formate species at $\delta = 166$.^[151]

4. EPR Spectroscopy

Electron paramagnetic spectroscopy (EPR), also called electron spin resonance (ESR), is suitable for the characterization of paramagnetic species with one or more unpaired

electrons. The magnetic moment of the electrons responsible for the resonance transitions in a magnetic field is a function of the electron spin operator, the Bohr magneton, and the g -factor. If the nucleus of the atom at which the unpaired electron is localized possesses a nuclear spin an interaction of the magnetic moment of the electrons with the magnetic moment of the atom nucleus and thus a splitting of the EPR signal occur. This interaction is called hyperfine interaction (hf). Its parameter is the hyperfine coupling constant (a tensor). If there is also an interaction of the unpaired electron with the nuclear spin of atoms in the local structure a superhyperfine coupling (shf) occurs which causes a further splitting of the EPR signal. By determination and analysis of the g tensor, the a tensor, and the shf splitting, EPR spectroscopy can provide important information on paramagnetic species and their near structure.

A special feature of EPR spectroscopy is its high sensitivity, which allows active sites and intermediates of chemical reactions with very low abundance in the framework or on the surface of catalysts under investigation. In the catalyst framework the unpaired electrons necessary for detection by EPR spectroscopy are formed by defects or by a substitution of the framework atoms. The molecules accessible to EPR spectroscopy must exist as radicals on the surface of the catalyst, formed, for example by a spontaneous oxidation at a Lewis acid sites or through irradiation of the sample with γ -quanta.^[154–158] Short relaxation times of electron spin and exchange processes lead to a broadening of the EPR signals. The use of EPR spectroscopy is therefore often limited in its maximum measurement temperature.^[159] Frequently, EPR measurements are carried out at temperatures of 77 K or below after stopping the reaction. In situ cells differ in their construction according to whether they are used for investigations under batch conditions or under flow conditions.^[160–162] The EPR measurement cell for reactions under flow conditions illustrated in Figure 7 was designed for the X-band, that is a resonance frequency of $\nu_0 = 9.5\text{ GHz}$ or a wavelength of $\lambda = 3\text{ cm}$, and consists of a reactor tube with an internal diameter of 3 mm to which is attached a heating wire. It is charged with 200 to 400 mg catalyst powder through which reactants are passed in a vertical direction. Typical applications of EPR spectroscopy in heterogeneous catalysis are investigations on the preparation and formation of catalysts, the characterization of adsorbed species, and investigations of heterogeneously catalyzed reactions.^[159, 163–165] To the first two points count both the spectroscopic characterization of the framework building blocks which act as active sites, and the adsorbate molecules and reactants.

4.1. EPR Investigations of Metal Ions in Solid Catalysts and Their Interactions with Reactants

Isolated Cu^{2+} ions in zeolites exchanged with copper produce spectra with characteristic anisotropies of the g tensors and the hf splitting which are dependent upon the cation site which the Cu^{2+} ions occupy. Table 1 shows a list of these parameters for Cu^{2+} ions in A, X, and Y zeolites as well as chabasite and mordenite.^[166] After in situ calcination of

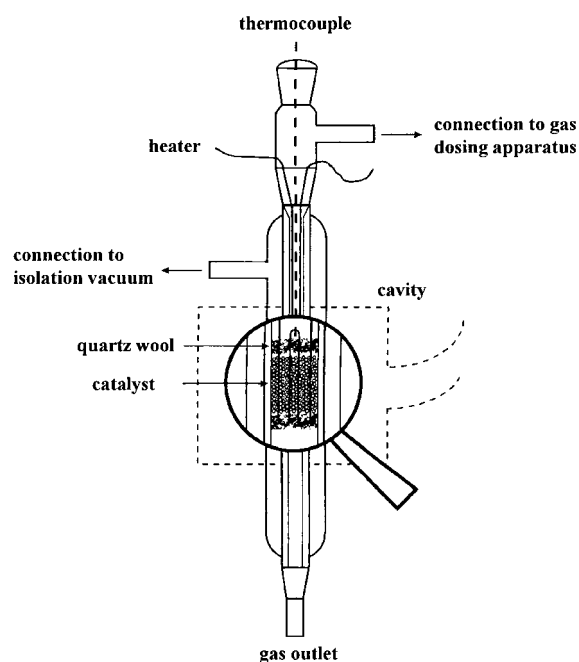


Figure 7. Schematic representation of an EPR resonator for in situ investigations of heterogeneously catalyzed reactions under flow conditions.^[162]

Table 1. EPR parameters of Cu^{2+} ions at different cation positions in various zeolites and Cu^{2+} in H-mordenite and H-ZSM-5 zeolite mixed physically with different copper compounds and calcined in situ at 823 K, 973 K, and 1073 K.^[166, 167]

Catalyst	Cation site	$g_{\parallel}$	$g_{\perp}$	$a_{\parallel}$ [mT]	$a_{\perp}$ [mT]
zeolite A	I	2.397	2.065	12.6	0.22
zeolites X and Y	II	2.387	2.073	12.4	1.34
zeolite X	III	2.354	2.068	14.0	1.60
zeolite Y	I'	2.332	2.067	15.7	1.86
chabasite	I'	2.340	2.073	16.0	2.00
mordenite	I	2.327	2.062	15.4	1.49
mordenite	VI	2.277	2.057	16.8	1.19
H-mordenite/CuO/823 K		2.325	2.055	14.4	1.9
HNa-ZSM-5/CuO/823 K		2.33	2.07	14.2	1.75
H-ZSM-5/CuO/1073 K		2.29	2.05	15.6	2.3
H-ZSM-5/CuF ₂ /973 K		2.31		15.3	
H-ZSM-5/Cu ₃ (PO ₄) ₂ /973 K		2.29		15.6	

mixtures of H-mordenite (H-M), HNa-ZSM-5 zeolite, and H-ZSM-5 zeolite with CuO, CuF₂, and Cu₃(PO₄)₂ at temperatures between 823 K and 1073 K (e.g. H-M/CuO/823 K), EPR signals were observed which correspond to Cu^{2+} at cation sites (see Table 1 below).^[167] This observation shows that a thermal treatment of physical mixtures of copper compounds with zeolites in their H form leads to a migration of the copper atoms into the zeolite pores and to a cation exchange. In the use of Cu-ZSM-5 zeolite as catalyst for the selective reduction of NO_x , the change in the oxidation state of copper in the interaction of reactants and trace gases (He , CH_4 , C_3H_6 , $\text{C}_2\text{H}_5\text{OH}$, CO , NO , NO_2 , H_2O , O_2) is important.^[168] Thus in a stream of CH_4 an extensive reduction in the intensity of the EPR signal of the Cu^{2+} cations ($g_{\parallel} = 2.28$ and $a_{\parallel} = 17$ mT) was observed which is again totally reversed by changing to an O_2 or NO stream.^[168] In a stream of He mixed with C_3H_6 , NO , and O_2 the reduction of some of the Cu^{2+} ions

of the CuH-ZSM-5 zeolite occurs even at room temperature.^[169]

Paramagnetic Pt species are formed by the exchange of Na-Y zeolite in an aqueous solution of $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ and subsequent reduction with H_2 .^[170] Ghosh and Kevan investigated Na-X and Ca-X zeolites exchanged with palladium as catalysts for the dimerization of ethene.^[171] Paramagnetic species with anisotropic g -tensors were found. These species were attributed to Pd^+ ions which act as catalytically active sites and coordinate ethene before it is converted into butene. A contribution of Pd^{3+} ions to the EPR spectrum was excluded.

A number of groups has investigated the incorporation of Ni, Co, and Mn into the framework of micro- and mesoporous aluminophosphates and the accessibility of these metal ions for probe molecules.^[172–175] After reduction of NiH-SAPO-5 and Ni-SAPO-5 with H_2 , different Ni^+ ions with symmetric g tensors are present.^[175] Electron spin echo experiments with ^{31}P modulation made it possible to detect different localizations of these nickel species in SAPO-5. In NiH-SAPO-5, the Ni^+ ions are located in the centers of hexagonal prisms (SI position), whereas the nickel atoms in Ni-SAPO-5 are preferentially tetrahedrally coordinated into the framework. Experiments on the adsorption of ethene showed that nickel incorporated into the framework forms adsorbed complexes consisting of two ethene molecules, whilst Ni^+ ions on SI positions coordinate only one ethene molecule.^[175]

EPR spectra of Fe^{3+} ions in zeolites often consist of two signals at $g = 2.0$ and $g = 4.2$ and allow a differentiation of the nature of the iron species present (see ref. [176–179] and references therein). Hexaaqua Fe^{3+} complexes in a Na-Y zeolite exchanged with iron lead to a strong signal at $g = 2.0$. After calcination of an Fe-ZSM-5 zeolite prepared by sublimation of FeCl_3 the signal of the Fe^{3+} ions at $g \approx 2.0$ disappears and signals at $g = 4.27$, $g = 5.6$ and 6.5 appear which Kucherov et al. assigned to tetrahedrally coordinated or distorted tetrahedrally coordinated extra-framework Fe^{3+} species.^[179] If this SCR catalyst is exposed to a mixture of NO , C_3H_6 , and O_2 a significant reduction of the extra-framework Fe^{3+} species at $g = 4.27$ occurs. Recent work has been concerned with the characterization of iron in aluminophosphates and silicoaluminophosphates. Fe^{3+} ions in aluminophosphate $\text{AlPO}_4\text{-5}$ ($\text{Fe}/\text{AlPO}_4\text{-5}$) also produce EPR signals with g -factors of 2.0 and 4.3 which are explained by octahedrally coordinated Fe^{3+} ions on framework positions and by Fe^{3+} ions on defect sites, respectively.^[180] The EPR spectra illustrated in Figure 8 were taken in steps of 20 K during the calcination of $\text{Fe}/\text{AlPO}_4\text{-5}$ in an air stream after synthesis (as-synthesized). The signal of Fe^{3+} ions on defect sites at $g = 4.3$ falls in the temperature range of 373 K to 573 K, whereas at the same time a considerable broadening of the signal of the Fe^{3+} on framework positions at $g = 2.0$ may be observed. A narrow signal occurring at $g = 2.0$ in the temperature range of 453 to 613 K is caused by the thermal decomposition of the template and the formation of paramagnetic coke species. After complete calcination of the template residues, spectra of Fe^{3+} ions are obtained which consist of three signal components. The analysis of the EPR

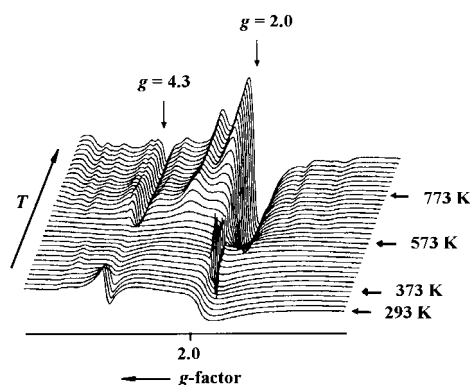


Figure 8. X-band EPR spectra ($0 < B_0 < 600$ mT) of Fe/AlPO₄-5 recorded during calcination ($293 \text{ K} < T < 773 \text{ K}$) in a stream of dry air. The measurement temperature was increased in stages of 20 K.^[180]

spectrum taken after calcination at 773 K gives a fraction of Fe³⁺ ions on framework positions of 45 %.

Other materials which have been investigated for their suitability as SCR catalysts are H-ZSM-5 zeolites modified with chromium and rhodium (Cr/H-ZSM-5, Rh/H-ZSM-5),^[181–184] and γ -Al₂O₃ modified with rhodium.^[183, 184] After calcination of the Cr/H-ZSM-5 zeolite at 773 K in O₂ and He a symmetrical signal of Cr⁵⁺ ions with an hf splitting of $a = 4.5$ mT and an shf splitting of 0.74 mT is formed.^[181] The shf splitting is an indication that the Cr⁵⁺ ions are isolated and incorporated into the neighborhood of framework aluminum atoms. During adsorption of propene onto the Cr/H-ZSM-5 zeolite under flow conditions an irreversible reduction of the Cr⁵⁺ signal occurs which indicates a reduction of the Cr⁵⁺ ions by propene.

Vanadium–phosphorus oxides (VPO) are materials which are used inter alia for the selective oxidation and oxidative amination of hydrocarbons. VO²⁺ ions which show an hf splitting of $a_{\parallel} = 20.2$ mT and $a_{\perp} = 7.2$ mT have been debated as the catalytically active sites of these VPO catalysts.^[185] If neighboring VO²⁺ ions are present spin–spin exchange and averaging of the hf splitting occurs. In investigations of the oxidative amination of toluene and the selective oxidation of *n*-butane on VPO catalysts under flow conditions, the spin–spin exchange behavior between VO²⁺ ions on the catalysts surface was determined by a signal shape analysis.^[162, 185, 186] The authors found a correlation between the catalytic activity of the VPO catalysts in the oxidative amination of toluene and the strength of the spin–spin exchange.^[185] It could thus be confirmed that isolated VO²⁺ sites, between which no spin–spin exchange occurs, do not participate in the catalytic process. The proposed reaction mechanism is based on vanadyl dimers with an oxidation state of the neighboring vanadium atoms changing between +4 and +3 during the oxidative amination of toluene and the concurrent reduction and reoxidation steps at the vanadium centers.

4.2. EPR Investigations of Adsorbate Molecules on Solid Catalysts

The time scale of EPR spectroscopy, which is of the order of nanoseconds, allows the determination of the mobility of

molecules in the pores of solid catalysts. By means of temperature-dependent EPR spectroscopy, Biglino et al. found a translation movement or a rotary diffusion for NO₂ on mordenite, ferrierite, and ZSM-5, Beta, L, X, and Y zeolites, dependent upon the respective structure.^[187] Roduner et al. investigated the mobility of organic radical cations in zeolites by a quantitative analysis of the anisotropy of the hf splitting and the *g* tensors.^[188] They established that the radical cation formed through spontaneous oxidation of 2,3-dimethylbut-2-ene in H-ZSM-5 zeolite at room temperature rotates about an axis vertically to the molecular plane, whereas the radical cation of 2,5-dimethylhexa-1,5-diene in H-mordenite undergoes rotational movement in the time scale of about 50 ns.

An important problem investigated by EPR spectroscopy of radical cations formed by spontaneous oxidation of organic molecules is that of the nature of the oxidizing sites. Harvey et al. carried out investigations on dealuminated H-mordenites which had been loaded with a number of different alkenes.^[189] The EPR signals of the radical cations formed on the dealuminated H-mordenites correlated well in their intensity with the quantity of extra-framework aluminum in the pores of the zeolites. This is a hint to the oxidative action of the Lewis acid sites associated with the extra-framework aluminum species. Since the calcination of the zeolites can also lead to dealumination and hence to a formation of Lewis acid sites precise control and definition of the calcination conditions is an important requirement for the implementation of reproducible experiments.

4.3. EPR Investigations of Heterogeneously Catalyzed Surface Reactions

There have been numerous EPR spectroscopic investigations of heterogeneous catalytic reactions.^[159, 164, 165, 190, 191] In a number of these works, zeolites have been used as matrix for the stabilization of radical cations formed by spontaneous oxidation. Crockett and Roduner have investigated the dimerization and transannular reactions of cycloalkenes and the oxidation of terpenes on H-mordenite.^[192, 193] After loading the H-mordenite with different terpenes (α -pinene, *trans*-isolimonene, α -terpinene) the EPR spectrum of the radical cation of α -terpinene was found in each case.^[193] With deuterated D-mordenite, whose deuterons were not incorporated into the product molecule, it was possible to establish that the above-mentioned reactions are not catalyzed by Brønsted acid centers but by Lewis acid sites. In contrast, Rhodes and Rhodes and Standing explained the formation of the radical cation of 1,2,3,4,5,6,7,8-octahydronaphthalene on H-mordenite after loading with cyclopentene and different acyclic dienes by an acid catalyzed conversion of the reactants prior to formation of the radical cations.^[194, 195] Similarly, the formation of the radical cation of 2,5-dimethyl-2,4-hexadiene after adsorption of 2,3-dimethyl-1-butene and 2-methylpropene on H-mordenite has been observed.^[196] On H-mordenite, the radical cations of 1,2,3,4,5,6,7,8-octahydronaphthalene undergo electron transfer reactions with other adsorbate molecules which act as electron acceptors.^[197] Similar electron

transfer reactions also occur with radical cations which are formed by adsorption of benzene and tetracyanoethene on H-mordenite.^[197]

A possible extension of the scope of EPR spectroscopy for the clarification of heterogeneously catalyzed reactions arises in the preparation of paramagnetic species through irradiation of the samples with γ -quanta, usually with ^{60}Co as γ -source. In this case, a one-electron oxidation takes place with formation of a radical cation or H addition with the formation of a neutral H adduct radical. In this way this technique is not dependent upon the spontaneous oxidation of reactants at Lewis acid sites which often only takes place at temperatures at which the paramagnetic species is very rapidly degraded again. Paramagnetic species can be produced at very low temperatures by the γ -radiation. This is of great importance because of the high reactivity of this species. With the radiation technique (radiolysis/EPR), reactions which are catalyzed at Brønsted sites are also accessible to EPR spectroscopy. However, in view of the reactivity of paramagnetic species investigations need to be performed during the use of radiolysis/EPR spectroscopy to establish whether or not the paramagnetic species themselves participate as reactive intermediates in the reaction under investigation. One suitable way is available through parallel investigations of the respective reaction on an inactive and an active catalyst (e.g. Na-ZSM-5 and H-ZSM-5 zeolite).^[198]

Radiolysis/EPR spectroscopy has been used for investigations of the isomerization of different olefins on H-ZSM-5 zeolite.^[199] In contrast to earlier work (see ref. [194–196]), no radical cations produced by the spontaneous oxidation of the reactants on the calcinated zeolites were observed in the radiolysis/EPR experiments, only radical cations formed by irradiation.^[199] Werst et al. explained these differences in radical formation by the different calcination of the zeolites in air and in vacuum.^[199] The formation of neutral radicals was found after radiation of cycloheptatriene and cyclohexane which had been adsorbed on Na-AZM-5 and H-ZSM-5 zeolites.^[200] Based on the observed relationship between the yield of neutral radicals and the aluminum content of the zeolite framework, a proton transfer from the adsorbed radical cations onto the zeolite framework was assumed as mechanism of the radical formation. Piocos et al. used the radiolysis/EPR technique for the investigation of the dimerization and isomerization of isobutene and other acyclic monoolefins on zeolites.^[198, 201] The authors established that the dimerization and isomerization of isobutene on H-ZSM-5 zeolite took place as low as 77 K, and cleavage reactions occurred even at below room temperature. In the reaction of different cyclic olefins on H-ZSM-5 zeolite the formation of radical cations of 1,2-dimethylcyclopentene was observed in each case.^[202] The formation of radical cations of differently alkylated bicyclododecenes occurred on H-mordenite under the same reaction conditions. This different catalytic behavior of the solid catalysts used was explained by the shape-selective action of the 10-ring pores of H-ZSM-5 zeolite.

A very interesting experimental approach to investigate the mechanism of heterogeneously catalyzed reactions is the MIESR technique (MIESR: *matrix isolation electron spin*

resonance). In this technique, developed by Lunsford and co-workers, the reactants flow through the catalyst bed arranged in a tubular flow reactor, and after their desorption from the catalyst surface they are trapped as radical cations in a solid argon matrix on a sapphire rod at a temperature of 16 K.^[203] For detection of the trapped radical cations, the sapphire rod is placed in a microwave resonator. This technique has already been used for the investigation of the oxidation of propene on Bi_2O_3 , MoO_3 , and bismuth molybdate catalysts,^[203] the oxidative coupling of methane on MgO ,^[204] and the formation of methyl radical cations on La_2O_3 , CeO_2 , Nd_2O_3 , Sm_2O_3 , Eu_2O_3 , and Yb_2O_3 .^[205]

Karge and co-workers have investigated the formation of hydrocarbon deposits (coke) during the reaction of olefins on H-mordenites under batch and flow conditions.^[160, 161] In the temperature range of 380 to 470 K, spectra with an hf splitting of $a = 1.6$ mT and a g -factor of $g = 2.0025$ were observed under both static and flow conditions (Figure 9) With a further

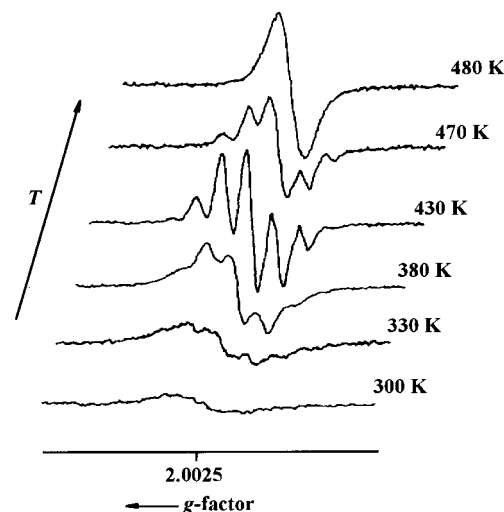


Figure 9. X-band EPR spectra of radical cations recorded during the formation of coke in the reaction of ethene on H-mordenite.^[161]

increase in temperature to 480 K, this hf splitting disappeared and a signal typical of high-temperature coke appeared. Olefinic and allylic hydrocarbons were suggested for the nature of the low-temperature coke ($T < 500$ K). The high-temperature coke formed at temperatures of ≥ 480 K, probably formed of polyaromatic radical cations, gave a narrow and structureless EPR spectrum.^[161] With radiolysis/EPR, Piocos et al. established that the EPR spectra produced in the oligomerization of olefins on dealuminated H-mordenite and H-ZSM-5 zeolite arose from radical cations of 2,3-dimethylbutene and larger branched olefins.^[198] Pradhan et al. investigated the formation of coke in the disproportion of ethylbenzene on H-ZSM-5 zeolite.^[206] In contrast to the work of Karge and co-workers,^[160, 161] Pradhan et al.^[198] were unable to find any hf splitting of the EPR signals. This was explained by the polycyclic and aromatic nature of the low-temperature coke formed from ethylbenzene.

5. UV/Vis spectroscopy

With UV/Vis spectroscopy it is possible to investigate electron transfers between orbitals or bands of atoms, ions, and molecules in the gas phase as well as in liquids and solids. The spectral region of 250 to 500 nm is of special interest for in situ UV/Vis spectroscopy. Investigations of solutions and crystals usually take place in transmission, whereas powder samples are often measured in diffuse reflections (DRS: *diffuse reflectance spectroscopy*). A significant advantage of UV/Vis spectroscopy lies in the individual detection of electron transfers without superimposition by neighboring vibrational bands. Unlike IR spectroscopy, where the use of Fourier transform techniques predominates, dispersive spectrometers are almost exclusively applied for UV/Vis spectroscopy because of the large band widths. Important applications of UV/Vis spectroscopy in heterogeneous catalysis are in situ investigations of the synthesis of solid catalysts, support interactions, and for the modification of solid catalysts during calcination and poisoning. Corresponding applications of UV/Vis spectroscopy have been summarized in reference [159]. The $\pi-\pi^*$ transitions observed in the investigation of surface reactions are an indication of the existence of unsaturated carbenium anions. The detection of these transitions under in situ conditions provides important information on the mechanism of the chemical reactions catalyzed by active sites.

Initial UV/Vis investigations on the reaction of methanol on H-ZSM-5 zeolite took place at the beginning of the 1980s.^[207, 208] Bands between 300 and 400 nm which occur even at 473 K indicate the presence of carbenium ions. The reaction of ethene on H-ZSM-5 zeolite was first investigated by Laniecki and Karge.^[209] These authors observed a band at 300 nm which they explained by allyl cations. The bands occurring at 280 to 330 nm, 360 to 380 nm, and 430 to 470 nm during the adsorption of olefins on acidic zeolites were attributed to unsaturated mono-, di-, and trienyl carbenium ions.^[210] Flego et al. investigated the alkylation of isobutane with 1-butene on LaH-Y zeolites under flow conditions.^[62] Directly after adsorption of 1-butene and an isobutane/1-butene mixture, the authors were able to detect UV/Vis bands of monoenyl carbenium ions (315 nm) and dienyl carbenium ions (370 nm). Since the intensity of these bands depended upon the activation temperature of the catalyst (Figure 10), the Lewis acid sites were held responsible for the formation of the carbenium ions. It was found that the formation of unsaturated carbenium ions was favored by reaction temperatures of up to 523 K, long contact times, and high concentration of Lewis sites.

Sulfated ZrO₂ is an interesting catalyst for skeletal isomerization of *n*-butane to isobutane at room temperature. Hitherto unexplained was the origin of the rapid rise and the subsequent slow decay in the activity of these solid catalysts during the initial phase of skeletal isomerization. To explain this effect, Knözinger investigated the formation of hydrocarbon depositions during the isomerization of *n*-butane on sulfated ZrO₂ under flow conditions.^[211] UV/Vis bands appearing at 366 and 400 nm suggested that immediately after the start of the reaction alkenyl and cycloalkenyl cations were

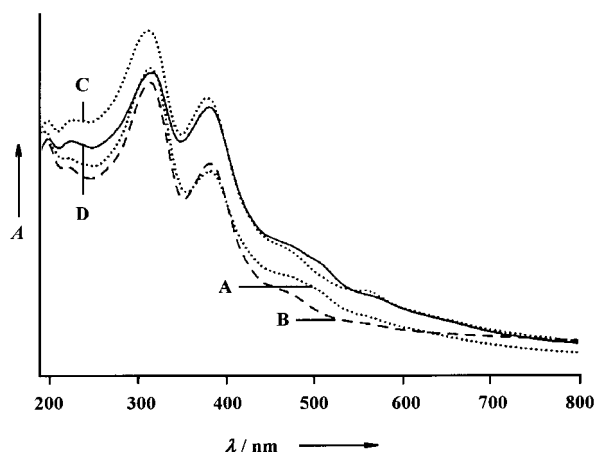


Figure 10. UV/Vis spectra of a LaH-Y zeolite recorded during the reaction of an isobutane/1-butene mixture (9:1) with LSHV = 4.07 h⁻¹ at a reaction temperature of 353 K and a pressure of 30 bar. Prior to the reaction the zeolite was activated at temperatures of 523 K (A), 623 K (B), 673 K (C), and 773 K (D).^[62]

formed. These carbocations are an initial step in the formation of arenes, which in their turn cause the later deactivation of the catalyst.

6. Outlook: The Role of In Situ Spectroscopy in Modern Catalyst Research

By means of examples this contribution has demonstrated that in situ IR, NMR, EPR, and UV/Vis spectroscopy are able to provide important novel information on the mechanisms of heterogeneously catalyzed reactions. Over the coming decade it is to be expected that our knowledge of heterogeneous catalysis will be improved significantly by the systematic application of these and other in situ techniques. A significant advantage of the in situ methods described in this contribution is that they can be operated under conditions of real catalysis, that is there is no "pressure gap" (or the in situ methods operate at least close to the pressures of industrial catalysis), and in many cases can be used at temperatures of up to 800 K.

In situ spectroscopy and the modern approaches of combinatorial catalysis research can thus complement each other in an ideal way (Figure 11): whereas the latter has as objective the empirical testing of new catalysts with high efficiency, in situ spectroscopy has the complementary, at least equally important and intellectually demanding role of providing the factual basis for an understanding of the processes on the surface of solid catalysts and for mechanistic models of heterogeneous catalysis. Only in this way will rational and scientifically sound catalyst design be generally possible. The cycle of combinatorial catalysis research and in situ spectroscopy will in many cases have to be run through many times before the catalyst necessary for industrial application is available.

Whereas combinatorial catalyst research and in situ spectroscopy will significantly contribute to an increase in efficiency, they will not be able to replace the traditional

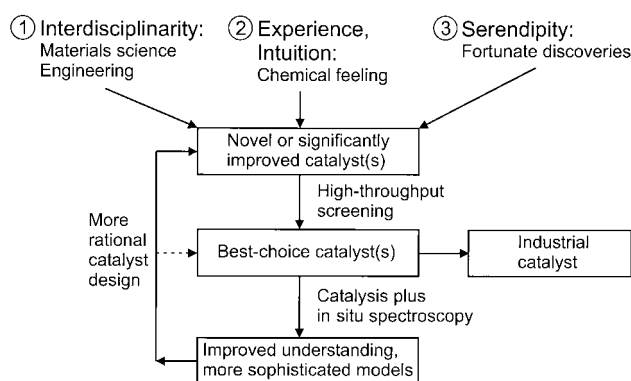


Figure 11. Elements of research in heterogeneous catalysis from innovation to the industrially used catalyst.

sources of materials innovation: 1) Fundamental research in the area of materials science and solid-state chemistry will provide novel materials with a potential for catalysis. It is imperative that these are put into practical applications in catalysis more rapidly and more professionally than hitherto. 2) Experience, intuition, and know-how will still play an important role in the search for novel catalyst for the reactions described. 3) Purely chance discoveries in catalysis which have led repeatedly to new industrial processes in the 20th century will become rarer. However, that in the future innovation in catalyst research will succeed through pure chance cannot be excluded.

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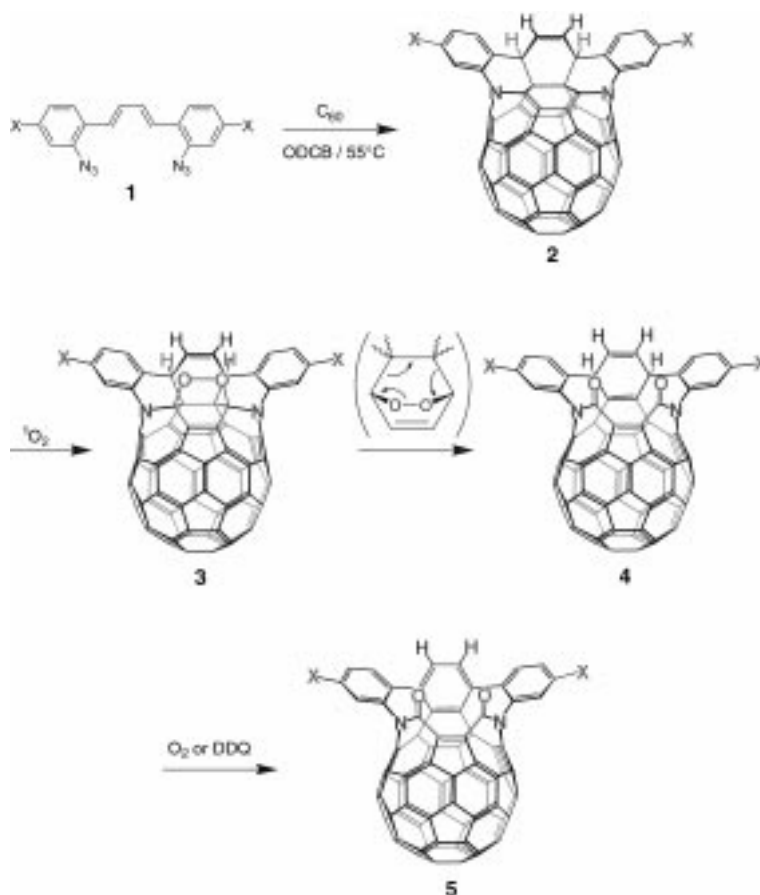
Ring-Opened Fullerenes: An Unprecedented Class of Ligands for Supramolecular Chemistry**

Jean-François Nierengarten*

Whereas the outer sphere chemistry of C_{60} and the higher fullerenes has been extensively investigated,^[1] the internal cavities of these carbon cages have been scarcely considered, even though they are large enough to hold any atom or small molecule.^[2] This situation is mainly associated with the difficulties of preparing endohedral fullerene complexes on a truly preparative scale. In the past few years, successful incorporation of rare gases or atomic nitrogen into the C_{60} cage has been achieved with incorporation ratios of up to 0.1 % by pressure/heating or plasma reactions. Several endohedral metallofullerenes have also been obtained by the evaporation of graphite/metal oxide or carbide composites. Although their preparation constitutes a remarkable achievement, the small quantities of material obtained after tedious HPLC separations appear to be a severe limitation for the exploration of this whole new world of fullerene chemistry. Therefore, a stimulating challenge for the organic chemists is the development of efficient synthetic approaches for the preparation of endohedral fullerene complexes. An immediate choice is to use the existing framework of fullerenes and create an opening wide enough to allow an atom or a small molecule to pass through before closing it back. However, the formation of an effective aperture on the surface of fullerenes has proven to be very difficult;^[3] the pioneering research in this area has been summarized by Rubin in an excellent review article.^[4]

In the last two years, key advances have been reported within this field by the research group of Rubin,^[5, 6] and this innovative work will be briefly presented here. It must also be mentioned that Stevenson et al. believed they were successful in the preparation of endohedral ring-opened fullerene complexes, however, further investigations revealed that it was actually not the case.^[7]

The formation of an effective opening within the C_{60} core has been achieved by an original reaction sequence in which four bonds of the fullerene cage are cleaved (Scheme 1).^[5] The reaction of diazidobutadiene **1** with C_{60} followed by N_2 extrusion afforded iminofullerene derivative **2**. Subsequent reaction with singlet oxygen yielded an endoperoxide intermediate **3**, which rearranged by spontaneous [2+2+2] ring opening to generate **4**. Finally, treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or oxygen afforded the dehydrogenation product **5**. This unique one-pot reaction provided efficient formation of the largest orifice created so far on the C_{60} molecule, thus providing the opportunity to test if small atoms or molecules could be made to pass through it.

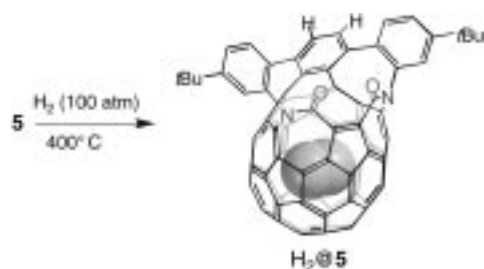


Scheme 1. Preparation of ring-opened fullerene **5**. ODCB = *ortho*-dichlorobenzene, X = *t*Bu.

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[**] Dedicated to Prof. François Diederich in recognition of his support and enthusiasm.

Incorporation experiments were first carried out with helium.^[6] Careful ³He NMR investigations provided a fully convincing demonstration of the incorporation of a He atom inside the ring-opened fullerene **5**. The formation of the host–guest complex He@**5** was found to occur even at low helium pressure, and the fraction incorporated under a pressure of 3–4 atm (0.05 %) approaches that of He@C₆₀ (0.1 %) prepared by the direct high-pressure method.^[8] Treatment of compound **5** at 400 °C under a H₂ pressure of 100 atm for 48 h also allowed the incorporation of molecular hydrogen inside a fraction of the ring-opened compound (Scheme 2).



Scheme 2. Incorporation of molecular hydrogen inside the ring-opened fullerene **5**.

Unambiguous demonstration of the presence of molecular hydrogen inside the ring-opened fullerene **5** was obtained from careful NMR experiments. It must be pointed out that the fraction of H₂@**5** thus produced was found to be as high as 5 %, which is by far the highest amount of direct incorporation obtained for any gas in a fullerene.

With the formation of a large orifice in the C₆₀ core and the successful insertion of He and H₂, two critical steps have been realized towards the development of an efficient synthetic approach for the preparation of endohedral fullerene complexes. The ring-opened fullerene **5** is also the first member of a new class of ligands for host–guest chemistry, and the preparation of endohedral ring-opened fullerene complexes paves the way for the preparation of original supramolecules. In addition, endohedral lanthanide–fullerene derivatives are good candidates for conventional diagnostic radiology (MRI contrast and X-ray imaging agents) as a consequence of the effective isolation of the internal metal from its surroundings and the absence of any release of the free metal in vivo.^[9]

Endohedral lanthanide ring-opened fullerenes could be, therefore, an interesting alternative to endohedral metallofullerenes for biomedical applications since they should be easier to prepare on a truly preparative scale.

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100 Years after Grignard: Where Does the Organometallic Chemistry of the Heavy Alkaline Earth Metals Stand Today?

Matthias Westerhausen*

The preparation of the alkylmagnesium halides by the direct synthesis from magnesium and haloalkanes 100 years ago^[1] led to a vast development of organometallic chemistry. Since then these Grignard compounds have been shown to be widely applicable as readily available metalation and metathesis reagents in organic and inorganic chemistry. Even geminal bismagnesiates alkanes have been isolated and structurally characterized.^[2] Although interest in corresponding compounds of the heavier alkaline earth metals has risen in the past few years,^[3] great difficulties are associated with their direct synthesis. On the one hand, the metals calcium, strontium, and barium are rather inert; on the other hand, the organometallic derivatives are extremely reactive. The high reactivity often leads to ether cleavage reactions; however, in apolar solvents, the organometallic compounds of the heavy alkaline earth metals are often insoluble. Consequently, two different synthetic strategies were developed in the last few years, namely the activation of the metals as well as the reduction of the reactivity of the organometallic reagent by steric shielding with bulky substituents and/or by shielding of the metals with multidentate Lewis bases.

Interest in the organometallic compounds of the heavier alkaline earth metals has increased recently not least due to the fact that an intermediate position between the derivatives of the alkali metals and of the metals of the scandium group is expected. Calculations showed already that monomeric dimethylstrontium and -barium should be bent,^[4] whereas dimethylcalcium is expected to be linear similar to the monomeric fluorides^[5] (Table 1). Also for the dimeric coligand-free alkaline earth metal dihydrides, uncommon structures of the bicyclic type $\text{HM}(\mu\text{-H})_3\text{M}$ for calcium and strontium as well as tricyclic $\text{Ba}(\mu\text{-H})_4\text{Ba}$ were predicted^[6]

Table 1. Calculated structural parameters of monomeric alkaline earth metal difluorides, -hydrides, and -methanides of calcium, strontium, and barium of the type R-M-R.

M	R	M-R [pm]	R-M-R [°]	Ref.
Ca	F	205	180	[5]
	H	206	180	[6b]
	Me	249	180	[4]
Sr	F	219	144	[5]
	H	220	140	[6b]
	Me	262	131	[4]
Ba	F	233	126	[5]
	H	231	119	[6b]
	Me	276	116	[4]

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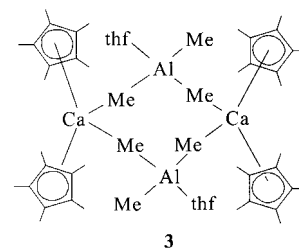
as a consequence of a small but structure-determining influence of the d orbitals.

Although numerous examples of alkaline earth metal-locenes^[7] and molecules with π -bonded anionic ligands are known,^[8] organocalcium chemistry has still only developed slowly since the report of the bis(1,4-dioxane) complex of bis[bis(trimethylsilyl)methyl]calcium (**1**), the first structurally characterized representative, by Lappert and co-workers.^[9] The Ca-C bond lengths of 248.3 pm lie in the region predicted by calculations. Whereas this research group chose the cocondensation of calcium with chlorobis(trimethylsilyl)methane to activate the metal, a few years later the synthesis of bis[tris(trimethylsilyl)methyl]calcium (**2**) (Ca-C 245.9 pm, C-Ca-C 149.7°), which surprisingly is bent at the metal atom, succeeded from the metathesis reaction of the corresponding coligand-free potassium derivative and calcium diiodide.^[10] This reaction was performed in benzene because the high reactivity of the organocalcium compound led to ether cleavage reactions. It was impossible to develop a chemistry based on **2** for the same reason.

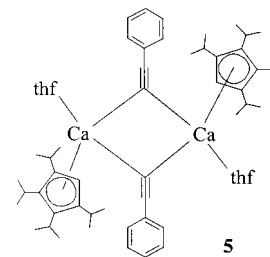
The reaction of decamethylcalcocene with trialkylalane in THF gave the loose adduct **3**,^[11] whereas the use of calcium bis[bis(trimethylsilyl)amide] led to the elimination of dialkylaluminum amide and to the formation of dimeric bis(trimethylsilyl)amidocalcium tetrakis(trimethylsilylmethyl)alanate (**4**).^[12] The alanate anions bind as bidentate ligands with Ca-C bond lengths of 263.8 and 267.8 pm. However, the elimination of another molecule of $(\text{Me}_3\text{Si})_2\text{NAl}(\text{CH}_2\text{SiMe}_3)_2$ and the formation of dialkylcalcium starting from **4** failed.

The pK_a values of the acids determine the equilibrium of metalation reactions. Since alkaline earth metal bis[bis(trimethylsilyl)amides] are soluble in common organic solvents and are commonly employed as soluble starting compounds, more H-acidic hydrocarbons such as cyclopentadienes or phenylacetylene are necessary. This route was used by Burkey and Hanusa^[13] to prepare dimeric tetraisopropylcyclopentadienylcalcium phenylacetylide **5**. The bridging phenylacetylide ligands display Ca-C distances of 252.1 and 255.1 pm.

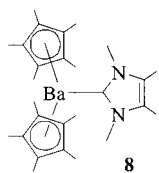
Molecules with Sr-C and Ba-C σ bonds have not been investigated as extensively. Carbene adducts of barium bis[bis(trimethylsilyl)amide] dissociate in solution;^[14] however, those



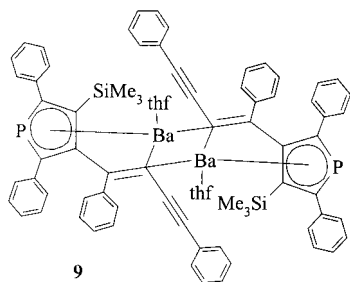
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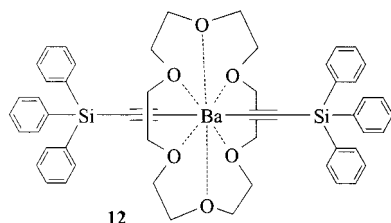
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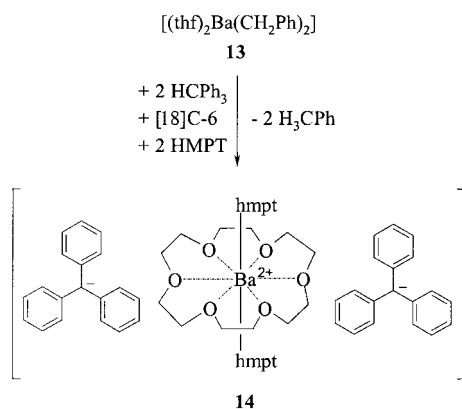
of decamethylcalcocene (**6**), -strontocene (**7**), and -barocene (**8**) have been structurally characterized (Ca–C 256.2, Sr–C 285.4 and 286.8, Ba–C 295.1 pm).^[15] The addition of diphenylbutadiyne to barium bis[bis(trimethylsilyl)phosphonide] yielded dimeric and intramolecularly shielded alkenylbarium phospholide **9** with an unsymmetric Ba₂C₂ cycle with Ba–C bond lengths of 288.1 and 307.1 pm.^[16] By shielding of the alkaline earth metal with crown ethers, Ruhlandt-Senge



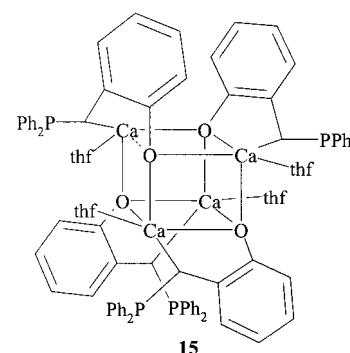
and co-workers^[17] succeeded in isolating crystalline triphenylsilylacetylides of calcium (**10**), strontium (**11**), and barium (**12**) with bent M–C≡C fragments and mean Ca–C, Sr–C, and



Ba–C bond lengths of 254, 272, and 285 pm, respectively. The similar acidity of acetylenes (pK_a values at 29) and of triphenylmethane ($pK_a=30$) also suggest their metalation; however, surprisingly the dependency on the metalating reagent was observed. Only the use of the recently published bis(tetrahydrofuran)dibenzylbarium^[18] (**13**) allowed the synthesis of the crown ether complex of bis(triphenylmethyl)barium (**14**), although as a solvent-separated ion pair ([18]C-6 = [18]crown-6; hmpt = hexamethylphosphoric acid triamide).^[19]

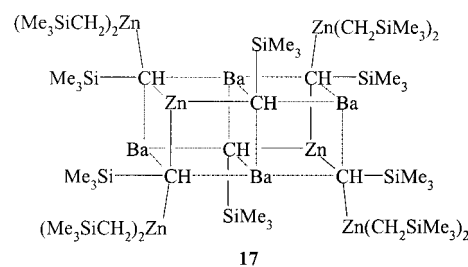


Other metalating reagents such as barium bis[bis(trimethylsilyl)amide] did not lead to this product, but allowed the isolation of the crystalline ether cleavage product which was already described earlier.^[20] With the intention of binding soft carbanions to the heavy alkaline earth metals and of avoiding ether cleavage reactions, substituted dibenzylcalcium^[21] and -barium^[22] have attracted more interest over the last few years. The alkaline earth metal cations avoid the formation of σ bonds, but prefer η^3 coordination to the π system of the carbanion. An interesting exception is compound **15** with a



Ca₄O₄ heterocubane moiety, which is available in rather poor yield from the ether cleavage reaction of calcium diiodide with (2-methoxyphenyl)(di-*p*-tolylphosphanyl)methyl lithium. The calcium atoms reside in a distorted octahedral environment; the Ca–C bond lengths are 259.1 pm.^[23]

The transmetalation of bis(trimethylsilylmethyl)zinc with distilled barium gave barium bis[tris(trimethylsilylmethyl)zincate] (**16**) in good yield, whereas the Ba–C–Zn bridges can be regarded as three-center, two-electron bonds (Ba–C 302.8 and 305.5 pm). Depending on the solvent, toluene or tetrahydrofuran molecules complete the coordination sphere of the soft metal center.^[24] The transmetalation under application of ultrasound leads to the metalation of the methylene groups and to the formation of the centrosymmetric cage compound [Ba₄Zn₆(CH₂SiMe₃)₈(CHSiMe₃)₆] (**17**).^[25] In this Ba₄Zn₆C₆



double cube the carbon atoms show a distorted octahedral coordination sphere. Compounds with bonds between the heavy alkaline earth metals and heavy tetrels are gaining interest, as shown for example, for the structurally characterized stannanides of barium.^[26]

The structural characterization of unsubstituted dialkyl-strontium and -barium compounds are still to be achieved. The increasing knowledge about the synthetic strategies and

the structures of these compounds is expected to be accompanied by an increase in the use of such compounds for industrial applications mainly as anionic polymerization catalysts, as has already been proven for selected derivatives.^[8d, 27] However, we are still at the beginning of a vastly developing chemistry of the heavy homologous Grignard reagents.

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The Light Shall Show the Way—Or: The Conformational Changes of the Retinal Chromophore in Rhodopsin upon Light Activation

Wolfgang Gärtner*

The Visual Pigment Rhodopsin—A Paradigm for the G-Protein-Coupled Receptors (GPCRs)

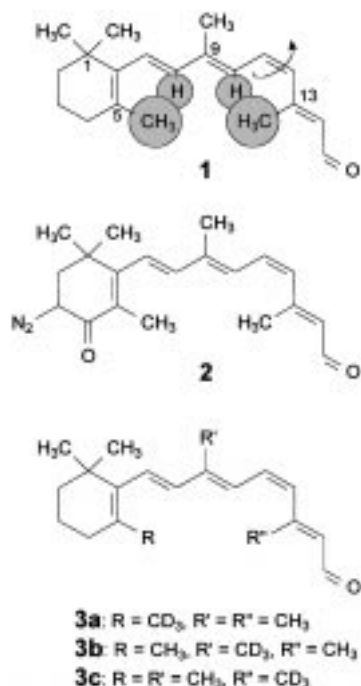
Only a few molecules can be considered as biochemical “classics” which have also made their way as distinctive examples into biochemistry textbooks. Amongst these is the archetype of visual pigments, bovine rhodopsin.^[1] The strong scientific interest in understanding the structure and function of rhodopsin is not only a consequence of its fascinating conversion of absorbed light into a biological signal, but is also a consequence of the fact that it serves as a paradigm for a large and continuously growing number of other receptors

with seven transmembrane α -helices. Common to all these membrane-intrinsic receptors is their capability to respond to external stimuli such as other sensory (olfactory and gustatory), hormone-mediated, or neurotransmitter-mediated signals, and to mediate the signal by activating a heterotrimeric G-protein in the interior of the cell.^[2]

Scientific investigation of visual pigments started more than a century ago with work by pioneers such as W. Kühne, who demonstrated image formation on the retina-embedded “Sehpurpur”^[3] when illuminated patterns were exposed to the eye. Since then, many multidisciplinary approaches have been used to understand the function of visual proteins, and have included the efforts of pharmacologists, biologists, biophysicists, medecians, neurologists, and even theoreticians. *Chemists* became involved in visual research through the identification of the vitamin A aldehyde retinal as the chromophoric principle of visual proteins by George Wald

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in the early 1930s.^[4a] He demonstrated that an 11-*cis* to all-*trans* photoisomerization was the initial reaction of rhodopsin, and was also able to show that—similar under physiological conditions—bleached visual proteins can be reconstituted in vitro by incubation with either the (naturally occurring) 11-*cis* (**1**, Scheme 1) or the 9-*cis* isomer of retinal.^[4b]



Scheme 1. Structural formula of 11-*cis*-retinal (**1**) and 3-diazo-4-ketoretinal (**2**), as well as 5-, 9-, and 13-trideuteriomethylretinal (**3a–c**).

The generation of modified chromophores by the then available broad repertoire of retinoid and carotenoid chemistry^[5] allowed detailed studies of the precise description of the chromophore conformation and of the interactions between the retinal molecule and the protein moiety.^[6] In particular, the wide range of wavelength sensitivity of visual proteins, which span the whole range of the “visible” (sic!) light and are based on only one type of chromophore,^[7] represented a challenge which evoked experimental as well as theoretical efforts. The identification of a covalent attachment resulting in the formation of a protonated Schiff base between the chromophore and the protein (Lys 296 in bovine rhodopsin) causes a high polarizability in the π -electron system of the polyene chain. This observation was fundamental for Honig and Nakanishi’s explanation of the wavelength tuning of visual pigments by an “external two point charge model”.^[8]

Chromophore–Protein Interactions in Rhodopsin at the Atomic Level

Despite the many attempts to obtain detailed knowledge of the structure and function of rhodopsin by employing chemical, spectroscopic, recombinant, and theoretical techniques, a description of the rhodopsin structure at the atomic level remained elusive for a long time. Only recently a three-dimensional (3D) structure with sufficient resolution was

presented^[9] which now allows the evaluation and critical revision of many of the results obtained and reported previously.

However, the crystal structure still does not allow a precise description of the interactions between the chromophore and protein, and also can not reflect the dynamics of the conformational changes which both components experience upon light activation. Two recent publications both address this aspect, namely, the conformation of the protein-embedded chromophore in the rhodopsin resting state and its conformational changes during the bleaching process.^[10, 11]

Investigation of Chromophore Movements by Cross-Linking Experiments

Borhan et al.^[10] make use of the chemically modified retinal derivative 3-diazo-4-ketoretinal (**2**; Scheme 1) to probe the “movement of retinal along the visual transduction path”, as their title says. Although the visual transduction still comprises more than just the light-induced reactions of the visual pigment itself, the results give a detailed and unexpected description of the interactions between the chromophore and its protein environment, and allow the conformational changes which the chromophore undergoes upon light excitation to be followed. The data highlighted in the *Science* paper^[10] are presented in greater detail in two accompanying publications^[12, 13] that deal with the same problem, namely, formation of new covalent bonds between the chromophore and protein by cross-linking during the bleaching process. This study is based on the structure of retinal derivative **2**, which is one of a series of retinoids of this type prepared by Nakanishi and his co-workers. All of these compounds can undergo several different reactions. Besides their ability to form a covalently linkage to the protein through a protonated Schiff base in an identical manner to the native chromophore, these compounds carry a diazo group on the cyclohexene ring as a second reactive group. This diazo group expels nitrogen upon irradiation with UV light and rearranges into a highly reactive carbene moiety, which intercalates into C–H bonds of nearby amino acids. The high reactivity of the carbene results in the covalent bond only being formed directly to adjacent molecules and thus probes the direct environment (for purposes of identification, these cross-linking molecules were furnished with a tritiated aldehyde group (–CTO) to enable the radioactivity in the cross-linked peptides to be followed more conveniently during the sequence analysis of the protein^[12]). Thus, these compounds in the opsin-bound form can be activated with respect to their photoisomerization (11-*cis* → all-*trans*) by long-wavelength irradiation, and can then be converted at any time after thermal reactions into the reactive carbene by exposure to ultraviolet light. The authors started their analysis of retinal–opsin interactions by first verifying that the artificial chromophore occupies the binding site of the native chromophore, and showed that the diazo group remains intact in the protein-bound chromophore in the dark. In order to probe physiologically relevant states of the light-activated rhodopsin, the photochemistry of rhodopsin assembled from **2** was initiated at low temperatures where the first stable photoproduct, bathorhodopsin, can be trapped,

and the resulting products of the reaction pathway can be obtained by a stepwise warming of the sample. By following this well established concept, bathorhodopsin was generated at -196°C and was then irradiated with UV light to form the carbene retinoid which was then allowed to react with amino acids in its proximity. Similarly, samples were subjected to the bleaching process at temperatures where lumi or alternatively a mixture of meta-I and meta-II rhodopsin can be trapped. The formation of the various stabilized intermediates was proven by difference UV/Vis spectroscopy (intermediate minus the parent state of rhodopsin) prior to the initiation of the cross-linking reaction.

Initiation of the cross-linking reaction in the dark (parent state of rhodopsin without long-wavelength irradiation of the pigment), and also cross-linking in the batho form yielded the same target amino acid, namely Trp265 in helix F. The position of the cyclohexene ring apparently remains nearly unperturbed, irrespective of the *cis*–*trans* photoisomerization of the chromophore (rhodopsin-to-batho). This finding, according to the authors, is in accordance with other results, such as those from vibrational spectroscopy studies, which indicate that despite adopting the *trans* geometry, the chromophore hardly changes its overall structure, presumably because of the fact that the photoisomerization of the 11,12 double bond is compensated to a large extent by conformational changes of single bonds.

When the protein was transformed into the lumi state, the authors found a surprising new target, Ala169, which is located in the fourth helix. This result at first glance causes confusion. Following the schematic drawings of the protein as given by the authors (Scheme 2; it should be kept in mind that

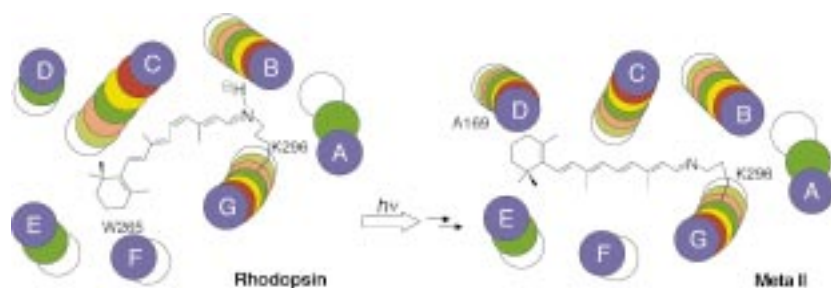
directed side of the rhodopsin protein, and requires not only the movement of the helix towards the reactive site of retinal, but also a rotation around the long axis of the helix. This same amino acid (Ala 169) is also found as the sole modified residue in the meta-I and meta-II forms, which indicates that no gross changes in the conformation of the chromophore occurs during the conversion into these intermediates. The finding of a selective cross-link of an amino acid, formerly believed as being “outward” directed, is a remarkable surprise, even more so when the experimental conditions reported by the authors are taken into account, that is, with only about 50% of the lumi intermediate generated. It is argued that apparently the movement of the ring site during formation of the lumi selectively directs the reactive carbene towards helix D. The authors claim that all these results from chemical studies, when taken together, indicate a remarkable structural and conformational flexibility of the chromophore and the protein during the light-induced bleaching of rhodopsin.

Characterization of the Chromophore Conformation by Using Solid-State Deuterium NMR Spectroscopy

The publication by Gröbner et al.^[11] also deals with the conformation of the chromophore in the resting state and its changes when the pigment is converted into the meta-I intermediate. This paper is the most recent in a series of publications dedicated to the determination of the conformation of the chromophore by NMR spectroscopy.^[14, 15] During their studies, the authors have applied solid-state NMR methods to several rhodopsin samples, each reconstituted with selectively deuterium-labeled retinal chromophores. The

principle of structure determination by this technique is based on the immobilization of the rhodopsin samples on solid supports (glass plates) within the NMR rotor at a precise angle with respect to the rotor axis. The orientation of the deuterium quadrupole coupling tensor of the deuterated substituents with respect to the membrane normal and the externally applied magnetic field (magic angle oriented sample spinning NMR, MAOSS-NMR) can be identified with this method. NMR analysis of rhodopsin samples assembled with retinals carrying trideuterated methyl groups (CD_3) at positions 5, 9, or 13 (**3a–c**, Scheme 1) thus allows the con-

formation of the chromophore within the binding site and its changes during the photobleaching process to be determined. Gröbner et al.^[11] give special emphasis to the movements of the cyclohexene ring during the rhodopsin–meta-rhodopsin conversion by investigating a 5- CD_3 chromophore. Similar as described by Borhan et al.,^[10] the meta-I state was prepared and stabilized below 0°C , and the conformation of its retinal chromophore was compared to that of the rhodopsin parent state. On the basis of a previously determined topological arrangement of the polyene chain (derived from 9- and 13- CD_3 -containing retinals^[14] which yielded angles of 44 and $30 \pm 5^{\circ}$ with respect to the membrane normal for these two substituents), the NMR data obtained with C5-



Scheme 2. Layout of the seven transmembrane helices in lumi- and metarhodopsin according to Borhan et al.^[10] (Copyright© American Association for the Advancement of Science 2000).

this publication was submitted, before the rhodopsin structure^[9] had been published), the fourth helix (D) in the parent state of rhodopsin is extruded from the chromophore binding pocket by the two helices C and E, which approach each other and are part of the chromophore binding site. Thus, the authors had to propose a remarkable change in the conformation of the chromophore and an even greater one of the protein during the conversion from the batho to the lumi form of rhodopsin in order to interpret their data. In their view, the helices C and E, formerly arranged close to each other, are now pushed aside allowing access for helix D (carrying the later cross-linked amino acid Ala169). Moreover, as the crystal structure shows, Ala169 is located on the outward

CD₃-labeled retinal indicate a 6-*s-trans* conformation of the cyclohexene ring for both the rhodopsin parent state and the Meta-I form (a value of $21 \pm 5^\circ$ was determined for the C5–CD₃ bond vector in the parent state, and of about 60° for the meta state). The polyene chain in the rhodopsin parent state exhibits a remarkable twist along its long axis (44 and 30° for the two substituents, see above) and an additional distortion between the cyclohexene ring and the polyene chain. The chromophore adopts a nearly completely relaxed all-*trans* conformation/configuration on arriving at the meta-rhodopsin state. In this intermediate, the bond vectors of all three methyl groups exhibit an angle of 60 – 65° to the membrane normal. These findings indicate that not only the cyclohexene ring, but also the polyene chain of the retinal undergoes conformational changes as a consequence of the photoisomerization of the 11–12 double bond. Besides the finding of a strong distortion of the polyene chain around the 11–12 *cis* double bond, which has already been postulated by other experiments, for example, circular dichroism,^[16, 17] to partially compensate the strong steric hindrance between the hydrogen atom at C10 and the methyl group at C13, the twist of the ring moiety and its overall 6-*s-trans* conformation is surprising. A steric interaction between the methyl group at C5 and the hydrogen atom at C8, which was first seen in crystals of retinoids, had also been suggested for the conformation of the chromophore in the rhodopsin binding pocket. Yet, in former studies the cyclohexene ring was assumed to adopt a distorted 6-*s-cis* conformation—this picture was interestingly also derived from solid-state NMR experiments.^[18] Gröbner et al.^[11] now argue that the former determination of the conformation as 6-*s-cis* had to rely on indirect evidence in which the NMR data from labeled retinal in rhodopsin were compared with model compounds measured in organic solvents, and the signals “were found at the upper end of the region for 6-*s-cis* model compounds, close to the range for the 6-*s-trans* derivatives” (footnote [18] in ref. [11]).

The arrangement of the chromophore in the binding pocket, based on the NMR-derived conformation, places the ring site of retinal as a sandwich between Trp265 (which was identified by the cross-link experiments, see above) and Tyr268. Both residues are highly conserved in all opsins. Whereas a conformational change of the ring moiety (from distorted 6-*s-transoid* to fully relaxed 6-*s-trans*), as proposed from the NMR work, appears feasible, such arrangement would render a rotation of the ring part, as postulated from the cross-linking experiments, highly improbable—a high energy barrier for such motion can be assumed because of the strong interactions between the three ring structures (cyclohexenyl and two aromatic amino acids).

The Three-Dimensional Crystal Structure of Rhodopsin: Consequences for the Proposed Chromophore Conformations

The results of both papers discussed here have to be considered using the knowledge provided by the recently presented structure of bovine rhodopsin.^[9] Although the structure is currently available only in the resting (Rho) state

at 2.8-Å resolution, the overall arrangement of the seven transmembrane helices is crucial and can not be neglected in the discussion of conformation and conformational changes. Whereas the proximity of the ring part of the chromophore and helix F, which was proposed from both the cross-link and the NMR experiments, is in agreement with the 3D structure, the strong conformational changes of the protein backbone as proposed by Borhan et al. when the receptor arrives at the lumi and meta state have to be discussed more critically. Not only, as outlined above, do the two helices C and E have to move apart from each other and allow helix D to approach the chromophore, but also helix D itself, which carries the cross-linked Ala 169 residue, has to rotate around its long axis, since the target alanine (in the parent state) points away (outwards) from the protein core and the chromophore binding site (Figure 1). The more gradual change of the ring part, as

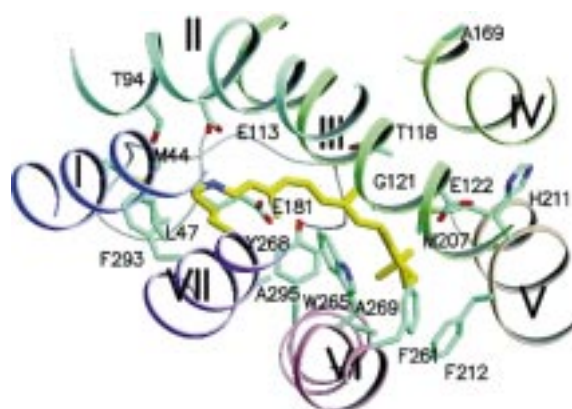


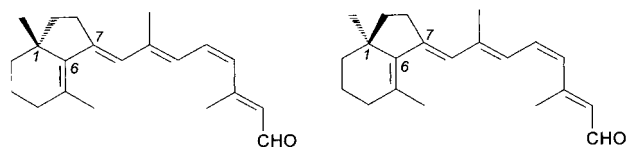
Figure 1. Three-dimensional crystal structure of rhodopsin from Palczewski et al.^[9] viewed from the cytoplasmic side of the protein. Note the strong tilt of helix III with respect to the membrane plane (Copyright© American Association for the Advancement of Science 2000).

suggested from the NMR studies by Gröbner et al.^[11] (from the formerly believed *cisoid* conformation into the *transoid* structure, and later in the meta state into the relaxed 6-*s-trans* conformation) is more agreeable with the crystal structure (but see below). One expects further NMR studies to be performed which may fill the gap between the parent state and the here-presented meta intermediate. Still, one might also expect slight changes of the protein backbone arrangement to be evident in a more refined crystal structure determination with higher atomic resolution, which probably might also indicate whether there is conformational freedom for large structural changes of the protein. It should be kept in mind that a large conformational flexibility is not unlikely for a photoreceptor such as rhodopsin, which has to be absolutely “silent” in the resting state and which undergoes large conformational changes in the cytoplasmic loops to generate a G-protein interaction site upon light activation. In fact, alternative methods can be used to detect conformational freedom in various regions of the protein. Steady-state and even time-resolved EPR measurements have been performed on rhodopsins, which carry spin labels at distinct positions. The most frequently used spin label is the 1-oxyl-2,2,5,5-tetramethylpyrrolidine group which can be covalently attached to cysteine residues, either at their naturally occurring

positions in the protein or at different sites by site-directed mutagenesis. This method yields information as to whether a particular domain of a protein gains or loses conformational flexibility during its function, and allows, upon the introduction of more than one label, distance measurements to be performed between adjacent protein regions.^[19]

Chemistry Strikes Back

The most recent report of modified chromophores, synthesized again by Nakanishi et al., however, offers a striking new viewpoint on the conformational flexibility between the ring and the chain in rhodopsin-bound retinal: The authors present the results of two retinal derivatives that were prepared in which the 6-*s-cis* configuration is fixed by a methylene bridge between one of the two geminal methyl groups of the ring and the C7 atom of the chain (Scheme 3).^[20] This new chemical



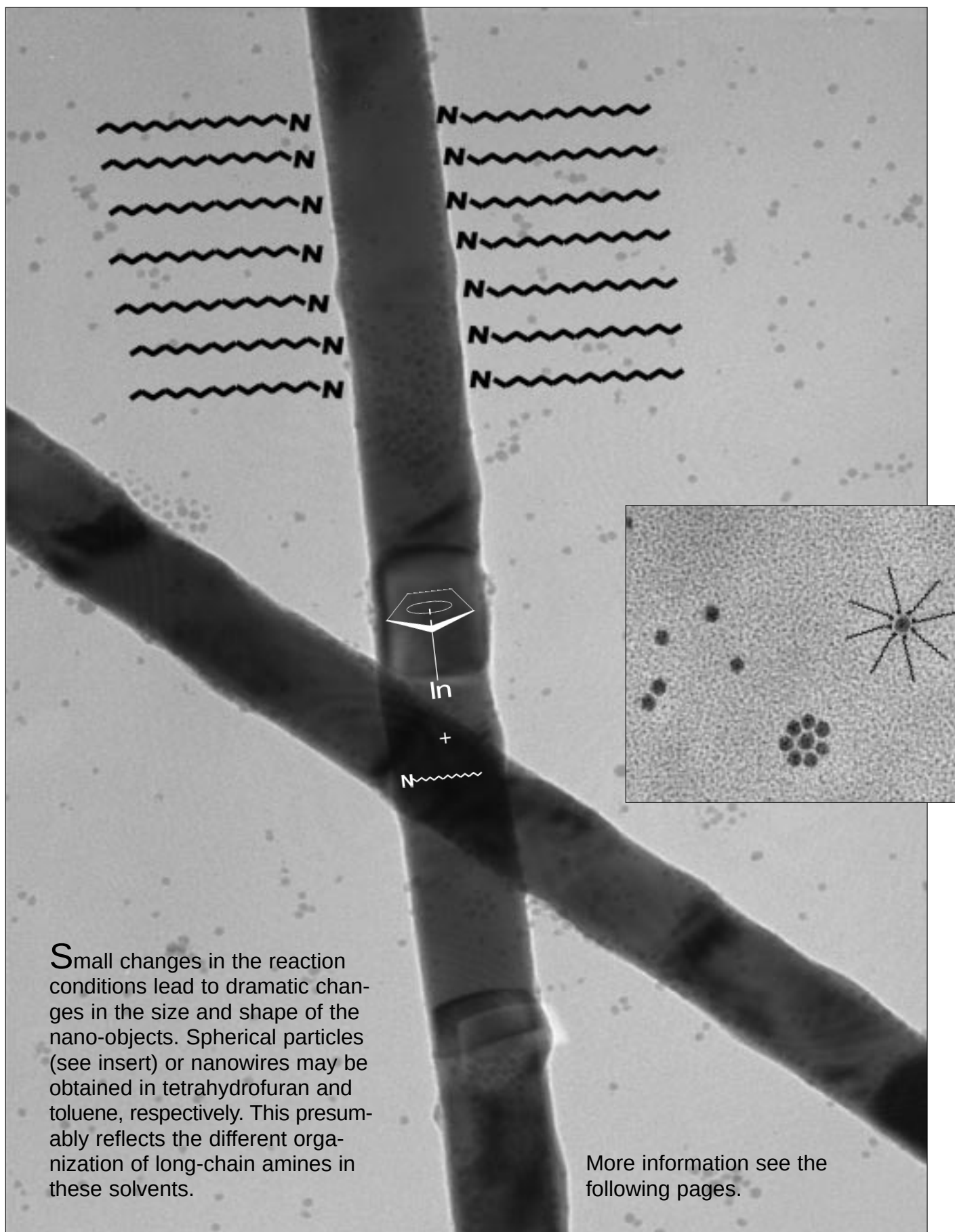
Scheme 3. Structural formula of the 11-*cis* isomers of 7,16- and 7,17-methanoretinol, the 1*R*- (left) and 1*S*-locked (right) analogues of retinal, respectively.

approach does not only allow unambiguous identification of the 6-*s-cis* conformation, but also provides even more subtle information. The authors succeeded in generating the methylene-bridged structure for both methyl groups (which are located below and above the ring plane) to yield the 1*R* and 1*S* enantiomer, of which only one (the 1*R* form) generates a visual pigment upon incubation with rhodopsin. The physiological activity of this new visual pigment, as determined by G-protein activation, reaches about 80 % of that of rhodopsin from natural sources.

It appears that although great progress has been made recently in the elucidation of the structure and function of

rhodopsin, the number of unanswered questions and unsolved problems is still growing.

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Selective Synthesis of Novel In and In₃Sn Nanowires by an Organometallic Route at Room Temperature**

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Controlling the shape of nanostructures at the mesoscopic level is one of the most challenging issues presently faced by synthetic inorganic chemists.^[1] This is especially true of nanowires, which are one-dimensional objects, for which the control of growth at the molecular level seems particularly difficult.^[2–7] Several methods have been used to grow nanowires, such as laser-assisted catalytic growth^[2] and the analogous SLS (solution–liquid–solid) method.^[3] Alternative methods use templating materials (mesoporous silica, alumina membranes, polymers, nanochannel glass, carbon nanotubes).^[4,5] Some recent papers propose the use of chelating agents to control the direction of nanowire growth in solution.^[6] Finally an elegant study by Alivisatos et al.^[7] emphasizes the importance of reaction conditions and, in particular, the concentration of the precursors and the ratio of surfactants for preparing CdSe nanocrystals of various shapes, including nanorods.

We have been interested for several years in using the tools of organometallic chemistry for preparing nanostructures.^[8] The next challenge appears to be the control of the mesoscopic structure. This can be achieved by self-assembly of spherical nanoparticles in one, two, or three dimensions, or by growing nanocrystals of controlled shapes (nanorods, nanowires, cubes, pyramids, etc.).^[1,7,9] We recently reported the synthesis of indium nanoparticles from the organometallic precursor InCp (Cp = C₅H₅[−]) and the two- and three-dimensional assembly of the particles by using trioctylphosphane oxide (TOPO) as a ligand.^[10] Here we report on the use of long-chain amines as templates for the synthesis of indium nanowhiskers^[11] from InCp (**1**) and the extension of this work to the preparation of unprecedented nanowires of In₃Sn.

In a typical experiment **1** (90 mg, 0.5 mmol) was dissolved in distilled and degassed toluene or THF (11 mL) containing hexadecylamine (HDA; 60 mg, 0.25 mmol). The reaction mixture was then allowed to react in a Schlenk tube in the absence of light or in a quartz cell in the presence of UV light ($\lambda = 365$ nm) and without stirring.

After irradiation for four days at room temperature, examination of a transmission electron microscopy (TEM) grid prepared from a drop of the crude solution revealed the presence of straight nanowires (or ribbons) with a high aspect ratio. The length of nanowires is difficult to estimate, but some were definitely longer than 40 μm , the distance between two copper wires of the grid, and others even exceeded 200 μm . The width of the nanowires was not controlled (most ranged between 100 nm and 1 μm ; Figure 1). These wires were shown by high-resolution electron microscopy (HREM) and X-ray diffraction (XRD) to consist exclusively of bct indium (Figure 2).

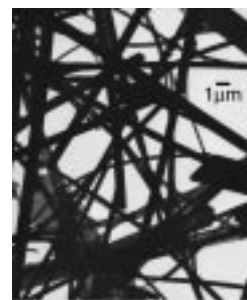


Figure 1. TEM micrograph of indium nanowires prepared in the presence of 0.5 equiv HDA (4-d reaction).

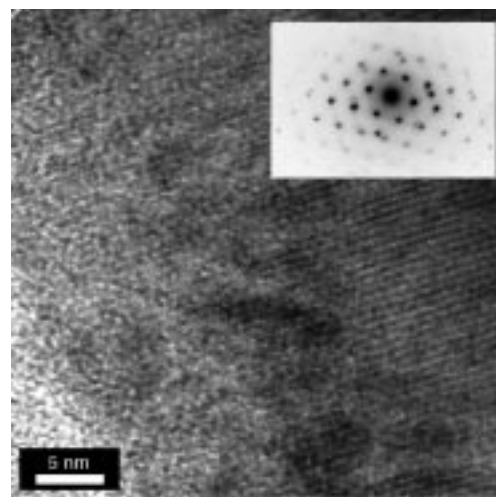


Figure 2. HREM micrograph of an indium nanowire revealing its monocrystalline (bct) structure. Inset: electron diffraction pattern of one wire corresponding to bct In (zone axis [100]).

Besides the wires, some nanoparticles were detected by TEM; they have the same size and shape as those prepared in the absence of UV irradiation (see below). Monitoring the reaction by NMR spectroscopy revealed decreasing intensity of the signal for **1** in solution and the production of CpH, and microscopy showed the predominant formation of wires.

Several parameters can affect the outcome of this decomposition reaction. To understand the formation of the wires and to achieve a better control over their diameter, we examined the influence of several parameters: nature of the amine and concentration relative to indium, rate of decomposition (UV irradiation), and the surrounding medium (nature of the solvent, concentration of the reagents in solution, and presence of water).

The amine/In ratio was varied while keeping the InCp concentration in solution and the reaction time (24 h) constant. In the absence of amine, the decomposition led to nanoparticles of about 11 nm in size without any formation of wires. However, most of these particles have an elongated

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shape, perhaps because of a preferred axis of growth. A small quantity of amine (amine/In 0.1/1) is sufficient for the formation, besides nanoparticles, of some nanowires with sizes between 100 and 800 nm. Increasing the amine/In ratio increases the yield of the reaction and leads to a better width distribution (most sizes lay between 150 and 350 nm and between 150 and 250 nm for amine/In ratios of 0.5 and 2, respectively; see Figure 3). With an amine/In ratio of 5, a width distribution centered at 250 nm was obtained.

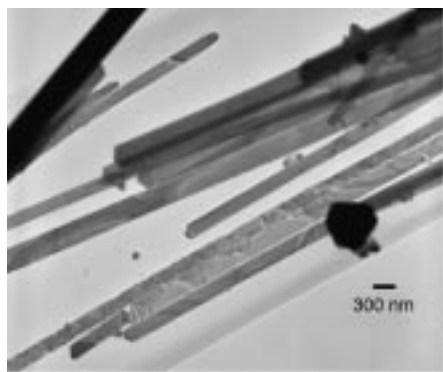


Figure 3. TEM micrograph of indium nanowires prepared in the presence of HDA (0.5 equiv).

For an amine/In ratio of 2, a microscopy grid was prepared after 90 min and after 24 h. The mean diameter of the wires was 150–200 nm and 200–250 nm, respectively. This suggests that increasing the time of the reaction increases the mean diameter of the wires, but not drastically. Changing the length of the alkyl chain from hexadecyl to dodecyl leads to the formation of thinner wires (diameter ca. 50–100 nm after 1.5 h of irradiation, and 100–150 nm after 24 h).

We compared reactions in the presence and in the absence of UV irradiation. In toluene at room temperature in the absence of UV irradiation, the reaction between **1** and 0.5 equiv HDA proceeded slowly to yield In particles of regular size centered near 3 nm. In addition to these particles, some larger crystals (10–20 nm) of various shapes were produced. However, for amine/In ratios greater than 2 in the absence of light, a few wires were obtained together with particles. Nevertheless, these experiments suggest that irradiation is crucial for the formation of the wires.

On decreasing the InCp concentration in toluene by a factor of five at an amine/In ratio of 0.5 or 2.5 nanoparticles of very narrow size distribution, centered at 6.7 and near 4.7 nm, respectively, were obtained. These particles, like the previously reported TOPO-stabilized particles, self-organize into hexagonal superlattices.^[10]

The water content of the reaction solution was previously demonstrated to play an important role in the regulation of the size of nanoparticles of indium or tin.^[8c, 10] In the present case, the water content of distilled toluene, measured by the Karl Fischer technique before experiments, was around 50 ppm. Increasing the water content to 250 or 450 ppm at an amine/In ratio of 2 led to the formation of nanoparticles and almost no wires. Carrying out the reaction in THF instead of toluene, either in the presence or in the absence of UV

light, does not produce wires, but nearly monodisperse nanoparticles of spherical shape and size centered near 7 nm (Figure 4). These particles also self-organize in two and three dimensions (see above and ref. [10]).

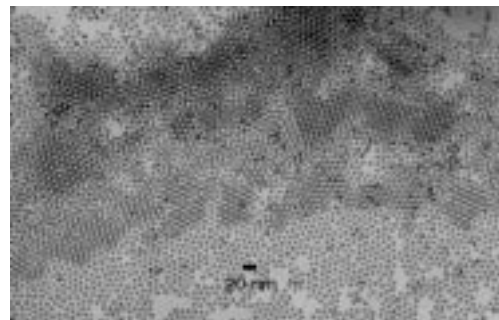


Figure 4. Mono- and multilayers of indium nanoparticles prepared in THF in the presence of 0.5 equiv HDA.

Finally, we previously reported that for late transition metals, the use of two organometallic precursors allowed the synthesis of bimetallic nanoparticles.^[8c,d] Since indium tin oxide wires appeared to be an attractive target, we investigated the co-decomposition of InCp and [Sn(NMe₂)₂]₂.^[12] In toluene at room temperature, with UV irradiation and in the presence of HDA (InCp/[Sn(NMe₂)₂]₂/HDA 1/0.41/0.5), nanowires were produced and were shown by XRD, HREM, and electron diffraction (ED) to consist exclusively of tetragonal β -In₃Sn. The mean diameter is smaller than in the case of pure In and lies near 100 nm (Figure 5).

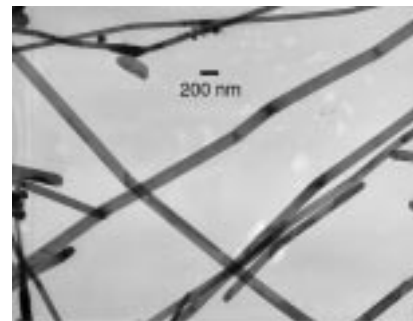


Figure 5. TEM micrograph of In₃Sn nanowires prepared in the presence of HDA.

In conclusion, we have described a simple method for obtaining indium nanowires at room temperature. This method can be easily extended to the controlled synthesis of nanowires of In₃Sn. The diameters of the wires are relatively large, near 200 nm for In and 100 nm for In₃Sn, but relatively narrow width distributions can be obtained. Clearly, the amine ligand is crucial for the formation of nanowires. However, the experiments varying the relative and absolute concentrations of InCp and the amine suggest that two other factors are important: the organization of the amine molecules in solution and the availability of indium atoms in the vicinity of the growing wire. These factors may explain the negative role of water, which may change both the organization of amine molecules in solution and cover the surface of

growing indium particles, as suggested previously for indium colloids in polyvinylpyrrolidone (PVP).^[10] The second factor is also in agreement with the need for photochemical decomposition of InCp, which presumably results in rapid production of indium atoms. The organization probably occurs in a similar way to that previously observed for gold–amine complexes.^[13] In contrast to other recently reported processes of nanowire growth, this process does not need a catalyst or even a continuous injection of precursors for growth to proceed. Hence, no seeds are visible at the ends of the wires. However, indium is a low-melting element, and a process involving “molten” indium may be operative. Amorphous, pseudomolten particles, such as those previously observed in PVP,^[10] may condense onto a preferred face of a preformed crystal. Finally, it is noteworthy that small changes in the reaction conditions allow the formation of spherical nanoparticles whose size depends upon the precise conditions, but which are uniform in each case and self-assemble in two and three dimensions.

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[2.2]Paracyclophane/Dehydrobenzoannulene Hybrids: Transannular Delocalization in Open-Circuited Conjugated Macrocycles**

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In memoriam Donald J. Cram

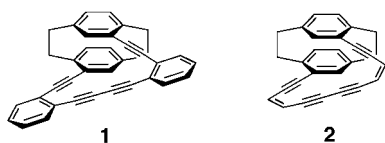
A variety of factors, such as conjugation length, planarity versus nonplanarity, double versus triple bonds, and introduction of donor and/or acceptor groups, can control the physical and chemical properties of an $[n]$ annulene.^[1] We have been involved extensively in developing the chemistry of a particular subset, namely dehydrobenzo $[n]$ annulenes (DBAs),^[2] and have shown the influence of many of the above-listed factors on DBA properties.^[3] Although the transmission of “electronic information” is easily accomplished throughout the macrocycle because of the closed circuit of conjugated π electrons, how would this transmission be affected if a small yet electronically bridgeable “gap” were installed? Inclusion in the DBA skeleton of a moiety capable of transannular interactions would provide such a test case. One particular segment that could provide a possible answer is a cyclophane.^[4] Small-sized cyclophanes are fascinating structures for annelation to a DBA skeleton in order to study transannular interactions in large macrocycles. Since the aromatic rings of a cyclophane can be fixed in a forced proximity and in a desired orientation, [2.2]paracyclophane

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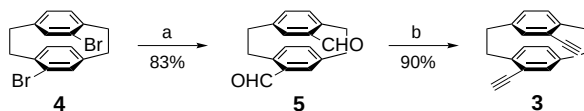
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(PC) in particular is characterized by a strong and unique transannular interaction between the two aromatic decks.^[5] Recent research has demonstrated how electronic communication in the PC system can be affected by varying the conjugation length of the appended substituents^[6] and by inclusion of electron-donating and -withdrawing moieties.^[7] These key model studies have provided insights into electronic delocalization between chromophores in the solid state, an important parameter to optimize when designing organic materials for optoelectronic applications.^[8] By incorporating the PC unit as part of an annulene core, we expect to observe enhanced electronic communication between the two fully conjugated decks of the annelated cyclophane. We report herein the synthesis of the novel [2.2]paracyclophane/dehydrobenzoannulene (PC/DBA) hybrids^[9] **1** and **2**, study of the transannular delocalization in these macrocycles using optical properties, and elucidation of solid-state structure of **2** by X-ray crystallography.

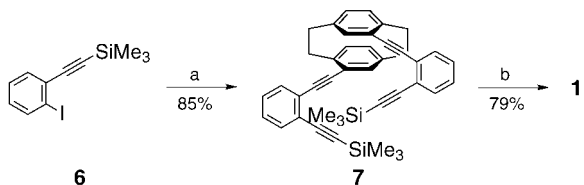


The key to PC/DBA synthesis was preparation of the parent dialkynylcyclophane **3** (Scheme 1). Metallation of dibromide **4**^[10] and treatment with *N*-formylpyrrolidine^[11] gave dialdehyde **5** in 83 % yield. This compound in turn was transformed into **3** (90 %) by using the mild alkylation procedure of Bestmann et al.^[12]



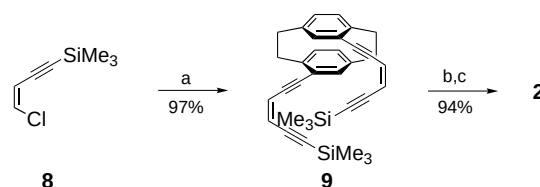
Scheme 1. Reagents: a) 1. BuLi, THF, 2. *N*-formylpyrrolidine; b) dimethyl 1-diazo-2-oxopropylphosphonate, K₂CO₃, MeOH.

PC/DBA **1** was assembled by Pd-catalyzed cross-coupling of iodoarene **6**^[3c] with diynylPC **3**, providing tetrayne **7** (Scheme 2). This material was desilylated and cyclized by using a one-pot procedure^[13] to give **1** in 67 % overall yield from **3**.



Scheme 2. Reagents: a) **3**, [Pd(PPh₃)₄], CuI, Et₃N, THF; b) K₂CO₃, CuCl, Cu(OAc)₂, py, MeOH.

The synthesis of PC/DBA **2** (Scheme 3) required a slight variation of the above route. Cross-coupling chloroalkene **8**^[14] with cyclophane **3** yielded tetrayne **9**. Use of the one-pot deprotection/cyclization method afforded **2** in a disappointing



Scheme 3. Reagents: a) **3**, [Pd(PPh₃)₄], CuI, PrNH₂, THF; b) K₂CO₃, MeOH, THF; c) Cu(OAc)₂, MeCN.

16 % yield. Stepwise deprotection and cyclization with Cu(OAc)₂ in MeCN, however, gave PC/DBA **2** in excellent overall yield (91 %).

Recrystallization from CH₂Cl₂ and pentane gave needles of **2** suitable for X-ray crystallography (Figure 1).^[15] Fusion of the annulene results in a small deviation from the normal

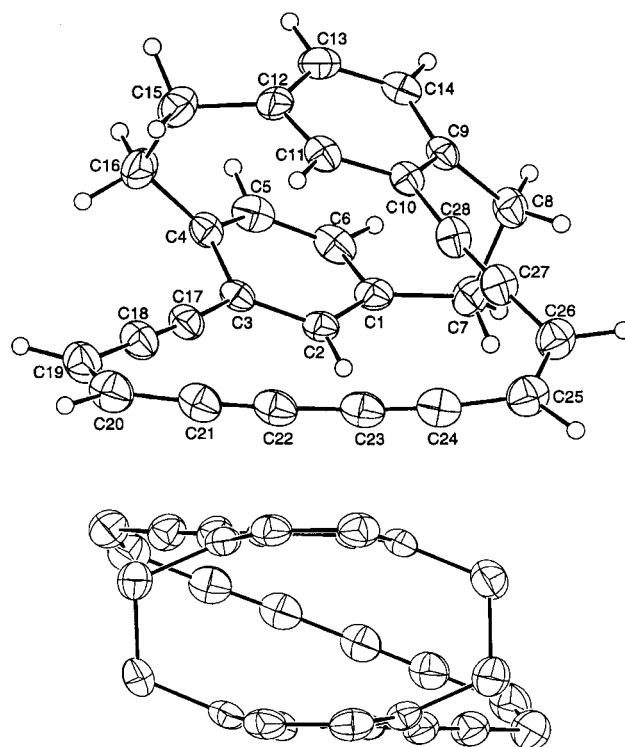
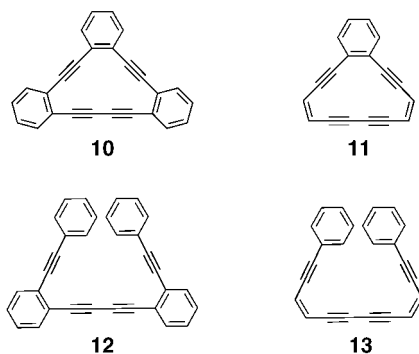


Figure 1. Structure of [2.2]paracyclophane/dehydrobenzoannulene hybrid **2**; ORTEP diagram, ellipsoids shown at the 30 % probability level.

[2.2]paracyclophane geometry. The interatomic distances between C2...C10 and C3...C11 are shortened slightly (3.06 Å) on the side of fusion, whereas the corresponding distances on the opposite side (3.09 Å) are typical for the PC skeleton. The small amount of strain in **2** caused by the 12-atom bridge is contained primarily in the diacetylene linkage, as the triple bonds deviate from linearity on the order of 5.2–9.0°. The remaining bond lengths and angles in **2** are characteristic of their respective subunits. Although Figure 1 depicts the *R* isomer of **2**, both enantiomers are present in the crystal. Given the tremendous interest in planar, chiral molecules,^[16] it should be possible to separate **1** and **2** on a chiral column or synthesize these molecules in optically active form. Such experiments are currently in progress.

To conclude whether or not “global” transannular delocalization was present in the PC/DBAs, the electronic absorption spectra of **1** and **2** were compared with those of **3**, α,ω -polyynes **7** and **9**, and dehydrobenzo[14]annulenes **10**^[17] and **11**^[18]. The most pertinent comparison, however, is with the appropriate “broken” analogues **12** and **13**^[19] because these systems contain the same chromophores found in **1** and **2**, yet lack the transannular effects enforced by the bridging ethylene units.



For the benzannelated series, the electronic absorption spectrum of PC/DBA **1** exhibits an appreciable bathochromic shift ($\Delta\lambda \approx 25-40$ nm) relative to both the “broken” analogue **12** and tetrayne **7** (Figure 2a). Interestingly, **1** shows approximately the same shift that is observed for **10**, but with greater absorption intensity. This suggests that the annelated cyclophane has similar electronic delocalization as the [14]annulene, but with increased electronic absorption attributable to the added electron density of the additional benzene ring in the cyclophane unit. In addition to the bathochromic shift, PC/DBA **1** also has an extended absorption cut-off relative to the other compounds in the series. These observations are most likely the result of “global” transannular delocalization occurring throughout the fully conjugated macrocycle by through-space electronic delocalization in the cyclophane.

Comparison of the electronic absorption spectra of PC/DBA **2** with diynyl cyclophane **3**, tetrayne **9**, annulene **11**, and “broken” analogue **13** (Figure 2b) did not show the expected bathochromic shift that **1** exhibited in the previous series. However, PC/DBA **2** does have an extended cut-off point ($\Delta\lambda \approx 50-75$ nm) in the absorption spectra relative to all other compounds used in this latter series, and shows greater absorption intensity at higher wavelengths than did annulene **11**. These observations are attributable to increased effective conjugation in the hybrid because of incorporation of alkene units in place of benzene rings.

The synthesis and study of [2.2]paracyclophane/dehydrobenzoannulene hybrids has led to strong qualitative evidence of “global” transannular delocalization between the fully conjugated decks of the annelated cyclophanes. The distinct bathochromic shift, increased absorption intensity, and extended absorption cut-off observed in PC/DBAs **1** and **2** when compared to other model systems can only be the result of delocalization through the open circuit of the PC/DBAs. We plan to continue this study through the synthesis of more

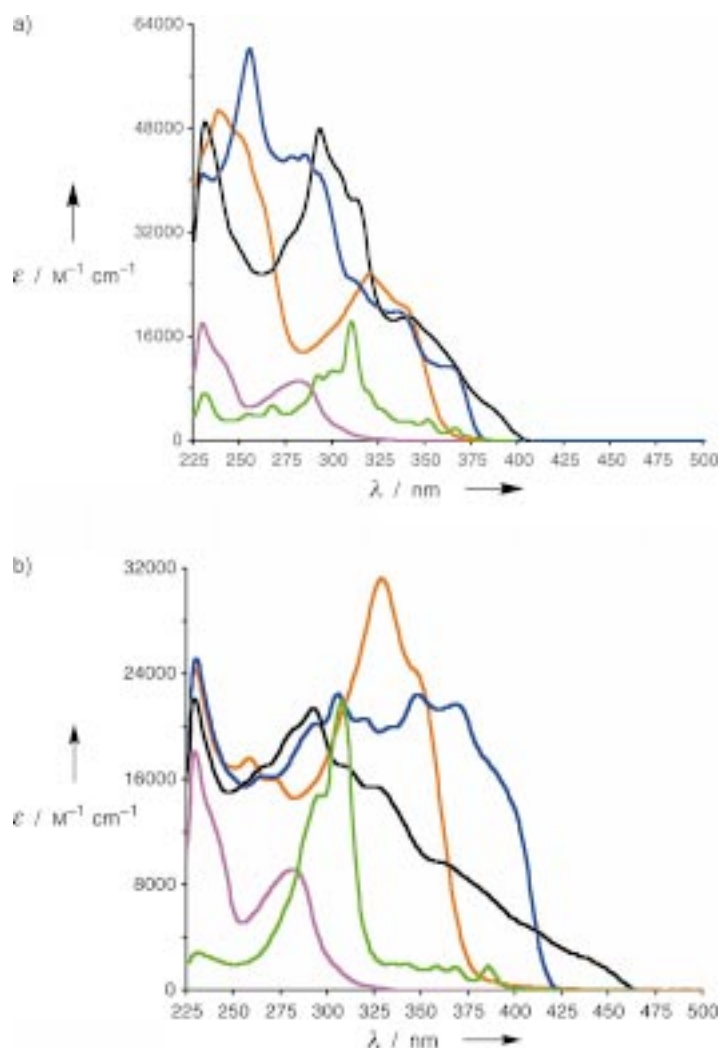


Figure 2. a) Electronic absorption spectra of PC/DBA **1** (black), diyne **3** (violet), tetrayne **7** (red), annulene **10** (green), and “broken” analogue **12** (blue). b) Electronic absorption spectra of PC/DBA **2** (black), diyne **3** (violet), tetrayne **9** (red), annulene **11** (green), and “broken” analogue **13** (blue).

complex PC/DBAs involving higher degrees of delocalization and possible “transannular aromaticity”. In addition, we are also exploring the use of these and other [2.2]paracyclophanes with polyunsaturated bridges as precursors to fullerenes and fullerene fragments, as beautifully demonstrated by the groups of Tobe^[9d] and Rubin.^[9e]

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- [15] C₂₈H₁₈, *M_r* = 354.45, yellow block, 0.13 × 0.16 × 0.20 mm, monoclinic, space group *P*₂₁/*n*, *a* = 9.505(2), *b* = 15.077(2), *c* = 13.687(2) Å, β = 98.608(8), *V* = 1939.3(6) Å³, *Z* = 4, ρ_{calcd} = 1.214 g cm^{−3}, Mo_{Kα} radiation (λ = 0.71073 Å), μ = 0.687 cm^{−1}, *F*(000) = 744, *T* = 23 °C, 2θ_{max} = 50°, 3583 independent reflections scanned, 1684 reflections in refinement (*I* = σ(*I*)), 325 parameters, *R* = 0.065, *R_w* = 0.047. Data were obtained on an Enraf-Nonius CAD-4 Turbo diffractometer. Structure refinement (C atoms anisotropic, H atoms isotropic) was accomplished with teXsan (version 1.7 for SGI workstations). 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-160412. 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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- [19] Prepared in two steps by coupling phenylacetylene with **6** and **8**, respectively, followed by in situ desilylation/homodimerization (as described in reference [13]).

The Remarkable Stable Emerald Green C₆₀F₁₅[CBr(CO₂Et)₂]₃: The First [60]Fullerene That Is also the First [18]Trannulene**

Xian-Wen Wei, Adam D. Darwish, Olga V. Boltalina, Peter B. Hitchcock, Joan M. Street, and Roger Taylor*

The fluorofullerene C₆₀F₁₈ is notable for having a fully delocalized and planar hexagonal ring and a normal curved “fullerene” region; it is also a hexa-substituted benzene.^[1] Derivatives that have been made include oxides, which are produced by oxygen insertion into long FC–CF bonds (the first examples of oxahomofullerenes),^[2] trifluoroalkyl derivatives produced by insertion of CF₂ groups into C–F bonds,^[3] and a triphenyl derivative produced by nucleophilic substitution of three fluorine substituents by phenyl groups.^[4]

Annulenes have long been of interest because of the question of whether the combination of [4*n*+2] π-electrons and approximate planarity leads to aromaticity. A number of examples are now known in which this latter condition is indicated, notably by small differences in bond lengths (which usually range from 1.38–1.40 Å) in a cyclic chain comprising either sp² or sp/sp² carbon atoms.^[5] The [18]annulenes have been of particular interest because of their presence in porphyrins, phthalocyanines, chlorophyll etc., and some have been prepared notably by Sondheimer et al., for example light brown plates of tridehydro[18]annulene.^[6] In these compounds, the C–C bonds *a* to the double bonds are in either an *all-cis* or a *cis,trans* relationship. Very recently, the feasibility of annulenes having *all-trans* relationships (“trannulenes”, which must have a hoop or barrel-like structure) has been

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conjectured.^[7] We now report the isolation of the first trannulene that is also a fullerene.

Because $C_{60}F_{18}$ may be a useful precursor for the formation of donor–acceptor dyads with enhanced acceptor properties, we are carrying out a preliminary small-scale exploration of reactions that have been used for the formation of dyads derived from [60]fullerene itself. One notable reaction of this type introduced by Bingel's group involves deprotonation of an α -bromoester by sodium hydride. The resulting anion then attacks the cage to give, after loss of bromide, a substituted methanofullerene;^[8] this reaction has been used very effectively for example in the fine work of Diederich et al. on tether-directed additions.^[9] A very recent example showing the versatility of the reaction is given in ref. [10].

With the intention therefore of obtaining a methanofullerene from $C_{60}F_{18}$ we carried out the reaction with diethyl bromomalonate by using an excess of ester together with DBU; the latter improved base was introduced by Camps and Hirsch.^[11] The reaction largely did not take place in the customary manner. The intermediate anion attacks the cage in the normal way, but instead of the bromide ion being lost, a fluoride ion is lost from a remote (δ) position. This results in either one, two, or three fluorine substituents being nucleophilically replaced by the $CBr(CO_2Et)_2$ moiety to give a mixture of mono-, di-, and trisubstituted derivatives (the relative extent can be controlled by the rate of addition of the base). We describe here the full characterization of the unique trisubstituted derivative $C_{60}F_{15}[CBr(CO_2Et)_2]_3$ (**1**) by ^1H and ^{19}F NMR spectroscopy, and by single-crystal X-ray crystallography.

The compound crystallizes as a toluene solvate giving emerald green diamond-shaped plates, and the single crystal X-ray structure is shown in Figure 1; for clarity the compound

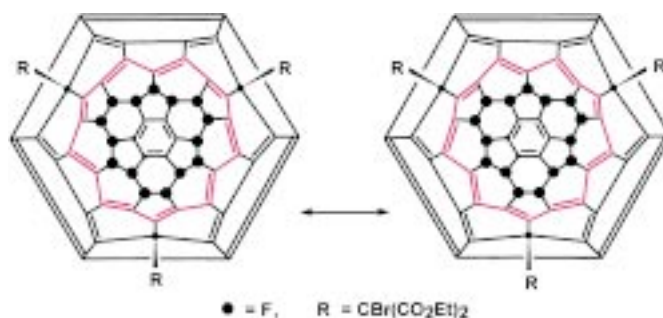
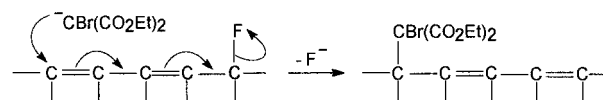


Figure 2. Schlegel diagrams for two canonical structures of **1** showing the resonance in the annulene belt.



Scheme 1. Mechanism of replacement of fluorine in $C_{60}F_{18}$ by $CBr(CO_2Et)_2$.

- The molecule contains an equatorial 18π annulene belt of electrons. It is thus not only an aromatic fullerene because of the planar delocalized hexagon that is surrounded by fluorine atoms, but is also aromatic by virtue of the annulene belt of $[4n+2]$ π electrons (magenta-highlighted in Figure 2); the *all-trans* relationship of the constituent bonds of the belt defines this as a *trannulene*.
- The electrons in this belt are highly delocalized as shown by the bond lengths for the constituent bonds (in bold, Table 1), which vary by a remarkably small $0.013 \text{ \AA}$. By this measure, this belt comprises the most delocalized annulene of any kind yet found, and because of the size of the cage, the deviations from planarity are small and apparently not detrimental.

Table 1. Bond lengths [$\text{\AA}$] in **1** (for notation, see Figure 3).

Bond	Length [$\text{\AA}$]	Bond	Length [$\text{\AA}$]
a	1.366	j	1.397
a'	1.378	k	1.468
b	1.484	l	1.530
c	1.608	m	1.428
d	1.565	n	1.376
e	1.655	p	1.539
f	1.490	q	1.420
g	1.506	r	1.402
h	1.410	s	1.448
i	1.392		

- The high level of conjugation in the belt is responsible for the intense emerald green color. By contrast, the mono- and di-substituted compounds have only the pale yellow-green color characteristic of $C_{60}F_{18}$ and its derivatives, because the unsubstituted fluorine atoms prevent formation of an annulene belt.
- The general structure may prove to be an outstanding candidate for investigation of donor–acceptor electronic interactions, through variation for example in the nature of the substituted alkyl addends. Such effects should for example be apparent from any observed color changes.

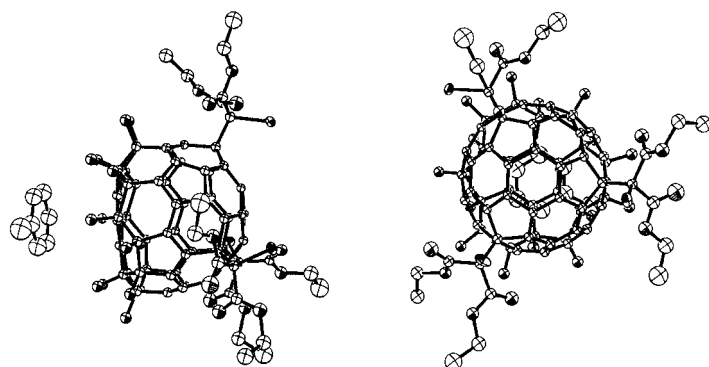


Figure 1. Two projections of **1**· C_7H_8 (ORTEP).

is shown as a Schlegel diagram (with two resonance structures) in Figure 2. The structure shows a number of most remarkable features:

- It is produced by a mechanism which is the first proven example of S_N2' substitution in a fullerene, a process we conjectured some years ago to account for the formation of epoxides on treatment of fluorofullerenes with moisture.^[12] In the present instance substitution occurs at the δ position relative to the departing fluorine atom, as shown in Scheme 1. This is evidently favored over substitution at the alternative β position for steric reasons.

- The hexagonal ring at the pole remote from the fluorine atoms has slightly enhanced aromaticity as shown by the bond length differences which are 0.046 Å compared to 0.060 Å for the corresponding hexagon in C₆₀F₁₈. This results from the presence of an sp³ carbon atom in the adjacent pentagons which reduces strain and facilitates bond-shortening in the bond common to the hexagon.^[13]
- The molecule appears to be particularly stable, and shows no evidence for long-term degradation.
- Compared to the corresponding bonds in the C₆₀F₁₈ precursor, bonds c, f, and h are shorter because of the reduced compression arising from the removal of adjacent fluorine atoms. By contrast, bonds l and p are lengthened because of the new adjacent addend.

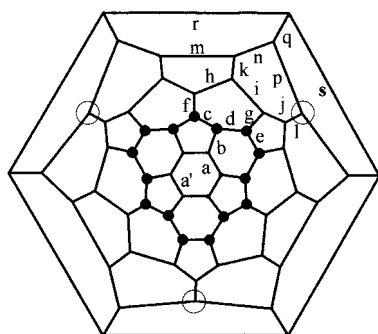


Figure 3. Schlegel diagram for **1** showing the notation for the symmetry-independent C–C bonds; ● = F, ○ = CBr(CO₂Et)₂.

The EI mass spectrum (70 eV) shows main fragmentation ions at m/z = 1481 and 1005 from loss of CBr(CO₂Et)₂ and [CBr(CO₂Et)₂]₃, respectively. A small peak at m/z = 1639/1641 results from the loss of one bromine atom from the parent molecule.

The ¹H NMR spectrum (Figure 4) comprises two resonances at δ_{H} = 4.13 (q, $J(\text{H,H})$ = 7.1 Hz, 2H; CH₂), 1.038 (t, $J(\text{H,H})$ = 7.1 Hz, 3H; CH₃) showing the molecule to have C_{3v} symmetry. An unusual feature is that on lowering the temperature (see inset to Figure 4) the signals broaden and move downfield (run in CS₂/CDCl₃). This is not due to a chemical exchange process, but could indicate formation of a radical, and is to be investigated further.

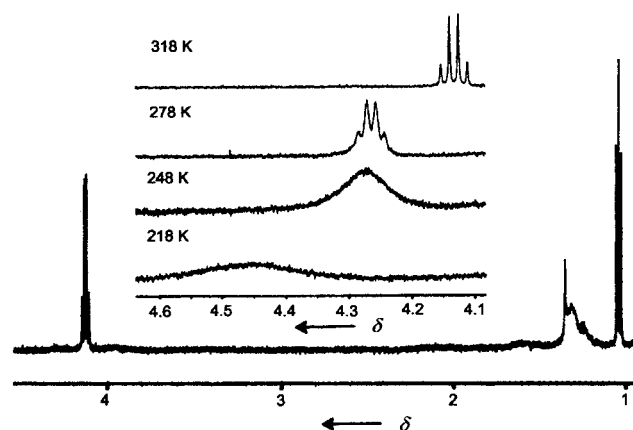


Figure 4. ¹H NMR spectrum (500 MHz, C₆D₆, 25 °C, TMS) of **1**.

The C_{3v} symmetry is confirmed by the ¹⁹F NMR spectrum (Figure 5) comprising three coupled lines (2D spectrum, not shown) at δ_{F} = –136.72 (1F), –143.87 (2F), –143.96 (2F). The notable difference in the spectrum from those for C₆₀F₁₈^[1] (and its derivatives) is that the upfield line of these latter which arises from the fluorine atoms attached to the carbon atom surrounded by the less electronegative sp³ carbon atoms is absent. This is consistent with the removal of the three outermost fluorine atoms.

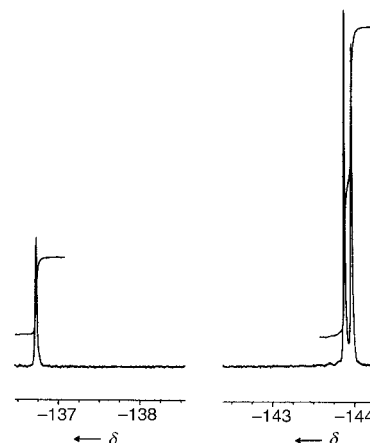


Figure 5. ¹⁹F NMR spectrum (376 MHz, CDCl₃, 25 °C) of **1**.

The UV/Vis spectrum (Figure 6) shows bands at 368, 380, 389, 395, 400, 437, 612, and 658 nm, these latter pair accounting for the observed color. Further work is in hand to prepare related derivatives and to investigate their spectroscopic and other properties.

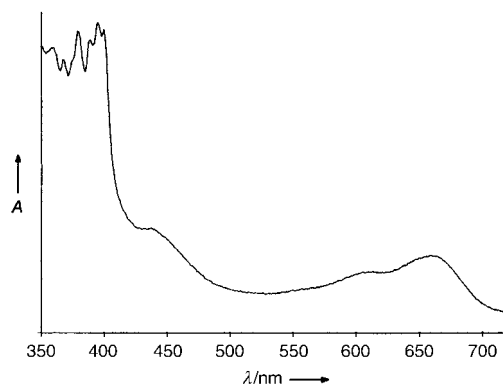


Figure 6. UV/Vis spectrum (hexane) of **1**.

Experimental Section

C₆₀F₁₈ (5 mg) was dissolved in toluene (15 mL) together with a slight molar excess of diethyl bromomalonate, and a ca. molar equivalent of DBU (dissolved in 0.4 mL of toluene) was added dropwise under argon. The solution turned green immediately and was stirred overnight at room temperature. A black precipitate was removed by filtration and the filtrate separated by HPLC (10 × 250 mm Cosmosil 5 Pye column, toluene elution at 4.7 mL min^{–1}). The fraction eluting at 3.55 min was repurified on a Cosmosil Buckprep column, (elution with 1:1 toluene:heptane at 4.7 mL min^{–1}) and **1** eluted at 5.0 min. The yield (ca. 30%) can be increased by using larger ester to DBU proportions to reduce the accompanying mono- and bis-substitution.

The structure was determined by mass spectrometry, ^1H and ^{19}F NMR spectroscopy as well as by X-ray diffraction.^[14]

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Catalytic Enantioselective Addition of Nitro Compounds to Imines—A Simple Approach for the Synthesis of Optically Active β -Nitro- α -Amino Esters**

Nagatoshi Nishiwaki, Kristian Rahbek Knudsen, Kurt V. Gothelf, and Karl Anker Jørgensen*

The development of C–C bond-forming reactions that create two new stereogenic centers with high diastereo- and enantioselectivity in a single step can open new routes to highly valuable optically active compounds. The catalytic enantioselective addition to imines^[1] belongs to this class of important reactions, and recently new catalytic asymmetric processes, for example, for Mannich-type,^[2] ene,^[3] allylation and alkylation,^[4] aza-Diels–Alder,^[5] Strecker,^[6] and aromatic electrophilic substitution reactions^[7] have appeared. Another important reaction of imines is the nitro-Mannich (aza-Henry) reaction, which is a powerful C–C bond-forming method that leads to 2-nitroamines.^[8] To the best of our knowledge there is only one other paper that describes the catalytic enantioselective version of the nitro-Mannich reaction.^[9] Although this reaction was catalyzed by a hetero-bimetallic complex, it has been described for nitromethane only and requires 60 mol % of the chiral ligand. In a very recent paper, we described the first catalytic asymmetric reaction of silyl nitronates with imines.^[10] The reaction proceeds at -100°C in the presence of bisoxazoline–copper catalysts to give 2-nitroamines with high enantioselectivities.

Herein we present a new and significantly simplified approach to the catalytic diastereo- and enantioselective nitro-Mannich reaction of an N-protected α -imino ester **1** with nitro compounds **2**. The reactions can be performed under ambient conditions to give β -nitro- α -amino esters **3** in good yields, and with high diastereo- and enantioselectivities [Eq. (1); Pg = protecting group].

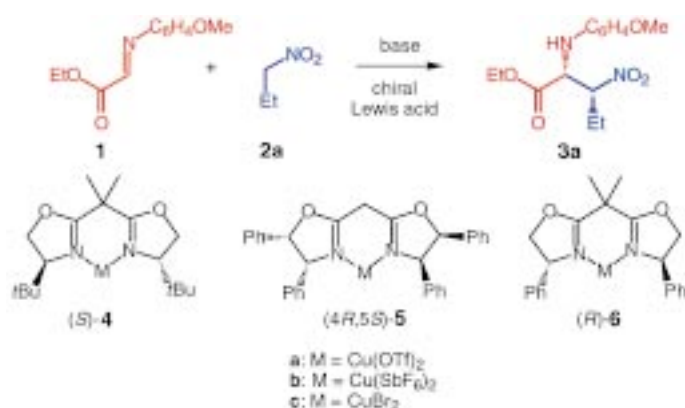


In the screening process, the reaction of *N*-(*p*-methoxyphenyl)- α -imino ester **1**^[11] with nitropropane **2a** in the presence of NEt_3 as the base has been studied at room temperature by using different combinations of chiral ligands and Lewis acids (Scheme 1). The use of chiral bisoxazoline

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.



Scheme 1. Reaction of imino ester **1** and nitropropane **2a** in the presence of NEt₃ with different combinations of chiral ligands and Lewis acids (see Table 1).

(BOX) ligands^[12] and copper salts gave the most promising results and some of them are presented in Table 1.

The BOX–Cu^{II} catalyst (*S*)-**4a** (20 mol %) affords only a small amount of the nitro-Mannich adduct **3a** in a high diastereomeric ratio and with 89 % *ee* of the major *erythro*-diastereomer (Table 1, entry 1). The use of the phenyl-substituted BOX ligands revealed a significant improvement in the catalytic activity to give **3a** in good to high yields. Excellent enantioselectivity of the β -nitro- α -amino ester **3a**, isolated in 80 % yield, was achieved by using catalyst (*R*)-**6a**, whereas (*4R,5S*)-**5a** afforded a racemic mixture of diastereomers (Table 1, entries 2,3). The reaction is highly solvent dependent and in CH₂Cl₂ adduct **3a** was formed in higher yield than in THF and Et₂O (Table 1, entries 3–5). The diastereo- and enantioselectivities are also dependent on the counterion, as observed by the application of triflate versus hexafluoroantimonate (Table 1, entries 3,6). A good conversion and high diastereo- and enantioselectivity was also obtained when using the CuBr₂–BOX catalyst **6c** (Table 1, entry 7), which is a poorer Lewis acid than **6a,b**.

The influence of amines on the reaction was also screened. For example, in the presence of cyclohexyldimethylamine, the

reaction proceeded in good yield and with high diastereo- and enantioselectivity (Table 1, entry 8). Secondary amines can also be used and give almost the same stereoselectivity (Table 1, entry 9), whereas *N,N*-dimethylaniline, 1,8-diazabicyclo[5.4.0]undec-7-ene, 1,2-bis(dimethylamino)ethane, and (–)-sparteine did not afford adduct **3a**. Among the amines tested, NEt₃ gave the best results.

The reaction is affected by the amount of amine and the order in which the reagents are added. An increase in the amount of NEt₃ to 40 mol % led to a nondiastereoselective reaction (Table 1, entry 10), whereas no reaction took place when the amount of amine was reduced to 10 mol %. When NEt₃ was added before the imine to a solution of the catalyst, a complicated product mixture was formed, and a low yield of **3a** with excellent selectivity was obtained (Table 1, entry 11).

The catalyst loading can be reduced to 10 mol %, while still maintaining the catalytic activity and the excellent diastereo- and enantioselectivity, although longer reaction times are required (Table 1, entry 12). The reaction can be performed under very user-friendly conditions by just mixing the reagents under ambient conditions in CH₂Cl₂ (not dried or distilled). Under these conditions, no decrease in catalytic activity was observed, and adduct **3a** was formed in high yield and excellent diastereo- and enantioselectivity (Table 1, entry 13). Furthermore, at 0 °C the reaction takes place with an improvement in both diastereo- and enantioselectivity (Table 1, entry 14). These results are important for the practical application of this reaction.

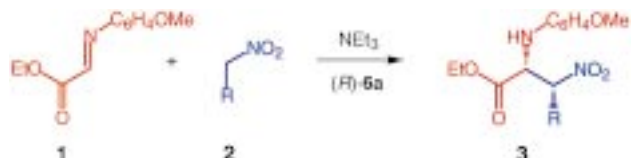
The scope of the catalytic enantioselective nitro-Mannich reaction of **1** is presented in Table 2. Nitropropane **2a** reacted with **1** to give **3a** in 81 % yield and with excellent diastereo- and enantioselectivity (Table 2, entry 1). The reaction with nitromethane **2b** proceeded with high enantioselectivity; however, the yield of the isolated nitro-Mannich adduct **3b** was moderate (Table 2, entry 2). The reactions of nitroethane and nitrohexane, **2c** and **2d**, respectively, gave the nitro-Mannich adducts **3c** and **3d**, in good yields, with very high diastereoselectivities and excellent enantioselectivities (97 % *ee* for both reactions; Table 2, entries 3,5). The stereoselec-

Table 1. Catalytic enantioselective nitro-Mannich reactions of *N*-(*p*-methoxyphenyl)- α -imino ester **1** with nitropropane **2a** under various reaction conditions at room temperature.^[a]

Entry	Catalyst	Amount [mol %]	Solvent	<i>t</i> [days]	Conversion [%] ^[b]	dr ^[c] <i>erythro</i> / <i>threo</i>	<i>ee</i> [%] ^[c] <i>erythro</i> / <i>threo</i>
1	4a	20	CH ₂ Cl ₂	1	8	95/5	89/70
2	5a	20	CH ₂ Cl ₂	1	62	84/16	2/2
3	6a	20	CH ₂ Cl ₂	1	85	92/8	94/85
4	6a	20	THF	1	14	96/4	97/90
5	6a	20	Et ₂ O	1	26	97/3	97/83
6	6b	20	CH ₂ Cl ₂	1	85	60/40	26/21
7	6c	20	CH ₂ Cl ₂	1	69	95/5	93/86
8 ^[d]	6a	20	CH ₂ Cl ₂	1	83	82/18	95/95
9 ^[e]	6a	20	CH ₂ Cl ₂	1	40	95/5	97/91
10 ^[f]	6a	20	CH ₂ Cl ₂	1	80	54/46	72/76
11 ^[g]	6a	20	CH ₂ Cl ₂	1	43	95/5	96/86
12	6a	10	CH ₂ Cl ₂	7	83	92/8	91/85
13 ^[h]	6a	20	CH ₂ Cl ₂	1	86	93/7	95/79
14 ^[i]	6a	20	CH ₂ Cl ₂	5	87	95/5	97/87

[a] The reactions were performed on a 0.2 mmol scale with 150 mol % of nitropropane. The catalyst and NEt₃ were used in a 1:1 ratio. For further details, see Supporting Information. [b] Determined by ¹H NMR spectroscopy of the crude product. [c] The diastereomeric ratios and enantiomeric excesses were determined by HPLC. [d] *c*-HexNMe₂ was used. [e] HNet₂ was used. [f] 40 mol % of NEt₃ was used. [g] NEt₃ was added before imine **1**. [h] Open-air reaction. [i] At 0 °C.

Table 2. Nitro-Mannich reactions of *N*-(*p*-methoxyphenyl)- α -imino ester **1** with different nitro compounds **2a–f** catalyzed by (*R*)-**6a** at room temperature or 0 °C.

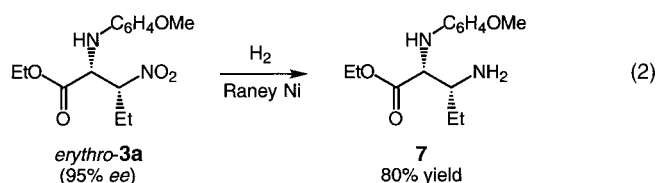


Entry	R	Yield [%] ^[a]	dr ^[b] erythro/threo	ee [%] ^[b] erythro ^[c] /threo
1 ^[d]	Et (2a)	81 (3a)	95/5	97/87
2	H (2b)	38 (3b)	–	87
3	Me (2c)	61 (3c)	70/30	97/95
4 ^[d]	Me (2c)	50 (3c)	73/27	99/98
5	Pentyl (2d)	52 (3d)	93/7	97/89
6	PhCH ₂ (2e)	80 (3e)	95/5	95/88
7	Ph (2f)	59 (3f)	55/45 ^[e]	74/77

[a] Yield of isolated products. [b] Diastereomeric ratios and enantiomeric excesses were determined by HPLC. [c] *erythro*: (2*R*,3*R*). [d] The reaction was performed at 0 °C. [e] Determined by ¹H NMR spectroscopy.

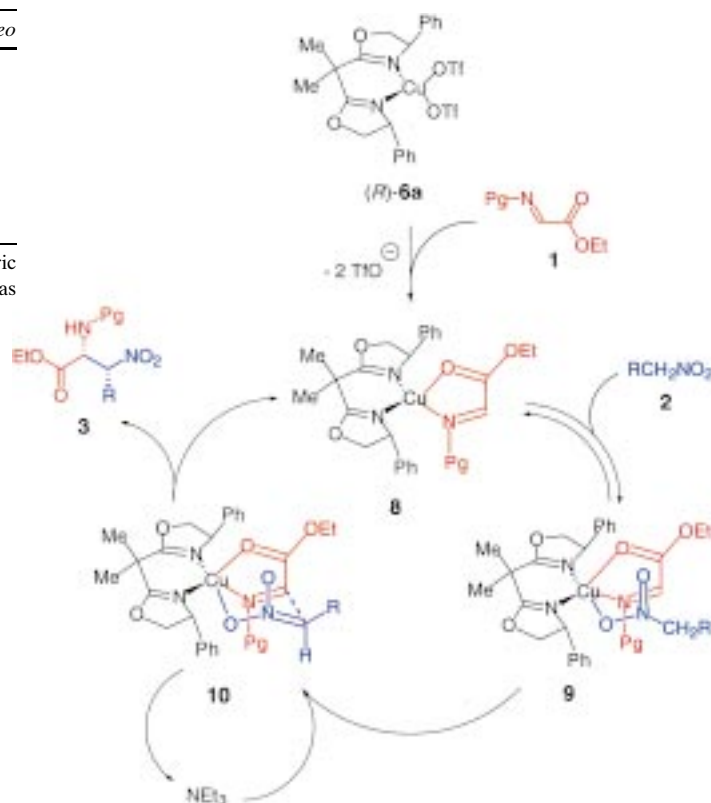
tivity can be improved by lowering the reaction temperature. The reaction of **2c** gave a diastereomeric ratio of 73/27 and 99 % *ee* of *erythro*-**3c** when the reaction was performed at 0 °C (Table 2, entry 4). The present reaction is not only restricted to simple 1-nitroalkanes, as shown by the fact that 2-phenyl-1-nitroethane **2e** reacted with **1** to give the nitro-Mannich adduct **3e** in 80 % yield and with excellent diastereo- and enantioselectivity (Table 2, entry 6). α -Nitrotoluene **2f** reacted with **1** under the present catalytic conditions to give **3f** in moderate yield and diastereoselectivity, but with good enantioselectivities of both diastereomers (Table 2, entry 7). The reactions of methyl nitroacetate and 2-nitroethanol with **1** catalyzed by (*R*)-**6a** gave the corresponding nitro-Mannich adducts for both compounds in good yields; however, moderate diastereo- and enantioselectivities were observed, which might be a result of a competitive binding of the acetate and hydroxy functionality to the Cu^{II} center (see below). For the reaction of 2-nitroethanol, the diastereomeric ratio is temperature dependent and at room temperature the ratio was 67:37, whereas at –15 °C the ratio was 14:86 with > 70 % *ee* of the major diastereomer of the adduct.

The present new catalytic enantioselective nitro-Mannich reaction leads to a simple procedure for the formation of optically active N-protected β -nitro- α -amino esters, a new class of α -amino acid derivatives. The optically active N-protected β -nitro- α -amino esters such as *erythro*-**3a** can easily be reduced to the corresponding N-protected α,β -diamino ester **7** in high yield [Eq. (2)].^[10] For example, further



reaction of the N-protected α,β -diamino ester has been used for the determination of the absolute configuration of the products.^[10]

In Scheme 2 a proposal for the catalytic cycle of the new nitro-Mannich reaction is suggested to account for the catalytic activity, and for the diastereo- and enantioselectivity. It is important for the outcome of the reaction that catalyst **6a** is mixed with the α -imino ester **1** first to give intermediate **8**. Nitro compounds coordinate relatively weakly to the copper center, as it has been reported that some BOX–Cu^{II}–



Scheme 2. Proposed catalytic cycle of the new nitro-Mannich reaction, which accounts for the catalytic activity, and for the diastereo- and enantioselectivity

catalyzed reactions can be performed in nitromethane as the solvent.^[13] Thus, it is proposed that intermediate **9** is in a fast equilibrium with **8**. In intermediate **9**, the acidity of the nitro compound is increased by the coordination of the nitro group to Cu^{II}. This facilitates the catalytic deprotonation of the nitro compound with Et₃N to give intermediate **10**, which is proposed to be the key intermediate in the reaction. This intermediate would lead to a cyclohexane-like cyclic transition state with additional coordination of the carbonyl group of **1** to the metal center in a pentacoordinated fashion. In this structure the nitro substituent occupies the less sterically crowded and more stable equatorial position, which accounts for the diastereoselectivity of the reaction. The chiral (*R*)-Ph-BOX ligand in **10** favors the formation of the (2*R*,3*R*)-enantiomer of the product, since the intermediate that leads to the opposite enantiomer would lead to steric repulsion between the bulky *N*-(*p*-methoxyphenyl) substituent of the α -imino ester and the chiral phenyl substituent of the ligand.

In summary, a new enantioselective catalytic addition reaction of nitro compounds to an α -imino ester in the presence of chiral bisoxazoline–Cu^{II} complexes has been

presented. Even under ambient conditions, the reaction gives optically active β -nitro- α -amino esters with excellent diastereo- and enantioselectivity.

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Catalytic Asymmetric Direct Mannich Reactions of Carbonyl Compounds with α -Imino Esters**

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The Mannich reaction is an important class of C–C bond-forming reactions in organic chemistry.^[1] A number of methods for the diastereoselective reaction of imines with enolates has been reported,^[1a, 2] and recently the first examples of catalytic enantioselective addition of enolates to imines were reported.^[1c, 3] One disadvantage of these stereoselective Mannich reactions is the preparation and stability of the enolate, and a major step forward for this important class of reactions would be a catalytic enantioselective version that uses carbonyl compounds rather than the enolates.^[4]

Recently, we demonstrated that simple chiral Lewis acids such as the bisoxazoline (BOX)–Cu^{II} complexes^[5] can mimic aldolase enzymes, and a highly enantioselective homo-aldol reaction of pyruvate which gave diethyl-2-hydroxy-2-methyl-4-oxo-glutarate in up to 96% *ee* was developed.^[6] In this homo-aldol reaction, the chiral Lewis acid acts both as a catalyst for the in situ generation/stabilization of the enol-pyruvate from pyruvate, and as a catalyst for the enantioselective addition step. This new aspect of Lewis-acid catalysis led us to try to develop other reactions based on this concept.

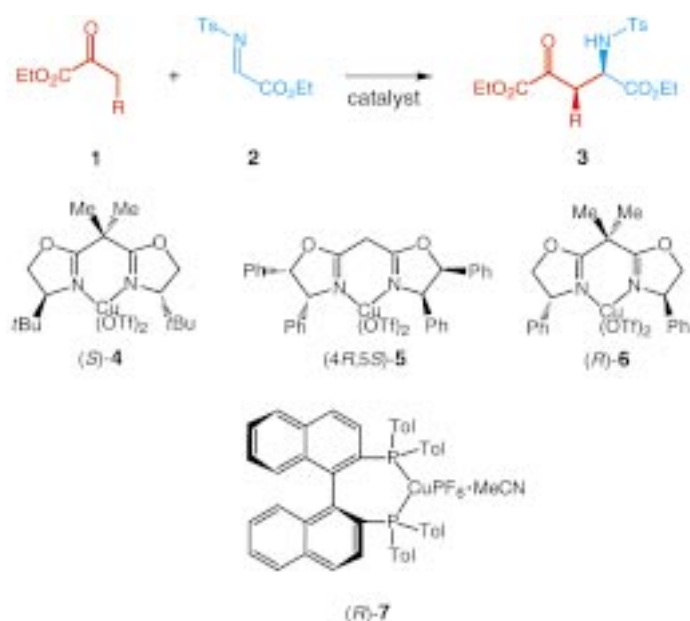
Herein we present the first catalytic diastereo- and enantioselective Mannich reaction of activated carbonyl compounds **1** with the α -imino ester **2** catalyzed by chiral Lewis acids (Scheme 1). This new development leads to a simple synthetic procedure for the formation of optically active highly functionalized α -amino acid derivatives **3** by using readily available carbonyl compounds rather than the often troublesome silyl enol ethers or silyl ketene acetals.

The reaction between ethyl pyruvate **1a** (R = H) and *N*-tosyl- α -imino ester **2** has been used for screening the reaction conditions for the chiral Lewis-acid catalyzed direct Mannich reaction. The metal ion is crucial for the success of this reaction. It has been found that copper(II) possesses the properties necessary for both the in situ generation of the enol species from **1a** and, in combination with chiral C_2 -symmetric ligands, the stereoselectivity of the reaction. Table 1 presents some results for the reaction of **1a** with **2** in the presence of bisoxazolines and BINAP (2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl) as chiral ligands. The use of the *t*Bu-BOX-Cu(OTf)₂ catalyst (*S*)-**4** in CH₂Cl₂ led to the formation of the Mannich adduct **3a** (R = H) in reasonable yield and 33% *ee* (Table 1, entry 1), whereas the di-Ph-BOX-Cu(OTf)₂ catalyst

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Scheme 1. Diastereo- and enantioselective Mannich reaction of activated carbonyl compounds **1** with α -imino ester **2**, catalyzed by chiral Lewis acids **4–7**. **1a**, **3a**: R = H; **1b**, **3b**: R = CH₃; **1c**, **3c**: R = Bn (Bn = Benzyl); **1d**, **3d**: R = Br.

Table 1. Catalytic enantioselective Mannich reaction of ethyl pyruvate **1a** (R = H) with N -tosyl- α -imino ester **2** using different catalysts under various reaction conditions.^[a]

Entry	Catalyst	Solvent	<i>t</i> [h]	Yield [%] ^[b]	<i>ee</i> [%] ^[c]
1	(<i>S</i>)- 4	CH ₂ Cl ₂	40	76	33
2	(4 <i>R</i> ,5 <i>S</i>)- 5	CH ₂ Cl ₂	40	44	12
3	(4 <i>R</i> ,5 <i>S</i>)- 5	THF	16	16	33
4	(<i>R</i>)- 6	CH ₂ Cl ₂	40	70	89
5	(<i>R</i>)- 6	THF	16	45	76
6	(<i>R</i>)- 6	Et ₂ O	40	< 5	–
7	(<i>R</i>)- 7	CH ₂ Cl ₂	40	< 10	–

[a] Experimental conditions: The reactions were performed with 0.25 mmol of **1a** and 0.375 mmol of **2**, and with 10 mol% catalyst. For further details see Supporting Information. [b] Yield of isolated product. [c] Enantiomeric excesses were determined by HPLC.

(4*R*,5*S*)-**5** gave lower yields and enantiomeric excesses of **3a** in CH₂Cl₂ and THF (Table 1, entries 2, 3). The application of the Ph-BOX-Cu(OTf)₂ catalyst (*R*)-**6** in the reaction was much more successful and led to **3a** in good yield and up to 89% *ee* (Table 1, entries 4, 5). However, catalyst (*R*)-**6** is solvent dependent, as <10% of **3a** was obtained in Et₂O (Table 1, entry 6). The BINAP–Cu^I catalyst (*R*)-**7**, which has been successfully applied as a catalyst in several different types of addition reactions to **2**,^[3d,e, 7] gave only traces of **3a** in the present reaction (Table 1, entry 7).

The combination of copper(II) and the Ph-BOX-ligand turned out to be an excellent catalyst for the Mannich reaction of α -carbonyl esters **1a–d** with the N -tosyl- α -imino ester **2**. The reactions proceeded in good yield, high *syn* selectivity, and excellent enantioselectivity. The best result for the reaction of ethyl pyruvate **1a** with **2** catalyzed by (*R*)-**6** is shown in Table 2, entry 1. Ethyl 2-oxo-butylate **1b** reacts with **2** to give **3b** in 89% yield and excellent enantioselectivity

(>98%) of the *syn* diastereomer when (*R*)-**6** was used as the catalyst in CH₂Cl₂ (Table 2, entry 2). A decrease in the catalyst loading to 5 mol% improved the yield to 98%, with the same high diastereo- and enantioselectivity (Table 2, entry 3). The reaction can also be performed in THF with similar results (Table 2, entry 4). The application of catalyst (4*R*,5*S*)-**5** to the reaction of **1b** with **2** gave a slightly lower yield of **3b**; however, the Mannich adduct was obtained with good enantioselectivity (Table 2, entry 5). The reaction of the benzyl compound **1c** with **2** is sluggish at room temperature. However, the Mannich adduct **3c** was obtained in 94% yield with 97% *ee* of the major diastereomer when the reaction mixture was heated to 40 °C (Table 2, entry 6). Ethyl bromopyruvate **1d** reacts with **2** to give the Mannich adduct **3d** in good yield and enantioselectivity (Table 2, entry 7).

Table 2. Catalytic diastereo- and enantioselective Mannich reaction of a series of different α -carbonyl esters **1a–d** with N -tosyl- α -imino ester **2** (see Scheme 1).

Entry	Catalyst	R	Solvent	<i>t</i> [h]	Yield [%] ^[a]	dr ^[b]	<i>ee</i> [%] ^[c]
1	(<i>R</i>)- 6	H (1a)	CH ₂ Cl ₂	40	70 (3a)	–	89
2	(<i>R</i>)- 6	Me (1b)	CH ₂ Cl ₂	40	89 (3b)	> 10:1	> 98/ > 90
3	(<i>R</i>)- 6 ^[d]	Me (1b)	CH ₂ Cl ₂	40	98 (3b)	> 10:1	94/ > 90
4	(<i>R</i>)- 6	Me (1b)	THF	40	71 (3b)	> 10:1	91/69
5	(4 <i>R</i> ,5 <i>S</i>)- 5	Me (1b)	CH ₂ Cl ₂	40	58 (3b)	> 10:1	63/29
6	(<i>R</i>)- 6	Bn (1c)	CH ₂ Cl ₂ ^[e]	40	94 (3c)	> 10:1	97/–
7 ^[f]	(<i>R</i>)- 6	Br (1d)	CH ₂ Cl ₂	20	79 (3d)	3:1 ^[g]	78 ^[h]

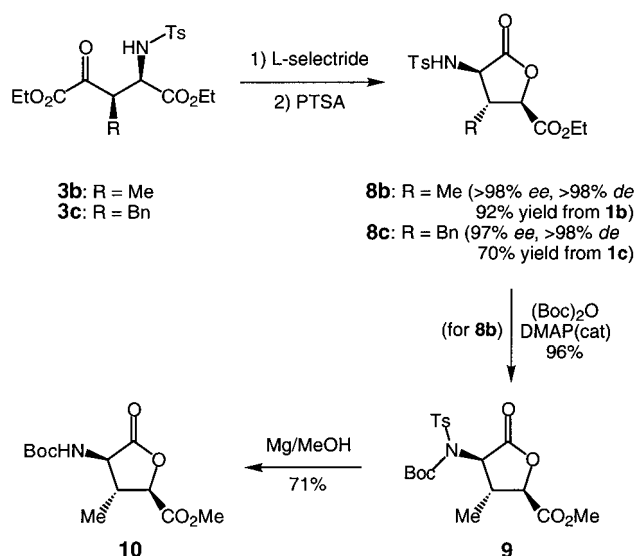
[a] Yield of isolated product. [b] Diastereomeric ratio measured by ¹H NMR spectroscopic analysis of the crude reaction mixture. [c] Enantiomeric excesses were determined by HPLC. [d] 5 mol% of catalyst applied. [e] Reaction temperature 40 °C. [f] 2 Equiv of the N -tosyl- α -imino ester **2** used. [g] A 1:1 diastereomeric ratio was obtained after flash chromatography. [h] Enantiomeric excess measured after dehalogenation.

The results in Table 2 show the scope of the reaction; the Mannich reaction of different α -carbonyl esters gives highly functionalized 4-oxo-glutamic acid ester derivatives in high yield and diastereoselectivity, and with excellent enantioselectivity. Different substituents can be attached to the α -carbonyl functionality, for example, the presence of bromine allow for further functionalization at this position. Some epimerization occurs at the stereogenic center C3, to which the substituent is attached, during purification by flash chromatography. This problem can be overcome by a direct reduction of the 4-oxo functionality (see below).

Herein we present further potential applications of this new Mannich reaction: preparation of highly functionalized optically pure lactones by selective reduction of the 4-oxo functionality and removal of the N -tosyl substituent.

The Mannich adducts **3b,c** underwent a diastereoselective reduction of the 4-oxo functionality with L-selectride to give a mixture of the corresponding alcohols and lactones and treatment of the reaction mixture with PTSA (*p*-toluenesulfonic acid) led to the smooth formation of the highly functionalized lactones **8b,c** (Scheme 2). The lactone **8b** was isolated in 92% overall yield from **1b**, and only one diastereomer was obtained. In a similar manner, the lactone **8c** was isolated with excellent diastereo- and enantioselectivity.

One of the major drawbacks in using the N -tosyl- α -imino ester **2** for catalytic enantioselective addition reactions has



Scheme 2. Formation of lactones **8** from Mannich adducts **3**. The tosyl protecting group was then exchanged for the synthetically more useful Boc group. Boc = *tert*-butoxycarbonyl.

been that the tosyl protecting group is very difficult to remove. We show that we can now remove the tosyl group and exchange it with the much more attractive Boc group,^[8] and one example is presented in Scheme 2. Treatment of the lactone **8b** with di-*tert*-butyldicarbonate and DMAP gave the Boc-protected lactone **9** in 98% yield. The reaction of **9** with Mg/MeOH resulted in the selective removal of the *N*-tosyl substituent, and the Boc-protected lactone **10** was isolated in 71% yield.

The lactones **8c** and **9** were isolated as crystalline products and the structures were characterized by X-ray analysis (see Supporting Information). These gave the absolute configuration for the three stereogenic centers in the product as (2*R*,3*R*,4*R*). The absolute configuration allows us to suggest that **11** accounts for both the diastereo- and enantioselectivity of the reaction. The first crucial step is the generation of the enol form (green in **11**) of the carbonyl compound and it is proposed that the copper(II) Lewis acid acts as the catalyst for the formation of the enol.^[9] The enol formed can undergo the Mannich reaction after further coordination of the *N*-tosyl- α -imino ester **2** (red in **11**) to the chiral BOX-Cu^{II} catalyst. In the proposed cyclohexane-like transition-state model with a chair conformation, the enol coordinates to metal in a bidentate fashion, with the R substituent of the enol in the less sterically crowded equatorial position, which is required to obtain the observed diastereoselectivity. We also propose a bidentate coordination of the imine **2**, with the tosyl substituent pointing away from the ligand.

In conclusion, a new direct catalytic highly enantioselective Mannich reaction of carbonyl compounds with an *N*-tosyl- α -imino ester has been presented. The reaction gives highly functionalized 4-oxo-glutamic acid ester derivatives in high

yield and diastereoselectivity, and excellent enantioselectivity which were converted into highly functionalized, optically active α -amino- γ -lactones.

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Pentagonal-Dodecahedral La₂ Charge Density in [I_h-C₈₀]Fullerene: La₂@C₈₀**

Eiji Nishibori, Masaki Takata,* Makoto Sakata, Atsushi Taninaka, and Hisanori Shinohara

New types of endohedral metallofullerenes^[1] have been synthesized and structurally characterized in the past few years.^[2] In particular, interesting behavior of metal atoms trapped inside carbon cages were revealed by structural studies, e.g. giant thermal motion,^[3] formation of metal clusters,^[2a] metal nitrides,^[2b] and metal carbides,^[2c] and violation of the isolated pentagon rule (IPR).^[2c,d] These characteristic features were specific to metallofullerenes and closely related to the cage symmetry and the electrostatic interaction between the metal atoms and the carbon cage, which results from charge transfer from metal atoms to the carbon cage.

La₂@C₈₀ was one of the first endohedral metallofullerenes, and was produced in macroscopic amounts as early as 1991. It was also the first endohedral dimetallofullerene.^[4] Although the cage symmetry of C₈₀ was determined to be I_h, similar to that of C₆₀, from ab initio theoretical calculations^[5] and ¹³C NMR spectroscopy,^[6] the endohedral structure of La₂@C₈₀ remained unknown. Here we report a novel feature of La₂@C₈₀, in which a perfect pentagonal-dodecahedral charge density of La₂ was detected in an icosahedral I_h-C₈₀ cage by a synchrotron radiation (SR) structural study. The characteristic charge density results from a highly selective trajectory of the two La atoms, which hop along the hexagonal rings of the I_h-C₈₀ polyhedral network.

The structural analysis was performed by means of SR powder diffraction. The experimental data were analyzed by the MEM/Rietveld method,^[7] a self-consistent iterative analysis of a combination of the maximum entropy method (MEM) and Rietveld analysis. The structures of endohedral metallofullerenes such as Y@C₈₂,^[7a] Sc@C₈₂,^[8] La@C₈₂,^[3] Sc₂@C₈₄,^[9] Sc₃@C₈₂,^[2a] Sc₂@C₆₆,^[2c] and Sc₂C₂@C₈₄^[2c] were successfully visualized as charge densities by using synchrotron radiation powder-diffraction data. Details of this method are described elsewhere.^[7, 9]

The cell parameters (monoclinic, space group *P*₂₁) were determined as *a* = 18.2872(8), *b* = 11.2120(4), *c* = 11.1748(4) Å, and β = 107.91(1)° by Rietveld analysis. The

reliability (*R*) factor based on the Bragg intensities *R*_i and the weighted profile *R* factor *R*_{wp} were 6.3 and 2.6 %, respectively. Figure 1 shows the result of Rietveld fitting. The number of structure factors derived in this analysis was 502. The MEM charge density was derived from these structure factors by dividing the unit cell into 128 × 72 × 72 pixels. The *R* factor of the final MEM charge density was 3.8 %.

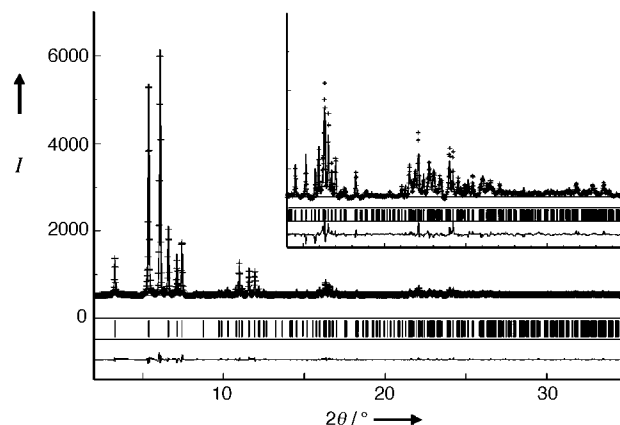


Figure 1. The result of Rietveld fitting for La₂@C₈₀. A closeup of the region between 14.0 and 35.0° is shown in the insert. The reliability factors are *R*_i = 6.3 % and *R*_{wp} = 2.6 %.

A three-dimensional representation of the La₂@C₈₀ molecule is shown in Figure 2 as a surface of equal charge density (1.7 e Å⁻³). The MEM charge density clearly reveals six- and

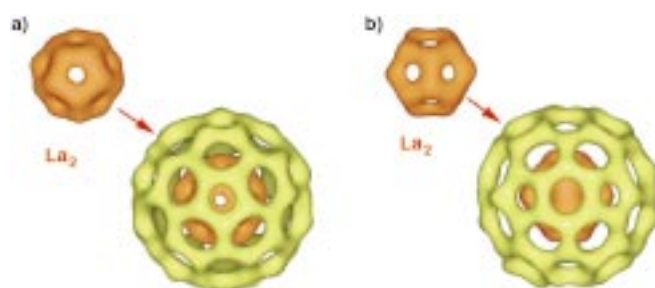


Figure 2. MEM charge densities of La₂@C₈₀ as the equal-density (1.7 e Å⁻³) contour surface along the a) *S*₁₀ and b) *C*₂ axes. The La₂ dodecahedral charge density is colored brown and is additionally shown beside the fullerene molecule.

five-membered rings consistent with the reported I_h symmetry of the C₈₀ cage. Figures 2a and b represent the top views along *S*₁₀ and *C*₂ axis of I_h-C₈₀, respectively. A pentagonal-dodecahedral cage (brown), which is presumably due to the charge density of the encaged La atoms, is clearly recognizable inside the C₈₀ cage. The inner dodecahedral cage is also shown separately.

To confirm that the charge density of this dodecahedral cage stems from the La atoms encapsulated in the C₈₀ cage, we counted the total charge of this internal polyhedron. The total charge, calculated on the basis of the MEM results, is 108.8 e, which is very close to the number of electrons for two La³⁺ ions (108 e). We therefore conclude that the internal dodecahedron represents the distribution of two La³⁺ encapsulated in

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the C_{80} cage; that is, the formal electronic structure is $(La^{3+})_2@C_{80}^{6-}$. The dodecahedral internal polyhedron therefore represents a time-averaged trajectory of the motion of two La atoms in the C_{80} cage. The entire $La_2@C_{80}$ molecule possesses the same I_h symmetry as I_h-C_{80} , since each pentagon of the dodecahedral charge density is faces a pentagonal ring of the C_{80} cage. This is consistent with previous ^{139}La and ^{13}C NMR studies, which suggested that the molecule has I_h symmetry.^[6] The observed longest La–La distance and the shortest La–C distance are 3.84(2) and 2.39(3) Å, respectively. These values can be compared with reported theoretical values (La–La 3.665, La–C 2.56 Å)^[5] for the D_{2h} -symmetric structure of $La_2@C_{80}$, in which La atoms are assumed to be symmetrically located on the C_2 axis.

The section of the charge density through the six-membered rings of the I_h-C_{80} cage is shown in Figure 3. The section through the internal dodecahedral cage is totally homogeneous in density. This indicates that the two La atoms,

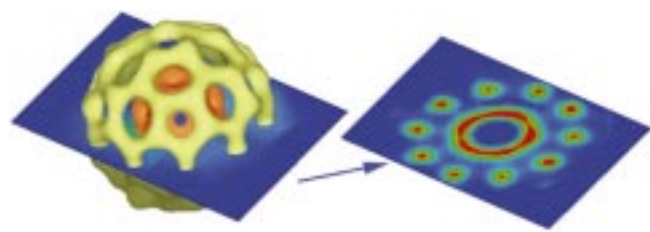


Figure 3. Section of charge density through the hexagonal rings of C_{80} . The homogeneous ring of charge density indicates an La_2 trajectory that connects the hexagonal rings of I_h-C_{80} .

separated by 3.84 Å, hop rapidly around the I_h-C_{80} cage with a trajectory that connects the hexagonal rings but not the pentagons of C_{80} within the timescale of NMR measurements (ca. 10^{-6} s) at room temperature. Such a highly selective trajectory for the internal motion of the encaged metal atoms has never been observed in any other endohedral metallofullerenes.

In the La_2 dodecahedral feature, the presence of covalent bonding between the two La atoms might be expected. However, in the central region of the cage, there is no significant localized charge density (see Figure 3). This suggests ionic rather than covalent bonding character; that is, La_2 is not encaged as a dimer in the C_{80} cage.

Of the five Platonic solids, the cube, the tetrahedron, and the octahedron appear in crystals in nature, and the dodecahedron and the icosahedron have been seen in fullerenes, proteins, and viruses. However, no molecules or crystals in the form of a double-shell Platonic solid have been discovered in

nature. Because of the I_h-C_{80} cage, a highly symmetrical charge density in the shape of a pentagonal dodecahedron results for the encaged La atoms, and this is the first example of a double-shell Platonic solid. This double-shell polyhedral structure is a novel form of endohedral metallofullerene and may be realized in other highly symmetrical fullerene cages such as C_{60} .

Experimental Section

A soot sample containing $La_2@C_{80}$ and other La metallofullerenes was produced in dc (300–400 A) spark mode under He flow at 50–80 Torr. $La_2@C_{80}$ was isolated by two-stage HPLC. The details of production and separation of $La_2@C_{80}$ is the same as for $Y@C_{82}$ and $Sc@C_{82}$ and described in detail elsewhere.^[1,2c] The purity of $La_2@C_{80}$ relative to other fullerenes was greater than 99.9%, as determined by LD-TOF mass spectrometry and HPLC. The $La_2@C_{80}$ powder specimen was grown from toluene solution. The powder sample was sealed in a silica glass capillary (0.3 mm internal diameter).

The synchrotron radiation X-ray powder experiment with imaging plate (IP) detectors was carried out on the large Debye–Scherrer camera at SPring-8 BL02B2. The exposure time was 6 h. The wavelength of incident X-rays was 1.0 Å. The X-ray powder pattern of $La_2@C_{80}$ was obtained in 0.02° steps in 2θ from 3.0 to 35.0° , which corresponds to 1.66 Å resolution in d -spacing.

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Spontaneous Phase Transfer of Nanoparticulate Metals from Organic to Aqueous Media**

David I. Gittins and Frank Caruso*

The synthesis and use of nanoparticles constitute a major research area that attracts both academic and industrial interest.^[1] Many applications require these particles to be water-dispersible and to remain suspended in water with no loss of physical or chemical properties over extended periods of time.^[2–4] However, water-based syntheses of nanoparticles are fraught with problems as a result of ionic interactions, which are typically overcome by using low reactant concentrations (about 5×10^{-4} M),^[5] or because the synthesis is carried out in the presence of stabilizers that are subsequently difficult to remove.^[6] In contrast, particles synthesized in organic solvents can be made at relatively high concentrations (up to 1 M of reactant)^[7] with predefined size and shape,^[8, 9] and with improved monodispersity compared to those prepared in aqueous solutions. Such particles are, however, water-immiscible, which limits their range of applicability.

A range of methods for the phase transfer of particles has been reported, with most studies focusing on the transfer of particles from aqueous to organic media.^[10–16] Previous research into the phase transfer of nanoparticulate material from organic to aqueous solutions has involved irreversible covalent capping of the nanoparticles with amphiphilic molecules. This process permanently changes the chemistry of the particle surface,^[17] and results in only a small proportion of transferred material.^[18–21] Herein we report a facile, rapid, one-step method for the direct and complete transfer of nanoparticles across the phase boundary (organic to aqueous) by use of a readily available organic compound, 4-dimethylaminopyridine (DMAP). The transferred particles displayed no signs of degradation or aggregation after six months, and are expected to be indefinitely stable. Unlike previous attempts,^[18–21] no precipitation steps or solvent exchanges are required, and the particles transferred by this method are not stabilized by a strong, covalently attached ligand: this is vitally different to thiol-stabilized particles when considering future applications. The availability of such concentrated nanoparticle solutions opens up new avenues for cyto-labeling,^[22] hetero- and homogenous catalysis,^[2, 3, 7] solid-state physics,^[23] and colloidal crystal applications.^[24]

Gold and palladium nanoparticles were synthesized in toluene by using tetraalkyl ammonium salts as the stabilizing agent.^[25] Such particles are commonly used in catalysis, while the more commonly studied thiol-passivated nanoparticles possess a surface monolayer of metal sulfide which makes them less efficient catalysts.^[7] To effect phase transfer an aqueous 0.1 M DMAP solution was added to aliquots of the as-prepared nanoparticle mixtures. Phase transfer of both the gold and palladium nanoparticles was initiated instantaneously upon the addition of the DMAP solution, with direct transfer across the phase boundary completed within one hour (no stirring or agitation was required, see the Supporting Information). The synthetic procedure produces high concentrations of nanoparticles, and so samples were diluted with solvent by a factor of approximately 1000 for subsequent analysis and photography.

The complete phase transfer of metallic nanoparticles was achieved (Figure 1). The UV/Vis spectra of the gold nanoparticles in solution before and after phase transfer were also

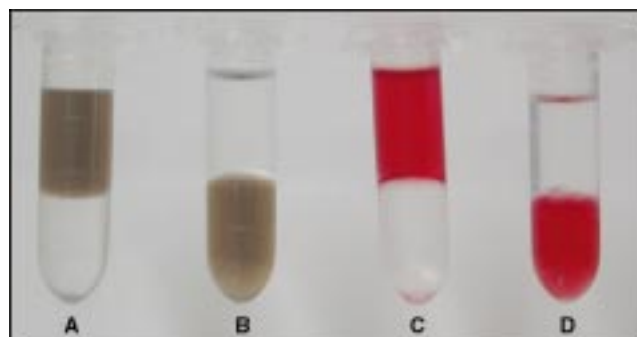


Figure 1. Photograph of a diluted aliquot of palladium nanoparticles in toluene above water (A), and a similar aliquot subsequently transferred into water by the addition of DMAP (0.1 M, pH 10.5; B); a diluted aliquot of gold nanoparticles in toluene above water (C), and a similar aliquot subsequently transferred into water by the addition of DMAP (0.1 M, pH 10.5; D).

recorded since particle aggregation (reversible or irreversible; flocculation or coagulation) and changes in the dielectric surroundings of the nanoparticles can be detected optically.^[26, 27] The as-synthesized gold nanoparticles (in toluene) displayed a maximum in the surface plasmon band (λ_{max}) at 518 nm (Figure 2). A 6-nm (± 0.2 nm) blue-shift ($\lambda_{\text{max}} = 512$ nm) in the wavelength of the surface plasmon band peak maximum was observed after the phase transfer (toluene to water) of the gold nanoparticles. The observed shift could arise from the combined effect of the change in refractive index of the medium on transfer (1.47 to 1.33)^[26] and exchange of the adsorbed molecules on transfer. Any particle aggregation would lead to a red-shift and broadening of the plasmon band absorption.^[28] The UV/Vis spectra obtained clearly indicate that the DMAP-induced phase transfer of the gold nanoparticles yields well-dispersed nanoparticles in aqueous solution. UV/Vis experiments on the palladium nanoparticles were not undertaken as they do not display a strong surface plasmon band absorbance.^[29]

Transmission electron microscopy (TEM) analysis also showed that there were no discernable differences in the

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

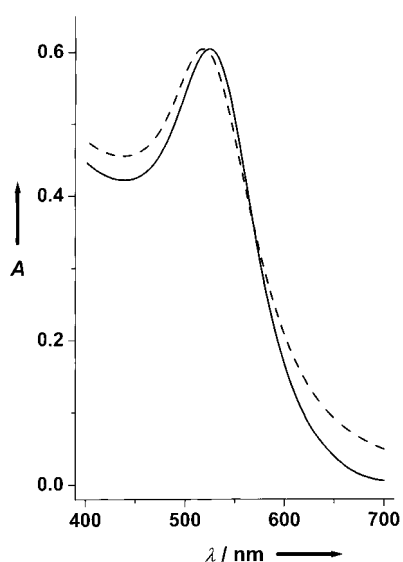


Figure 2. UV/Vis spectra of a diluted solution of gold nanoparticles in toluene (solid line) and the same sample transferred into an equal volume of 0.1M DMAP solution at pH 10.5 (dashed line).

morphology of both the gold and palladium nanoparticles after phase-transfer (Figure 3 and see the Supporting Information). Analysis of the gold nanoparticle samples yielded

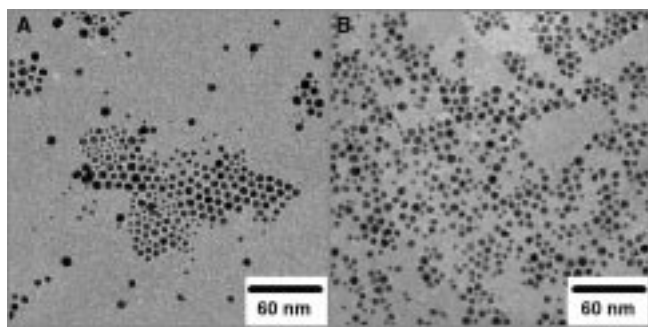


Figure 3. Transmission electron micrographs of gold nanoparticles synthesized in toluene (A) and the same sample one month after being transferred into water by the addition of DMAP (B).

average diameters of 5.5 nm (standard distribution (SD) = 0.7, 153 particles counted) and 5.5 nm (SD = 0.8, 115 particles counted) for the nanoparticles in toluene and aqueous media, respectively. Analysis of the palladium nanoparticle samples gave average diameters of 4.5 nm (SD = 0.9, 145 particles counted) and 4.8 nm (SD = 1.2, 122 particles counted) for the nanoparticles in toluene and aqueous media, respectively. In addition, energy-dispersive X-ray fluorescence analysis (EDAX) spectra of the particles transferred to the aqueous solution and dried on a TEM grid showed there were no bromide ions present (the tetraalkylammonium counterion; see the Supporting Information); however, traces of the organic salt may still remain adsorbed to the particle surface.

As TEM provides information on the morphology of the nanoparticles in the dry state, analytical ultracentrifugation (AU) measurements were conducted to determine the size distributions of the nanoparticles in solution (see the Experimental Section).^[30–32] Analysis of the two samples shown in

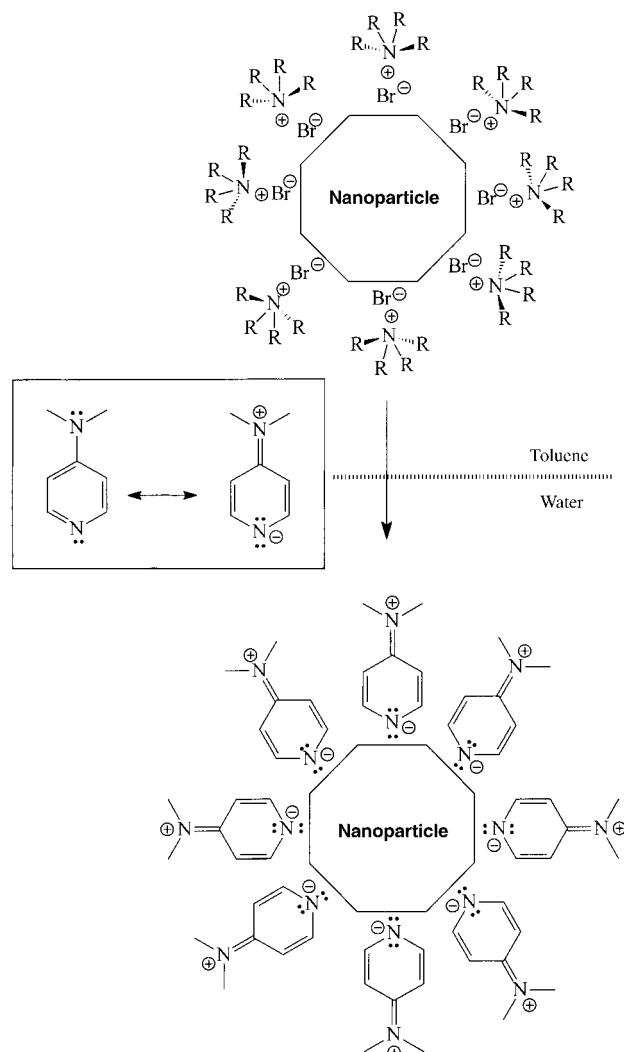
Figure 3 by AU resulted in average diameters of 5.1 (SD = 1.1), and 5.2 nm (SD = 1.1) for the nanoparticles in toluene and aqueous media, respectively. The average diameters of the palladium nanoparticles in toluene and water were found by AU to be 2.8 (SD = 1.5) and 3.1 nm (SD = 1.6), respectively. These data are in good agreement with the TEM values and further confirm that no significant aggregation of the transferred nanoparticles occurred.

The stability of the DMAP-stabilized particles was examined with respect to salt concentration and pH. All samples (Au, Pd, pH 10.5) were stable for a period of at least six months in 3M NaCl solution (for gold, $\lambda_{\text{max}} = 514$ nm). Measurements on the DMAP-stabilized nanoparticles in aqueous solution (pH 10.5) by microelectrophoresis gave an average ζ -potential of +25 mV (five measurements on three different transfer experiments), which confirmed there was a positively charged particle surface. The particles were found to be colloiddally stable within the pH range 7 to 12 (ζ -potential of approximately +35 mV), although the proportion of flocculated particles increased (as seen by eye; see the Supporting Information) as the pH value was lowered (from 10.5 to 3.0) by the stepwise addition of dilute acid (1 mM HCl, pH 3).^[28] This observation concurs with the postulated mechanism of phase transfer (see Scheme 1). Lowering the pH value would result in a greater proportion of the endocyclic nitrogen groups being protonated and unavailable for bonding to the nanoparticle surface (and hence stabilizing the nanoparticles). Areas of the nanoparticle surface subsequently become “deprotected”, which causes reversible aggregation. A decrease in the degree of particle flocculation (detected by a blue-shift in the peak plasmon band absorption) was achieved by the addition of dilute base (1 mM NaOH) to restore the pH value back to its original value (pH 10.5). The separation of aggregated particles was not instantaneous and was only detected after several days; however, this was always repeatable. As expected from simple acid/base equilibria, raising the pH value of the solution to greater than pH 13, by the addition of dilute base, led to particle aggregation as the proportion of charged DMAP molecules decreased ($\text{p}K_{\text{a}} = 9.6$).

Several compounds (as 0.1M aqueous solutions) were added to aliquots of gold nanoparticle solutions to arrive at an understanding of the mechanism for the spontaneous phase transfer of metal nanoparticles from organic to aqueous phases. Pyridine and 4-aminopyridine caused immediate aggregation of the particles suspended in toluene (seen as a color change from red to blue, followed by precipitation). Only the DMAP-containing vial resulted in the transfer of the nanoparticles to the aqueous phase; in the other samples the precipitate accumulated at the toluene/water interface. These results point to the necessity of the tertiary (highly basic) amine conjugated with an electron-donating (weakly basic) group to effect the phase transfer. Evidence against the formation of a strong covalent bond between the stabilizing molecule (DMAP) and the metal nanoparticle surface was proved by repeatedly washing the aqueous phase with toluene. The toluene washings continually decreased the amount of DMAP in the aqueous phase, finally causing the particles to aggregate. This effect is indicative of the removal

of DMAP from the particle surface. Covalently bound molecules cannot be removed by solvent washing.^[18]

A possible mechanism for the spontaneous phase transfer of nanoparticles in the presence of DMAP molecules is as follows (Scheme 1): The addition of an aqueous DMAP solution to the nanoparticle dispersion in toluene results in



Scheme 1. Proposed mechanism for the spontaneous phase transfer of gold and palladium nanoparticles from an organic reaction medium (toluene) to water by the addition of DMAP. $R = C_8H_{17}$.

the DMAP partitioning across the water/toluene phase boundary (detected by thin-layer chromatographic analysis of the organic phase), and physisorbing onto the nanoparticle surface. Simple acid-base calculations show that 98% of the DMAP is present in the free base form in a 0.1M aqueous solution. It is proposed that the DMAP molecules form a labile donor–acceptor complex with the metal surface atoms through the endocyclic nitrogen atoms, as previously reported for planar gold substrates,^[33] with the surface charge arising from partial protonation of the exocyclic nitrogen atom that extends away from the nanoparticle surface being required to transfer the particles into water.^[34]

In conclusion, a general method to efficiently transfer gold and palladium nanoparticles from an organic solvent (in this

case, toluene) to water has been described. The benefits of such a method are threefold. Firstly, by replacing the hydrosol synthetic methods that require high dilution and lengthy dialysis purification.^[22] Secondly, synthetic reactions in organic solvents produce high concentrations of nanoparticles with improved monodispersity relative to those prepared in water;^[25, 35] the method reported here offers such particles to researchers requiring water-based chemistries. Thirdly, the transfer of nanoparticles out of the organic phase (*without precipitation*) permits the recycling of the relatively expensive ammonium salts. Furthermore, the method efficiently produces water-dispersible metal nanoparticles as an isolable solid, which is important when highly concentrated particle solutions are required, for example, for colloidal crystal applications.^[24] The expected strong affinity of the DMAP-stabilized particles for negatively charged substrates, commonly used as supports in heterogeneous catalysis, also warrants further study.^[7]

Experimental Section

Nanoparticle synthesis: A 30 mM aqueous metal chloride solution ($HAuCl_4$ or Na_2PdCl_4 , 30 mL) was added to a 25 mM solution of tetraoctylammonium bromide in toluene (80 mL). The transfer of the metal salt to the toluene phase can be clearly seen visually within a few seconds. A 0.4M solution of freshly prepared $NaBH_4$ (25 mL) was added to the stirred mixture, which caused an immediate reduction to occur. After 30 min the two phases were separated and the toluene phase was subsequently washed with 0.1M H_2SO_4 , 0.1M NaOH, and H_2O (three times), and then dried over anhydrous $NaSO_4$.^[7, 35] Previous high-resolution TEM analysis confirmed similar nanoparticles to be crystals with a truncated octahedral morphology.^[36, 37]

Phase transfer: An aqueous 0.1M 4-dimethylaminopyridine (DMAP) solution (1 mL) was added to aliquots (1 mL) of the as-prepared nanoparticle mixtures. This concentration of DMAP was found to be sufficient to effect the complete and spontaneous phase transfer of the nanoparticles. Whilst not discussed here, nanoparticles from larger volumes of the reaction solution (up to 0.5 L) have also been successfully transferred into water (1 mL), with subsequent recycling of the tetraalkylammonium salt. Direct phase transfer across the organic/aqueous boundary was completed within 1 h, with no stirring or agitation required. Solid DMAP could also be added directly to the toluene solution to precipitate the particles, which could then be resuspended in water. Phase transfer was also possible for similar particles synthesized in chloroform but not for particles obtained from synthetic reactions using different organic stabilizers, for example, sodium 5,14-diethyl-8,11-dioxo-7,12-dioxaoctadecane-2-sulfonate (Na-AOT) or didodecyltrimethylammonium bromide.

All reagents were obtained from Sigma–Aldrich and used as received. UV/Vis spectra were recorded using a Cary Model 4E UV/Vis spectrophotometer (0.2 nm resolution). The ζ -potentials of the nanoparticles were determined with a Malvern Zetasizer 4 by taking the average of five measurements at the stationary level. The mobilities were converted into the electrophoretic potential by using the Smoluchowski relation. TEM measurements were performed on a Philips CM12 microscope operated at 120 kV. Particle-size distribution was estimated by analytical ultracentrifugation from sedimentation velocity experiments at 20 °C on a Beckman–Coulter Optima XL-I ultracentrifuge with absorption optics used for detection. 12-mm self-made double-sector titanium centerpieces were used. Analytical ultracentrifugation applies a constant centrifugal force to a dilute sample of nanoparticles. At the beginning of the experiment a single wavelength scan across the radius of the cell gives a constant absorbance value that is indicative of a constant colloid distribution. As the experiment continues, the time-dependent sedimentation of the particles can be detected by time-dependent radial scans of the local colloid concentration. Particle fractionation during the experiment allows the calculation of the distribution of the sedimentation coefficient from a series of radial scans over time. The particle-size distribution, together with the particle density,

solvent density, and viscosity, can be obtained for particles with sizes even in the Ångström range.^[31]

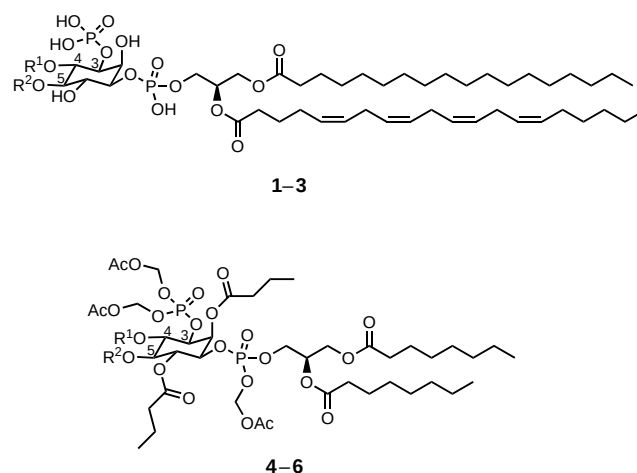
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Membrane-Permeant 3-OH-Phosphorylated Phosphoinositide Derivatives**

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Phosphoinositides that are phosphorylated at the 3-hydroxy group (Scheme 1) are important signaling molecules in eukaryotic cells, particularly because of their participation in the receptor-mediated activation of protein kinases and in



Scheme 1. Phosphoinositides PtdIns(3,5)P₂ (**1**): R¹ = H, R² = P(O)(OH)₂; PtdIns(3,4,5)P₃ (**2**): R¹ = R² = P(O)(OH)₂; PtdIns(3,4)P₂ (**3**): R¹ = P(O)(OH)₂, R² = H and the corresponding membrane-permeant derivatives which can be bioactivated: di-C₈-Bt₃PtdIns(3,5)P₂/AM (**4**, R¹ = Bt, R² = P(O)(OCH₂OAc)₂), di-C₈-Bt₂PtdIns(3,4,5)P₃/AM (**5**, R¹ = R² = P(O)(OCH₂OAc)₂), and di-C₈-Bt₃PtdIns(3,4)P₂/AM (**6**, R¹ = P(O)(OCH₂OAc)₂, R² = Bt). Bt = COC₃H₇.

the control of intracellular calcium levels. The key enzyme involved in the biosynthesis is the phosphoinositide 3-kinase (PI 3-kinase), which is activated by growth factors such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). The 3-hydroxy phosphorylated phosphoinositides formed by the enzyme have been associated with the mechanisms of oncogene transformation, cytoskeletal rearrangements, association of proteins to the plasma membrane, protein trafficking, endo- and exocytosis, the uptake of glucose by adipocytes, and the regulation of epithelial

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chloride transport.^[1,2] The biosynthesis relies on various isoforms and splice variants of PI 3-kinase, and although usually more than one 3-OH-phosphorylated phosphoinositide is found in cells, most of them appear to originate from the same biosynthetic pathway.^[1] PtdIns(3,5)P₂ seems to appear predominantly in yeast and to a lesser extent in mammalian cells. Because of its orphan status, PtdIns(3,5)P₂ is an interesting isomer for physiological tests.^[3]

Which phosphoinositide connects the growth factor receptor signal to a physiological response, in our case to epithelial chloride secretion (Figure 1), the deficiency associated with

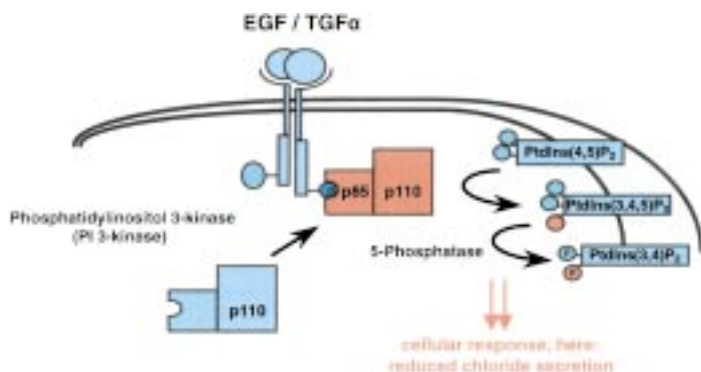
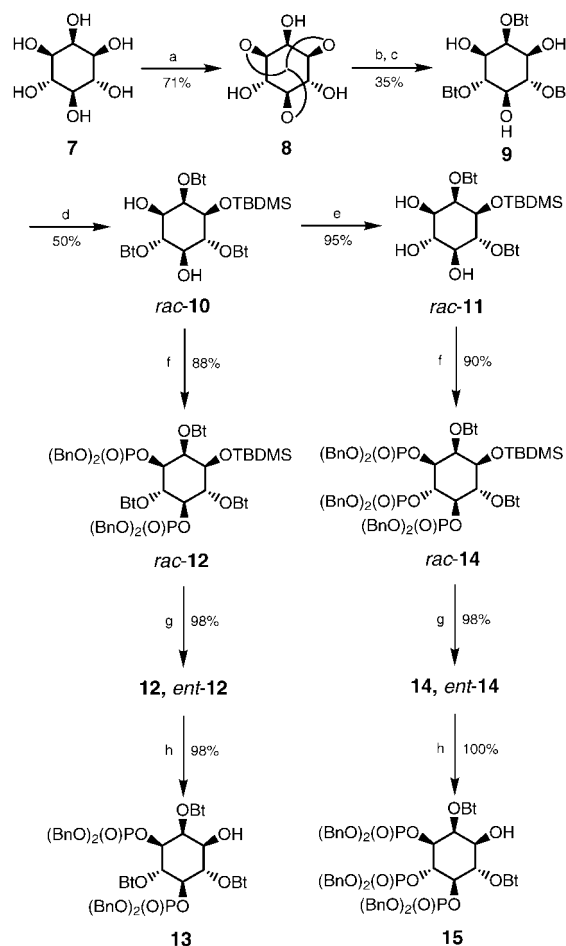


Figure 1. Partial growth factor signaling pathway: EGF or TGFα activate receptor tyrosine kinases, resulting in translocation of PI 3-kinase from the cytosol to the plasma membrane, where the heterodimeric enzyme complex p85/p110 phosphorylates phosphoinositides at the 3-OH group. Which of the resulting 3-OH-phosphorylated products mediates the signal towards the cellular response?

the deadly hereditary disease cystic fibrosis (CF)? To answer this question we synthesized membrane-permeant derivatives of three of the 3-OH-phosphorylated phosphoinositides, namely phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P₂, **1**), the triphosphate PtdIns(3,4,5)P₃ **2**, and its metabolite PtdIns(3,4)P₂ **3** (Scheme 1). Membrane-permeant derivatives of inositol phosphates and phosphoinositides have previously been shown to be useful tools to mimic intracellular signals without disrupting the plasma membrane when administered.^[2, 4, 5] The hydroxy groups of the previously used membrane-permeant PtdIns(3,4,5)P₃ derivatives were either not or only partially protected as butyrate esters.^[2] However, the presumably slowly cleaved butyrate groups will prevent scrambling of phosphate groups by intramolecular migration^[5] when the fast enzymatic hydrolysis of the acetoxymethyl ester groups takes place. This appears to be particularly important in the case of the closely related bisphosphate isomers **1** and **3**, which would otherwise be readily interconverted. Extracellular addition of the compounds should predominantly elevate the concentration of a single phosphoinositide when a short-term physiological response is observed. We therefore studied the ability of EGF and the three phosphoinositide derivatives to inhibit transepithelial chloride secretion of nasal epithelia from patients suffering from cystic fibrosis. It appears to be increasingly important to understand the growth factor mediated signaling in CF tissue,

because there is increasing evidence that growth factor levels are greatly enhanced in lung tissue of CF patients.^[6]

Similarly to the majority of syntheses of inositol phosphates, we started from *myo*-inositol (**7**). Tributyrat **9** was prepared in three steps via orthoester **8**.^[7] Monosilylation of the more reactive 1-/3-hydroxy group led to *rac*-**10**, which served as a precursor in the synthesis of the PtdIns(3,4,5)P₃ and PtdIns(3,5)P₂ derivatives (Scheme 2). Phosphitylation of



Scheme 2. a) HC(OMe)₃, PTSA, DMF, 120 °C, 18 h; b) Bt₂O, DMAP, pyridine, 25 °C, 18 h; c) TFA, H₂O, MeOH, 25 °C, 8 h; d) TBDMSCl, imidazole, DMF, 25 °C, 4 d; e) lipase from *Candida cylindracea*, 0.1 M phosphate buffer pH 8, MeOH, 25 °C, 5 d; f) (BnO)₂PNiPr₂, tetrazole, MeCN, 25 °C, 3 d, then MeCO₃H (32%), -30 → 25 °C, 1 h; g) separation of enantiomers on Chiradex (Merck), eluent: 100% MeCN; h) Et₃N·3HF, MeCN, 25 °C, 3 d. PTSA = *p*-toluenesulfonic acid, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, TBDMS = *tert*-butyldimethylsilyl, Bn = benzyl.

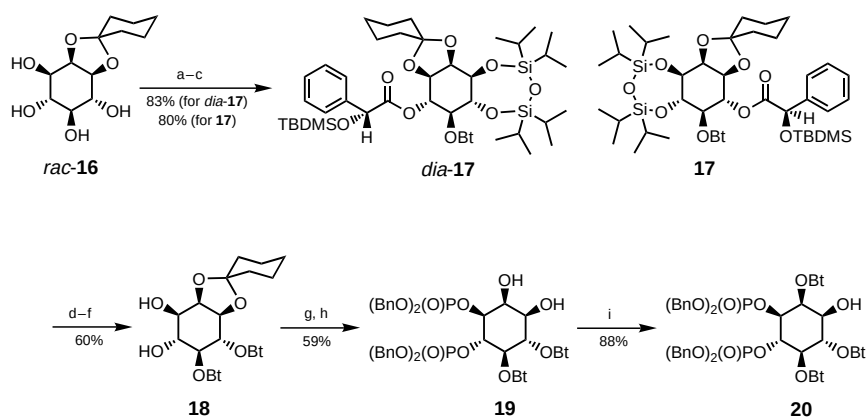
rac-**10** and subsequent oxidation of the phosphoric triester^[8] resulted in the fully protected inositol 3,5-bisphosphate derivative *rac*-**12**. The enantiomers were separated by preparative chiral HPLC (50 × 250 mm, Chiradex, Merck)^[9] and furnished the alcohols **13** and *ent*-**13** after cleavage of the silyl groups by treatment with triethylamine trihydrofluoride.

For the synthesis of the 3,4,5-phosphorylated precursor, we discovered that pig liver esterase and lipase from *Candida cylindracea* regioselectively cleaved the sterically less hin-

dered 4-O-butyrate group of *rac*-**10** in almost quantitative yield, but unfortunately not enantioselectively.^[10] The resulting diester *rac*-**11** was phosphorylated and the enantiomers **14** and *ent*-**14** were separated subsequently by chiral HPLC. Deprotection gave the 1- and 3-hydroxy compounds **15** and *ent*-**15**, respectively, which are suitable for the lipid coupling.

Early attempts to prepare enantiomerically pure precursors for the desired PtdIns(3,4)P₂ derivative **6** were set back by the discovery that a protected inositol bisphosphate similar to those described above did not separate on chiral HPLC. This hints towards the essential character of the 5-position in the molecular recognition by the chiral β -cyclodextrin resin. We therefore developed a facile three-step one-pot reaction to produce the diastereomeric mandelic esters **17** and *dia*-**17** starting from monoketal *rac*-**16** (Scheme 3).^[11] The diastereomers were separated on silica gel with a surprisingly high mass recovery (83 and 80%, respectively). Exchange of the chiral auxiliary for a butyryl group and subsequent desilylation gave dibutyrate **18**, which was subsequently phosphorylated. After removal of the cyclohexylidene ketal, the third butyryl group was introduced regioselectively at the axial 2-OH group of diol **19** via an intermediate orthobutyrate.^[12] The spontaneous hydrolysis by stirring in water-containing methanol furnished the 1-hydroxyinositol derivative **20** with high selectivity in 88% yield.

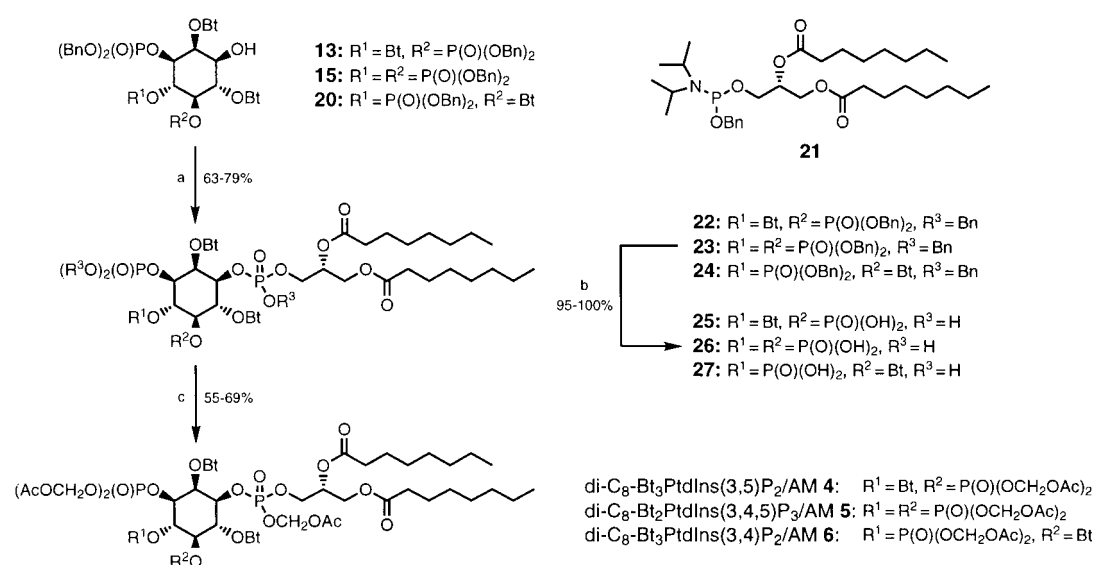
The final steps are shown in Scheme 4. Alcohols **13**, **15**, and **20** were coupled with the enantiomerically pure phosphoramidite **21**.^[13] Subsequent oxidation with peracetic acid gave the fully protected phosphoinositide derivatives **22–24**, respectively. Removal of the benzyl groups by hydrogenolysis and alkylation of the free phosphate groups with acetoxy-



Scheme 3. a) TIPDS/Cl₂, pyridine, 25 °C, 3 d; b) (–)-2-O-TBDMS mandelic acid chloride, pyridine, –20 → 25 °C, 1 d; c) Bt₂O, DMAP, pyridine, 80 °C, 5 d, separation of diastereomers on silica gel, petroleum ether (55–62 °C)/EtOAc 25:1, then 10:1; d) MeNH₂, EtOH, H₂O, 25 °C, 2 h; e) Bt₂O, DMAP, pyridine, 25 °C → 60 °C; f) TBAF (5 equiv), TBME, 25 °C, 2 h; g) (BnO)₂P(O)Cl, tetrazole, MeCN, 25 °C, 3 d, then MeCO₂H (32%), –30 → 25 °C, 1 h; h) TFA, MeOH, 25 °C, 1 h; i) MeCH₂CH₂C(OMe)₃, PTSA, 25 °C, 2 h, then aq. MeOH (95%), 25 °C, 18 h. TIPDS/Cl₂ = 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane, TBAF = tetrabutylammonium fluoride, TBME = *tert*-butyl methyl ether.

methyl bromide^[14] led to the membrane-permeant phosphoinositide derivatives **4–6** as pairs of R_{P1}/S_{P1}-diastereomers in 5–15% overall yield. The absolute configurations were determined by the preparation of known reference *myo*-inositol derivatives from the enantiomerically pure precursors *ent*-**13**, **15**, and **17**, respectively (see Experimental Section for details).

For membrane-permeant derivatives of inositol phosphates and phosphoinositides it was previously shown that the bioactivated modifications of the hydroxy and phosphate groups allow diffusion over the plasma membrane of living cells, that the original highly charged compound is formed intracellularly, and that the biological activity of the messenger molecules can be monitored.^[2, 4, 5] Phosphoinositides **4–6** provide us for the first time with the opportunity to distinguish whether the downstream signal transduction of growth factors



Scheme 4. a) **21**, tetrazole, CH₂Cl₂, 25 °C, 3 d, then MeCO₂H (32%), –30 → 25 °C, 1 h; b) H₂ (1 atm), Pd(C), MeCO₂H, 25 °C, 1 d; c) AcOCH₂Br, iPr₂NEt, MeCN, 25 °C, 3 d.

(Figure 1) relies on $\text{PtdIns}(3,4,5)\text{P}_3$ or its metabolite $\text{PtdIns}(3,4)\text{P}_2$. This is of particular interest in cystic fibrosis, in which chloride secretion of epithelial cells is greatly diminished as a result of a point mutation in the cAMP-regulated apical chloride channel.^[15] To overcome the resulting pathological conditions, the activation of alternative chloride channels, particularly those regulated by intracellular calcium, represents one of the possible therapeutic strategies.^[16] However, these channels are under the negative control of several external signals including growth factors.^[2, 17] Growth factors are abundant in the infected lung tissue of cystic fibrosis patients and are therefore suspected to hinder chloride secretion.^[6]

Herein we show that EGF suppresses ATP-induced chloride secretion of nasal epithelial cells in CF patients. ATP is an agonist that stimulates calcium-mediated chloride secretion. When cells were preincubated with di- C_8 - $\text{Bt}_2\text{PtdIns}(3,4,5)\text{P}_3/\text{AM}$ (**5**; 200 μM) the negative effect of EGF on the chloride secretion was mimicked (Figure 2). In contrast, when cells

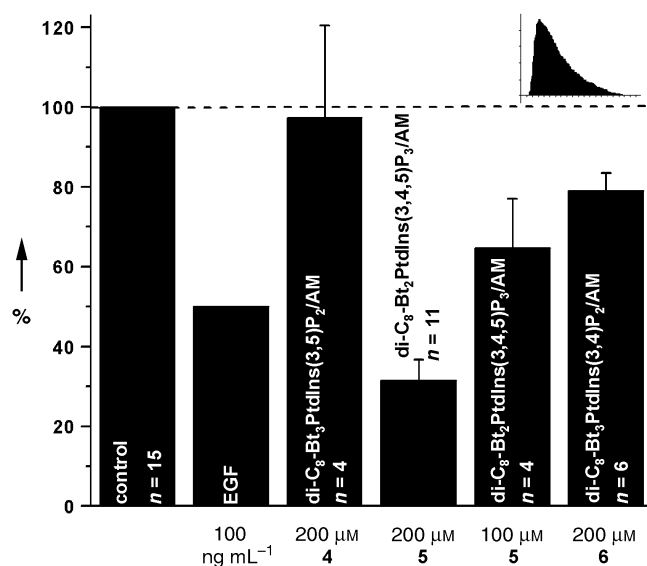


Figure 2. Effect of membrane-permeant phosphoinositide derivatives **4–6** on ATP-stimulated chloride secretion, measured as a short-circuit current (I_{SC}) of cultured human nasal epithelial cells from a cystic fibrosis patient; integral analysis (see insert) as percent of control.

were treated with the corresponding 3,5-bisphosphate **4** (200 μM), chloride secretion was in the range of the control experiments, whereas the 3,4-bisphosphate **6** showed only a marginally inhibitory effect. The results suggest that mainly $\text{PtdIns}(3,4,5)\text{P}_3$ (**2**) but not its metabolite **3** mediates the growth factor signal that limits calcium-mediated chloride secretion. These results make $\text{PtdIns}(3,4,5)\text{P}_3$ -binding proteins prime targets for the development of $\text{PtdIns}(3,4,5)\text{P}_3$ antagonists that could potentially become a treatment against cystic fibrosis.

Experimental Section

Nasal epithelial tissue from CF patients was provided by the Children's Hospital in Seattle, USA. Cells were held in culture for up to four passages and were grown to confluency on permeable supports.

Ion-transport measurements: chloride secretion was monitored by changes in short-circuit current (I_{SC}) after cells were placed in Ussing chambers as described before.^[18] Increases in chloride secretion were induced by adding 100 μM ATP to the apical side of the monolayer.

Delivery of phosphoinositides to the cytosol: cells were pre-incubated with the membrane-permeant phosphoinositides **4–6** for 45 min prior to mounting the cells. Butyryl groups and acetoxymethyl ester groups were previously shown to be readily removed when the compounds entered the cells by diffusion, but were moderately stable in the extracellular space.^[4]

Selected physical data for **5** ($R_{\text{P1}}/\text{S}_{\text{P1}}$ -diastereomers): ^1H NMR (600 MHz, $[\text{D}_8]\text{toluene}$, 20 $^\circ\text{C}$): δ = 0.793 (t, J = 7.4 Hz, 3H; CH_3), 0.804 (t, J = 7.6 Hz, 3H; CH_3), 0.817 (t, J = 7.3 Hz, 3H; CH_3), 0.820 (t, J = 7.1 Hz, 3H; CH_3), 0.822 (t, J = 7.1 Hz, 6H; $2 \times \text{CH}_3$), 1.050 (t, J = 7.5 Hz, 3H; CH_3), 1.097 (t, J = 7.5 Hz, 3H; CH_3), 1.087–1.232 (m, 32H; $16 \times \text{CH}_2$), 1.444–1.594 (m, 12H; $6 \times \beta\text{-CH}_2$), 1.732 (s, 3H; Ac), 1.741 (s, 3H; Ac), 1.767 (s, 3H; Ac), 1.768 (s, 3H; Ac), 1.779 (s, 3H; Ac), 1.780 (s, 3H; Ac), 1.782 (s, 3H; Ac), 1.784 (s, 3H; Ac), 1.800 (s, 3H; Ac), 1.875–2.009 (m, 4H; $2 \times \beta\text{-CH}_2$), 1.826 (s, 3H; Ac), 1.830 (s, 3H; Ac), 1.836 (s, 3H; Ac), 1.881 (s, 3H; Ac), 1.961 (s, 3H; Ac), 2.040–2.323 (m, 12H; $2 \times \alpha\text{-CH}_2$), 2.664 (ddd, J = 17.5, 8.1, 7.3 Hz, 1H; $\alpha\text{-CH}_2$), 2.723 (ddd, J = 17.6, 7.8, 7.5 Hz, 1H; $\alpha\text{-CH}_2$), 2.732 (ddd, J = 17.5, 8.2, 7.2 Hz, 1H; $\alpha\text{-CH}_2$), 2.779 (ddd, J = 17.6, 8.3, 7.2 Hz, 1H; $\alpha\text{-CH}_2$), 4.165 (dd, J = 12.1, 6.2 Hz, 1H; $\text{H}_\gamma\text{-sn1}$), 4.178 (ddd, J = 11.1, 7.7, 5.8 Hz, 1H; $\text{H}_\gamma\text{-sn3}$), 4.315 (ddd, J = 11.3, 7.1, 4.3 Hz, 1H; $\text{H}_\gamma\text{-sn3}$), 4.333 (dd, J = 12.2, 6.4 Hz, 1H; $\text{H}_\gamma\text{-sn1}$), 4.390 (dd, J = 6.8, 5.2 Hz, 2H; $2 \times \text{H}_\gamma\text{-sn3}$), 4.407 (dd, J = 12.3, 3.8 Hz, 1H; $\text{H}_\gamma\text{-sn1}$), 4.510 (dd, J = 12.3, 3.6 Hz, 1H; $\text{H}_\gamma\text{-sn1}$), 4.853 (ddd, J = 9.5, 9.5, 4.7 Hz, 1H; H-5), 4.868 (ddd, J = 9.6, 9.6, 4.7 Hz, 1H; H-5), 4.911–5.013 (m, 4H; $\text{H}_\gamma\text{-1}$, $\text{H}_\gamma\text{-1}$, $\text{H}_\gamma\text{-3}$, $\text{H}_\gamma\text{-3}$), 5.089 (ddd, J = 9.7, 9.7, 9.7 Hz, 1H; H-4), 5.105 (ddd, J = 9.6, 9.6, 9.6 Hz, 1H; H-4), 5.326 (dddd, J = 5.7, 5.7, 4.2, 4.2 Hz, 1H; $\text{H}_\gamma\text{-sn2}$), 5.460 (dddd, J = 6.2, 5.2, 5.2, 3.8 Hz, 1H; $\text{H}_\gamma\text{-sn2}$), 5.571 (dd, J = 11.6, 5.3 Hz, 1H; H-6), 5.593 (dd, J = 11.3, 5.4 Hz, 1H; H-6), 5.625 (dAB, J = 5.5 Hz, $\nu_{\text{A}} = 5.614$, $\nu_{\text{B}} = 5.636$, $J_{\text{PH}} = 7.2$ Hz, 2H; OCH_2O (P-1)), 5.647 (dAB, J = 5.5 Hz, $\nu_{\text{A}} = 5.635$, $\nu_{\text{B}} = 5.659$, $J_{\text{PH}} = 8.0$ Hz, 2H; OCH_2O (P-1)), 5.742–5.896 (m, 24H; $12 \times \text{OCH}_2\text{O}$), 6.207 (dd, J = 2.9, 2.9 Hz, 2H; $\text{H}_\gamma\text{-2}$, $\text{H}_\gamma\text{-2}$); ^{13}C NMR (50 MHz, $[\text{D}_8]\text{toluene}$, 20 $^\circ\text{C}$): δ = 13.61, 13.64, 13.94, 14.03, 14.38, 18.26, 18.34, 18.81, 20.22, 20.31, 23.15, 25.31, 29.54, 29.57, 32.22, 34.12, 34.18, 34.27, 34.36, 35.77, 35.81, 35.93, 36.07, 62.02, 62.29, 66.67, 69.58, 69.82, 70.03, 70.20, 73.72, 76.67, 76.98, 82.95, 83.06, 83.16, 83.47, 83.54, 168.85, 168.00, 169.17, 169.25, 169.32, 169.53, 172.14, 172.27, 172.57, 172.69, 172.90, 172.98, 173.08; ^{31}P NMR (81 MHz, $[\text{D}_8]\text{toluene}$, 20 $^\circ\text{C}$): δ = -1.30 (s, 1P), -2.10 (s, 1P), -2.84 (s, 1P), -2.86 (s, 1P), -3.13 (s, 2P), -3.77 (s, 2P); MS (DCI): m/z : 1397 [$M - \text{CH}_2\text{OAc}$]⁻, 1143 [$M - \text{C}_{10}\text{H}_{15}\text{O}_4$]⁻; HRMS (DCI, $R = 5000$) for [$M - \text{CH}_2\text{OAc}$]⁻: calcd: 1397.37720, found: verified (± 10 ppm).

Determination of absolute configurations: **4**: Compound **ent-13** was converted into *myo*-inositol 1,5-bisphosphate^[19] by means of complete deprotection by hydrogenolysis and subsequent debutyrylation ($[\alpha]_{\text{D}}^{20} = +9.8^\circ$, $c = 0.5$ in H_2O ; lit.:^[19] $[\alpha]_{\text{D}}^{20} = +6.0^\circ$, $c = 0.5$ in H_2O). **5**: Compound **15** was converted into *myo*-inositol 1,3,4,5-tetrakisphosphate by phosphorylation of the 1-hydroxy group and complete deprotection ($[\alpha]_{\text{D}}^{20} = -3.2^\circ$, $c = 1$ in H_2O ; lit.:^[20] $[\alpha]_{\text{D}}^{25} = -2.5^\circ$, $c = 1$ in H_2O). **6**: Compound **17** was deacylated and desilylated to form 1,2-*O*-cyclohexylidene *myo*-inositol ($[\alpha]_{\text{D}}^{25} = -33.8^\circ$, $c = 1$ in MeOH); lit.:^[21] $[\alpha]_{\text{D}}^{22} = -36.0^\circ$, $c = 1.05$ in MeOH, 96% ee).

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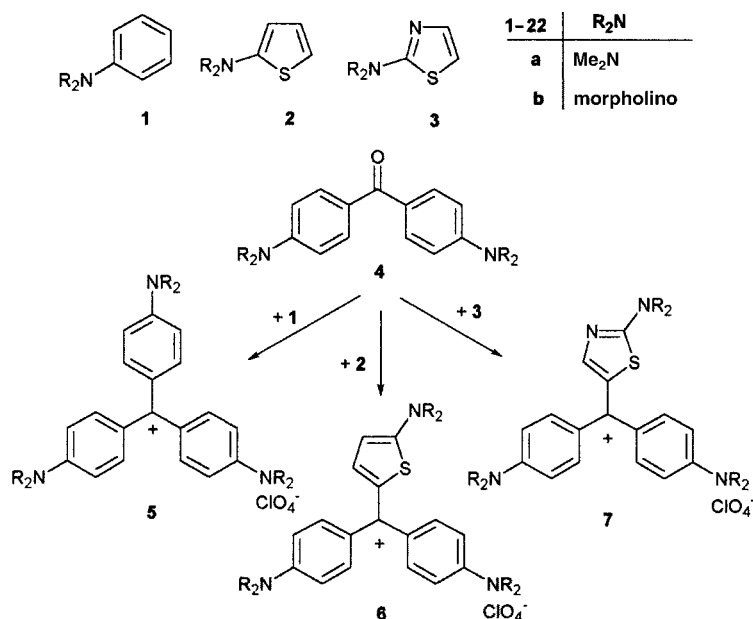
Synthesis and Spectral Characterization of a New Class of Heterocyclic Analogues of Crystal Violet Dyes**

Antje Noack, Anke Schröder, and Horst Hartmann*

Dedicated to Professor Siegfried Hünig on the occasion of his 80th birthday

Crystal Violet **5a** is a classical representative of synthetic dyes that is to some extent still used today. It attracted considerable attention immediately after its discovery by von Hofmann in 1873, because such a beautiful and colorfast

dye was unknown.^[1] The dye can be synthesized by various methods usually starting from dimethylaniline (**1a**).^[2] Some of these routes utilize Michler's ketone **4a** that is readily accessible from **1a** and phosgene (see Scheme 1).



Scheme 1. Synthesis of the Crystal Violet dyes **5–7**.

To improve the coloristic properties of Crystal Violet numerous experiments were performed to synthesize structurally modified derivatives. Among others, sulfonation leading to more water soluble, acidic Crystal Violet dyes, or the replacement of the benzenoidic rings by polycyclic or heterocyclic groups were tried successfully. Further attempts to make the dyes more colorfast by substituting the *N*-alkyl groups by different types of aryl substituents were carried out.^[3] With most of these dyes practical applications were rather limited, but from a theoretical point of view some of these compounds were of great importance.^[4] A few years ago it was shown that Crystal Violet derivatives with an extended π system exhibit extraordinarily high quadrupole-induced nonlinear optical (NLO) coefficients through a multidirectional intramolecular charge transfer from the periphery to the center of the molecule that makes them promising candidates for the synthesis of materials with NLO properties.^[5]

In recent years 2-dialkylaminothiophenes **2**^[6] and 2-dialkylaminothiazoles **3**^[7] have received special attention as heterocyclic analogues of the dialkylanilines **1**, since they are also able to form a large variety of different dyes. Thus, these heterocyclic amines can be used for, among other things, the preparation of diazo dyes,^[8, 9] methine and azomethine dyes,^[10, 11] as well as squaraine acid and croconine dyes.^[12, 13] They can also be used for the preparation of a series of compounds that, because of their donor–acceptor character, possess an intense absorption in the visible spectral range and a high dipolarity. Thus, compounds of this type can be used as indicators for the determination of solvent polarities^[14] or for the preparation of materials with NLO properties.^[15] However, to our knowledge, compounds of type **2** and **3** have not

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yet been used in the preparation of heterocyclic analogues of triphenylmethane dyes.

We found that this preparation can be carried out successfully if Michler's ketone (**4a**; or one of its derivatives which, like the bis(morpholino) derivative **4b**, is structurally modified at the nitrogen atoms) is treated either with one of the heterocyclic compounds **2** or **3** (which were generally used as their 2-morpholino derivatives) in presence of an acidic condensation agent such as POCl₃ or treated with a derivative of these aminoheterocycles that is lithiated at the 5 position. Whereas in the former case the reaction proceeds via a bis[4-(*N,N*-alkylanilino)]chloromethinium chloride, in the latter case it proceeds via the corresponding bis[(*N,N*-dialkylanilino)]-substituted carbinols^[2] from which the hitherto unknown heterocyclic Crystal Violet analogues **6** and **7**, which we isolated as perchlorates were obtained (Scheme 1, Table 1).

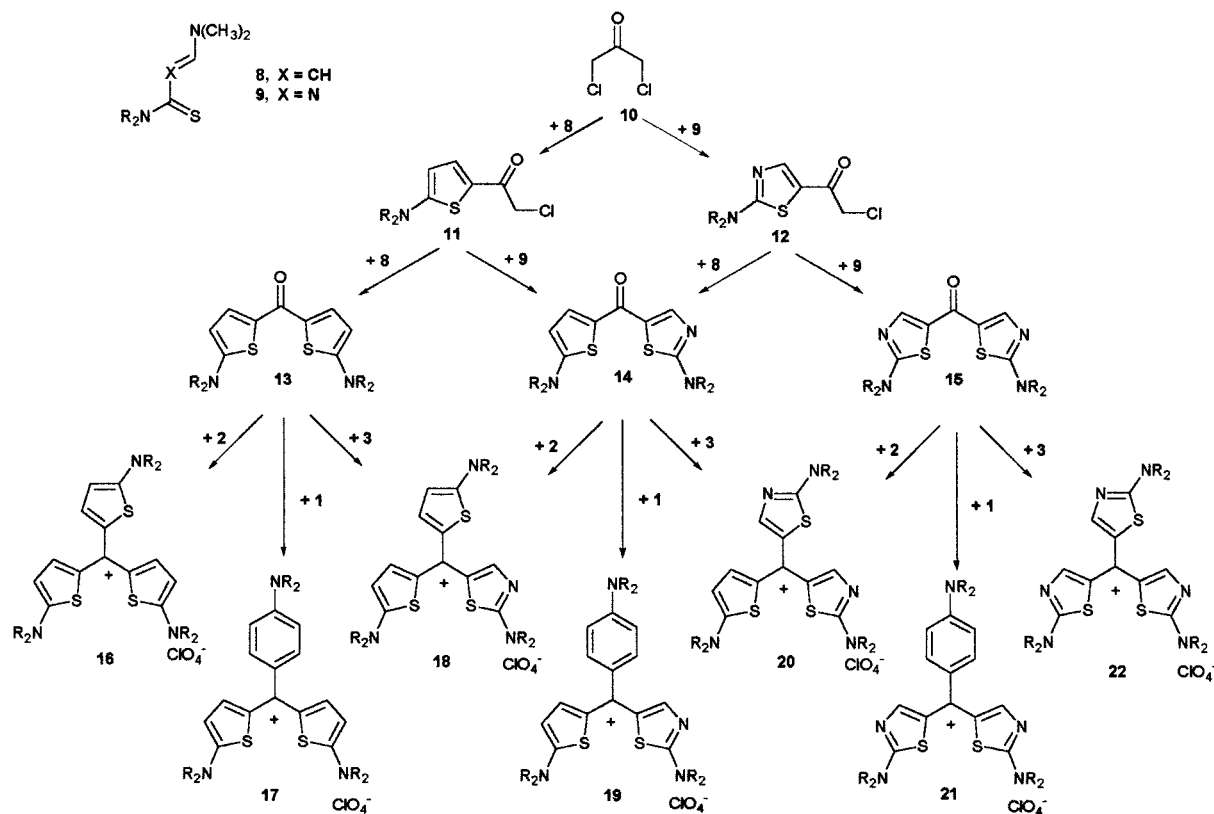
With both these methods further heterocyclic analogues of Crystal Violet can be prepared as long as suitable heterocyclic analogues of **4** are available. Such compounds are unknown at yet, but can be synthesized according to a method for the synthesis of 2-aminothiophenes and 2-aminothiazoles that are acceptor-substituted in the 5 position, recently described by us.^[16] This synthesis starts from *N,N'*-persubstituted thioacrylamides **8**^[17] or their aza analogues **9**^[18] which are allowed to react with 1,3-dichloroacetone (**10**) in the presence of a suitable auxiliary base. Depending on the reaction stoichiometry and conditions either *N,N*-disubstituted 2-amino-5-(chloroacetyl)thiophenes **11**, 2-amino-5-(chloroacetyl)thiazoles **12**, or *N,N'*-persubstituted bis(2-amino-5-thienyl)-ketones **13** as well as bis(2-amino-5-thiazolyl)ketones **15** were obtained. The two latter types of compounds are, as well as

the *N,N'*-persubstituted (2-amino-5-thiazolyl)-(2-amino-5-thienyl)ketones **14**, optionally accessible through the reaction of the 5-chloroacetyl-substituted 2-aminothiophenes and 2-aminothiazoles **11** and **12**, respectively, with the corresponding thioacrylamide derivative **8** or **9**.

By using one of the routes described above, the new heterocyclic Michler's-ketone analogues **13–15** were converted into the corresponding heterocyclic Crystal Violet analogues **16–22**. Thus, a complete series of thiophene and thiazole analogues of Crystal Violet is now accessible (Scheme 2, Table 1).

The identity of all the heterocyclic Crystal Violet analogues **6**, **7**, and **16–22** synthesized by us could be confirmed by their elemental analysis and NMR spectra (Table 1).^[19] The ¹H and ¹³C NMR spectra are rather straight forward in the aromatic region. Thus, in the ¹H NMR spectra only doublets or singlets (as in the case of thiazolyl-substituted derivatives) could be found. In the ¹³C NMR spectra the signals for the central methine atoms are found between $\delta = 140$ and 160 depending on the nature of the corresponding carbo- or heterocyclic moieties.

All heterocyclic Crystal Violet analogues **6**, **7**, and **16–22** described here are, as expected, deeply colored compounds with intensive absorption in the visible range of the spectrum (Table 1). Similar to the Crystal Violet derivative **5b** obtained by the reaction of three equivalents of *N*-(4-lithio-phenyl)-morpholine with one equivalent of diethyl carbonate and subsequent treatment with perchloric acid, **6**, **7**, and **16–22** usually have only one absorption band in the visible region (Figure 1), which is in contrast to the triphenylmethane dye, Malachite Green, which has only two dimethylamino groups on its three phenyl moieties. With the exception of **7b**, this



Scheme 2. Synthesis of the Crystal Violet dyes **16–22**.

Table 1. Characteristic data of the heteroanalogous Crystal Violet perchlorates **5b–7b** and **16b–22b**.

5b : m.p. >350 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 597 nm (4.97); ¹ H NMR ([D ₆]DMSO): δ = 3.63 (t, 12 H, CH ₂), 3.77 (t, 12 H, CH ₂), 7.21 (d, 6 H, CH), 7.32 (d, 6 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 46.56, 65.64, 113.42, 127.29, 139.25, 155.44, 176.53
6b : m.p. 161–163 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 582 nm (4.77); ¹ H NMR ([D ₆]DMSO): δ = 3.37 (t, 8 H, CH ₂), 3.75 (t, 8 H, CH ₂), 3.83 (t, 4 H, CH ₂), 7.07 (d, 4 H, CH), 7.19 (d, 2 H, CH), 7.34 (d, 2 H, CH), 7.43 (d, 1 H, CH), 7.86 (d, 1 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 46.72, 52.09, 65.17, 65.74, 113.43, 119.66, 127.61, 130.14, 134.23, 148.91, 158.81, 171.43, 179.66
7b : m.p. 180–185 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 579 nm (4.92); ¹ H NMR ([D ₆]DMSO): δ = 3.56 (t, 8 H, CH ₂), 3.76 (t, 8 H, CH ₂), 3.81 (t, 4 H, CH ₂), 3.85 (t, 4 H, CH ₂), 7.16 (d, 4 H, CH), 7.45 (d, 4 H, CH), 8.18 (s, 1 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 46.49, 49.61, 65.11, 65.61, 113.46, 126.51, 129.25, 136.79, 154.97, 162.86, 179.96, 179.99
16b : m.p. >370 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 615 nm (4.97); ¹ H NMR ([D ₆]DMSO): δ = 3.51 (t, 12 H, CH ₂), 3.77 (t, 12 H, CH ₂), 6.79 (d, 3 H, CH), 7.68 (d, 3 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 49.85, 65.15, 110.18, 123.23, 142.38, 142.89, 171.02
17b : m.p. 206–209 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 609 nm (4.83); ¹ H NMR ([D ₆]DMSO): δ = 3.32 (t, 4 H, CH ₂), 3.63 (t, 8 H, CH ₂), 3.76 (m, 12 H, CH ₂), 6.90 (d, 2 H, CH), 7.07 (d, 2 H, CH), 7.33 (d, 2 H, CH), 7.55 (d, 2 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 46.99, 50.52, 65.26, 65.96, 112.54, 113.50, 125.13, 127.02, 133.17, 145.03, 152.82, 171.80, 173.56
18b : m.p. 235–242 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 623 (4.77), 541 nm (4.42); ¹ H NMR ([D ₆]DMSO): δ = 3.62 (t, 12 H, CH ₂), 3.76 (t, 12 H, CH ₂), 6.92 (d, 2 H, CH), 7.74 (s, 1 H, CH), 7.83 (d, 2 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 48.26, 50.45, 65.27, 112.48, 122.52, 124.18, 139.99, 144.25, 150.36, 172.90, 174.82
19b : m.p. 163–165 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 582 nm (4.50); ¹ H NMR ([D ₆]DMSO): δ = 3.33 (t, 4 H, CH ₂), 3.64 (t, 4 H, CH ₂), 3.75 (m, 8 H, CH ₂), 3.81–3.86 (m, 8 H, CH ₂), 6.92 (d, 1 H, CH), 7.09 (d, 2 H, CH), 7.34 (d, 2 H, CH), 7.55 (d, 1 H, CH), 7.66 (s, 1 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 46.67, 48.36, 51.87, 65.08, 65.68, 66.17, 113.42, 124.99, 126.16, 131.33, 132.95, 133.41, 148.27, 152.88, 168.29, 175.58, 177.62, 179.56
20b : m.p. 265–268 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 600 nm (4.44); ¹ H NMR ([D ₆]DMSO): δ = 3.64 (m, 8 H, CH ₂), 3.75 (m, 8 H, CH ₂), 3.84 (8 H, m, CH ₂), 7.30 (d, 1 H, CH), 7.87 (s, 2 H, CH), 8.12 (d, 1 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 48.30, 51.80, 65.15, 104.54, 118.18, 122.82, 127.51, 147.58, 151.66, 175.05, 176.72
21b : m.p. 114–115 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 558 nm (4.24); ¹ H NMR ([D ₆]DMSO): δ = 3.25 (t, 4 H, CH ₂), 3.76 (t, 8 H, CH ₂), 3.80–3.82 (m, 12 H, CH ₂), 7.31 (d, 2 H, CH), 7.58 (d, 2 H, CH), 8.18 (s, 2 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 46.37, 49.36, 65.06, 65.60, 113.40, 125.07, 126.99, 134.79, 154.29, 161.65, 169.28, 178.28
22b : m.p. 171–173 °C; UV/Vis (CH ₂ Cl ₂): $\lambda_{\max}$ (lg ϵ) = 570 nm (4.42); ¹ H NMR ([D ₆]DMSO): δ = 3.78 (m, 24 H, CH ₂), 8.35 (s, 3 H, CH); ¹³ C NMR ([D ₆]DMSO): δ = 49.02, 65.09, 124.63, 142.93, 158.74, 177.18

single band in the visible region usually has a more or less well-defined shoulder towards shorter wavelengths; for **7b** there is a shoulder on the longer wavelength side as well. The position of the maximum of these bands is independent of the substituents on the amino groups, but markedly dependent on the heterocyclic moieties. From a theoretical point of view these absorption bands arise from two transitions polarized perpendicularly to each other.^[4] Clearly, in all the compounds (the heterocyclic analogues and Crystal Violet) these transitions have very similar energies. Thus, even compounds that have two or three (e.g. **19**) different side groups, display comparatively small band splittings.

Because of the small size of the crystals obtained it was not possible to determine the structures of the Crystal Violet

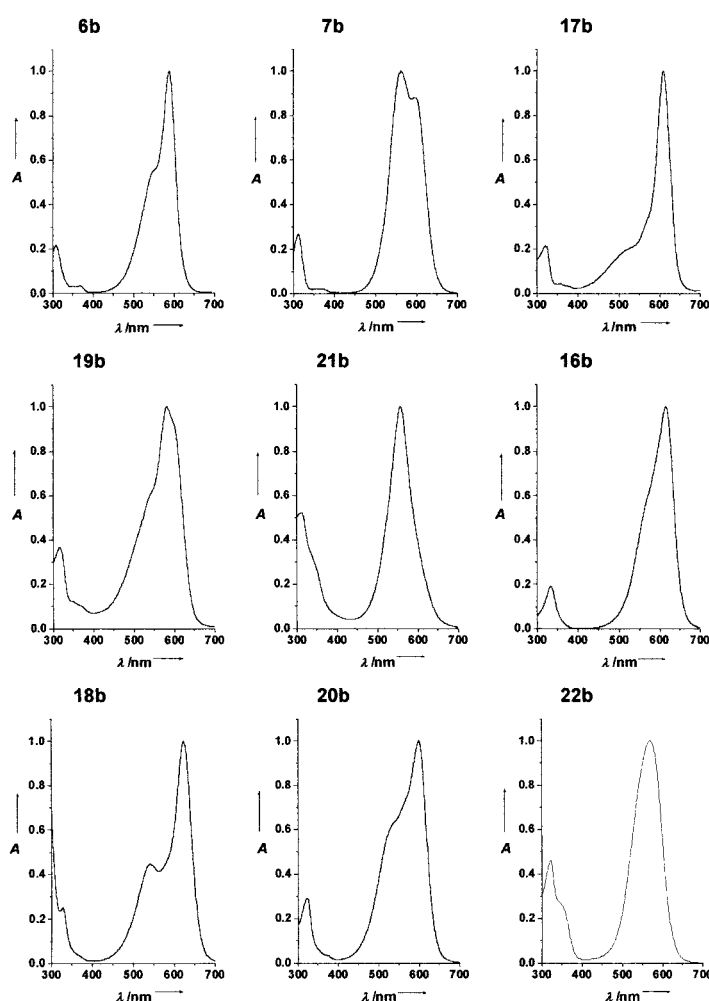


Figure 1. Absorption spectra of heteroanalogous Crystal Violet derivatives.

analogues by X-ray analysis. Therefore, no unambiguous description of the three-dimensional arrangement of the side groups can be given. However, force-field calculations^[20] demonstrate that, because of the smaller steric demand of a heterocyclic five-membered ring compared to a carbocyclic six-membered ring, all the compounds that possess two heterocyclic side groups have them in a coplanar conformation. Thus, the heterocyclic derivatives differ from Crystal Violet **5a**, which has, following force-field calculations performed by us and in agreement with the published X-ray data,^[21] a propellerlike structure, and also from the recently published structures of tris(bithienyl)methinium and tris(trithienyl)methinium ions that are also of the propeller type.^[22]

To illustrate these findings the molecular structures of Crystal Violet **5a** and the tris(2-dimethylamino-5-thienyl)methinium ion **16a** calculated using the MM2 method are given in Figure 2. As can be seen **5a** has a propellerlike structure with almost identical torsion angles for the three dimethylamino-substituted phenyl groups relative to the central trimethylene methane unit, whereas the heterocyclic Crystal Violet analogue **16a** exhibits an almost planar bis(2-dimethylamino-5-thienyl)methinium unit and a 2-dimethylamino-5-thienyl unit almost perpendicular to it. Because force-field calculations have some uncertainties with large π -systems,

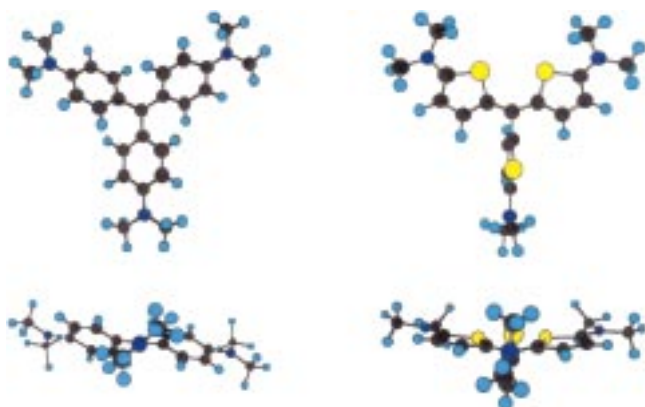


Figure 2. MM2-optimized structures of the cation of Crystal Violet **5a** (left) and of the tris(2-dimethylamino-5-thienyl) methinium ion **16a** (right).

quantum-chemical ab initio calculations were performed. The results obtained demonstrate the existence of flat energy minima between different conformers of the sulfur-containing compounds which makes it more difficult to find the global energy minimum and, hence, to find the most-stabilized conformation.^[23]

It has to be seen whether the unusual structures of the heterocyclic analogues of Crystal Violet dyes described here have a marked effect on their NLO behavior, which is determined by the molecular symmetry and the character of the excited states of the compounds.^[24] According to theoretical studies molecules with two energetically almost degenerate electronic transitions that are perpendicular to each other should display a special NLO behavior indicative of an octopolar charge distribution in the corresponding molecular system.^[25]

Experimental Section

General: The UV/Vis absorption spectra were recorded with a UV-2501 spectrometer, Shimadzu, Tokyo, and the NMR spectra with a Gemini 300 spectrometer, Varian, Zurich.

Synthesis of the bis(heteroaryl)ketones **13b–15b** and their precursors **11b** and **12b**: To 3-dimethylaminothioacrylmorpholine (**8b**; 10 mmol) or *N*-(dimethylaminomethylene)morpholino thiourea (**9b**; 10 mmol) in acetonitrile (50 mL) were added 1–5 equivalents of 1,3-dichloroacetone (**10**), the mixture was then heated to boiling for a short time. The mixture was cooled to room temperature, triethylamine (30 mmol) was added then the mixture was heated to boiling again before being poured into water (100 mL). The precipitate was collected by filtration and recrystallized from ethanol or DMF: **11b**: yield 23%; m.p. 156–158 °C (ethanol); ¹H NMR (300 MHz, CDCl₃): δ = 3.29 (t, 4H, CH₂), 3.81 (t, 4H, CH₂), 4.40 (s, 2H, CH₂Cl), 6.05 (d, 1H, CH), 7.54 (d, 1H, CH); **12b**: yield 63%; m.p. 164–166 °C (ethanol); ¹H NMR (300 MHz, CDCl₃): δ = 3.59 (t, 4H, CH₂), 3.78 (t, 4H, CH₂), 4.39 (s, 2H, CH₂Cl), 7.91 (s, 1H, CH); **13b**: yield 74%; m.p. 286 °C (DMF); ¹H NMR (300 MHz, CDCl₃): δ = 3.28 (t, 8H, CH₂), 3.84 (t, 8H, CH₂), 6.10 (d, 2H, CH), 7.61 (d, 2H, CH); **14b**: yield 98%; m.p. 306–307 °C (DMF); ¹H NMR (300 MHz, CDCl₃): δ = 3.29 (t, 4H, CH₂), 3.60 (t, 4H, CH₂), 3.83 (m, 8H, CH₂), 6.10 (d, 1H, CH), 7.60 (d, 1H, CH), 7.90 (s, 1H, CH); **15b**: quantitative yield; m.p. 312–313 °C (DMF); ¹H NMR (300 MHz, CDCl₃): δ = 3.57 (t, 8H, CH₂), 3.73 (t, 8H, CH₂), 8.11 (s, 2H, CH).

Synthesis of the heterocyclic Crystal Violet analogues **16b–22b**: To a cooled suspension, prepared by addition of a solution of butyllithium (1.6 M) in hexane (10 mL) to a solution of 2-morpholiniothiophene **2b** or 2-morpholiniothiazole **3b** (10 mmol), respectively, in absolute 1,4-dioxane (50 mL), a bis(heteroaryl)ketone **13b–15b** (10 mmol) was added under

argon and the resulting mixture heated for 8 h on a water bath at 90 °C. After cooling to room temperature the mixture was filtered and perchloric acid (70%, 0.5 mL) added to the filtrate. The resulting precipitate was collected by filtration, dried, and recrystallized from glacial acetic acid.

The Crystal Violet analogues **6b** and **7b** were prepared similarly by the reaction of *N*-(4-lithio-phenyl)morpholine with methyl 2-morpholiniothiophene-5-carboxylate or 2-morpholiniothiazole-5-carboxylate,^[17c] respectively.

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A Stable DNA Duplex Containing a Non-Hydrogen-Bonding and Non-Shape-Complementary Base Couple: Interstrand Stacking as the Stability Determining Factor

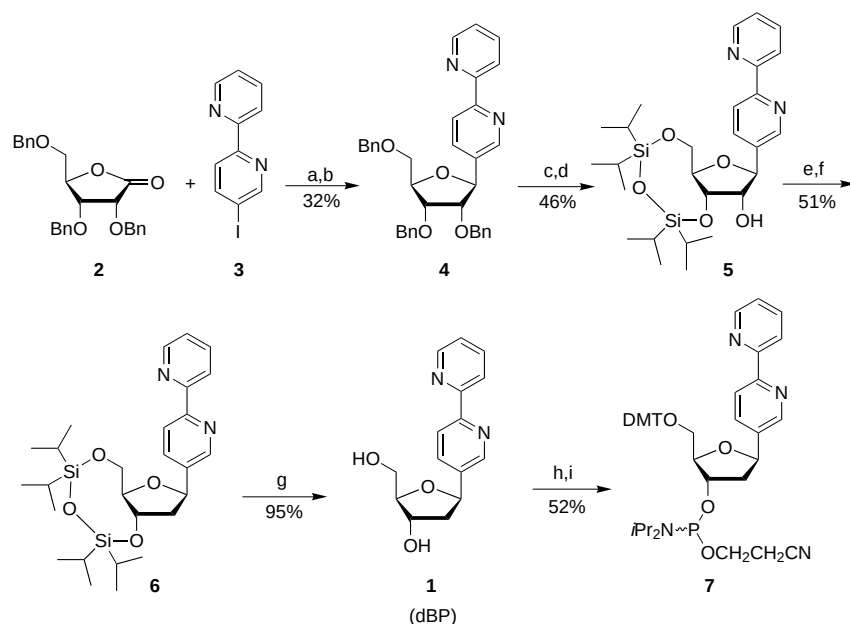
Christine Brotschi, Adrian Häberli, and Christian J. Leumann*

Hydrogen-bonding and stacking interactions between nucleobases are the major components of the noncovalent forces that stabilize the DNA and RNA double helix.^[1, 2] The relative contribution of each factor to the stability has been a matter of debate since the discovery of the structure of the double helix. Recent reports on stable duplex formation and selective DNA-polymerase-mediated replication of oligodeoxynucleotides that contain hydrophobic, non-hydrogen-bonding base pairs led to a new twist in this discussion,^[3–14] and have triggered a re-evaluation of the importance of aromatic stacking interactions and shape selectivity in the base-pair formation.^[15]

In a research program devoted to the introduction of specific metal-binding sites into DNA,^[16–18] we became interested in the synthesis and incorporation into oligonucleotides of the nucleoside analogue **1** (dBP; see Scheme 1), which contains a 2,2'-bipyridyl (BP) unit as a base-surrogate.^[19] Herein we report the efficient self-recognition of dBP within a DNA duplex, with high selectivity and stability in the absence of transition metal ions.

The synthesis of the corresponding deoxy-C-nucleoside **1** started from 2,3,5-tri-*O*-benzyl-D-ribonolactone **2**^[20] and 4-iodo-2,2'-bipyridine **3**^[21, 22] (Scheme 1) and followed established routes in C-glycoside synthesis (Scheme 1).^[23, 24] Lithiation of **3** followed by addition to lactone **2** resulted in the intermediate formation of the corresponding hemiacetals, which upon reduction with Et₃SiH afforded selectively the β -isomeric C-glycoside **4** (¹H NMR NOE). After a series of typical transformations for the selective removal of the 2'-hydroxy group, C-glycoside **1** was obtained and subsequently converted into the corresponding phosphoramidite building block **7**.

The incorporation of **7** into the duplex **8** (Table 1) was effected by standard protocols in automated DNA synthesis.^[25] The thermal stability of the duplexes **8** (X, Y = dBP, dA, dG, dC, dT, and the abasic site analogue ϕ) was assessed by determination of the melting temperatures (*T*_m) by using UV spectroscopy (10 mM NaH₂PO₄, 150 mM NaCl, pH 7.0; Figure 1a; Table 1). Inspection of the *T*_m data



Scheme 1. Reagents and conditions: a) **3** (1.0 equiv), *n*BuLi (1.0 equiv), THF, -78 °C, 2 h, then **2** (0.9 equiv), -78 °C → RT, 16 h; b) Et₃SiH (5 equiv), BF₃·OEt₂ (5 equiv), CH₂Cl₂, -78 °C → RT, 16 h; c) BBr₃ (3.4 equiv), CH₂Cl₂, -78 °C, 4 h; d) 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (1.0 equiv), pyridine, RT, 5 h; e) 1,1'-thiocarbonyl diimidazole (1.2 equiv), CH₃CN, RT, 18 h; f) Bu₃SnH (1.6 equiv), AIBN (1.5 equiv), toluene, 80 °C, 2 h; g) (HF)₃·NEt₃ (1.0 equiv), THF, RT, 18 h; h) 4,4'-dimethoxytrityl (DMT) chloride (1.2 equiv), pyridine, RT, 16 h; i) iPr₂NNEt (3 equiv), [(iPr₂N)(NCCH₂CH₂O)P]Cl (1.3 equiv), THF, RT, 3 h. AIBN = α,α' -azobisisobutyronitrile.

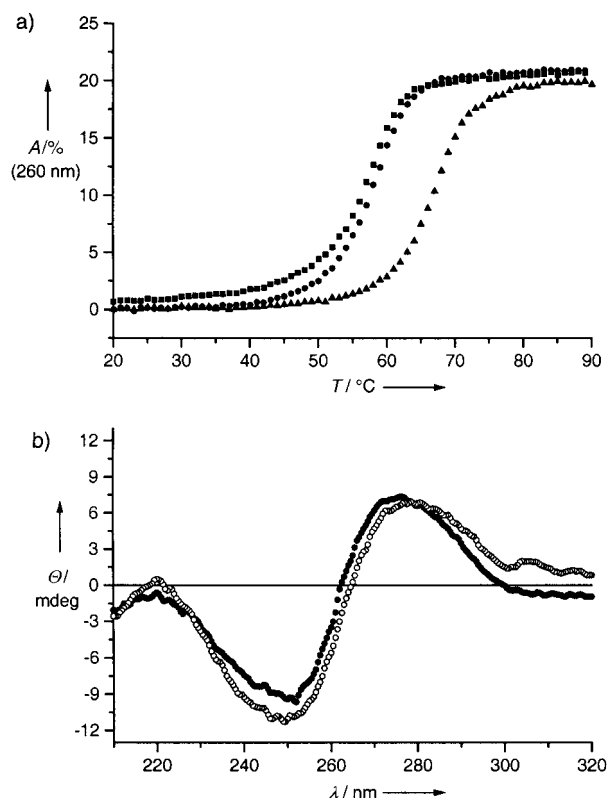


Figure 1. a) UV melting curves of the duplexes **8** with X = Y = dBP (▲), X = dBP, Y = ϕ (●), and X = dBP, Y = dT (■); A = standardized absorption; experimental conditions are described in Table 1; b) CD spectra of duplexes **8** with X = Y = dBP (○) and with X = dA, Y = dT (●); c) **8** = 2.4 μ M, buffer described in Table 1).

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Table 1. Sequence of the 19-mer DNA duplex **8** and T_m data for various combinations of X and Y, as extracted from UV melting curves (260 nm, 1.2 μ M, in 10 mM NaH_2PO_4 , 150 mM NaCl, pH 7.0).

Y	X	BP	5'-GATGAC X GCTAGCTAGGAC 3'-CTACTG Y CGATCGATCCTG				8
			ϕ	A	G	T	C
BP		67.4	58.5	n.d. ^[a]	n.d. ^[a]	n.d. ^[a]	n.d. ^[a]
T		57.9	50.1	64.0	60.0	58.5	55.7
C		59.7	52.0	55.6	66.7	56.1	54.6
A		61.4	52.5	57.0	59.2	64.1	55.8
G		62.0	54.8	59.2	58.6	60.1	66.0

[a] Not determined.

reveals that the unnatural base pair dBP:dBP in the given sequence ($T_m = 67.4^\circ\text{C}$) is more stable than a dA:dT base pair by 3.4 K, and of similar stability to a dG–dC base-pair (+0.7 K). The self-recognition of dBP is selective. When each of the natural bases is placed opposite a dBP residue in the duplex, T_m decreases by 5.4–9.5 K, which corresponds to the differences in the free enthalpies of duplex formation ($\Delta\Delta G^\circ$) of 1.7–3.6 kcal mol^{−1} (Table 2) relative to the dBP:dBP duplex. Thus the effect of a dBP:dN base-pair on duplex stability is similar to that resulting from a mismatched natural base pair. Importantly, if the dBP residue is located opposite to an abasic site analogue, a decrease in thermal stability of 8.9 K is observed.

Table 2. Two-state model dependent thermodynamic data of duplex formation obtained from curve fitting of the experimental UV melting curve.^[27] Estimated error: $\pm 5\%$.

Y (X = BP)	ΔH [kcal mol ^{−1}]	ΔS [cal K ^{−1} mol ^{−1}]	$\Delta G^{25^\circ\text{C}}$ [kcal mol ^{−1}]
A	−115	−317	−21.0
T	−106	−293	−19.1
G	−108	−296	−20.5
C	−112	−308	−20.4
BP	−113	−303	−22.7

To exclude traces of transition metal ions bound to dBP, which would thus interfere with the T_m experiments, EDTA (0.1 M) was included in the buffer in control experiments. No differences in T_m were observed in these cases. Given the low pK_a of the bipyridyl unit (4.3) and the unfavorable geometry of the ring nitrogen atoms for interresidue hydrogen bonding, we also exclude the formation of a protonated dBPH⁺:dBP base pair at pH 7.0.

A structural model of a DNA duplex with two opposing dBP units clearly shows that the two bases can only be accommodated in the double helix without major backbone distortion if the distal pyridyl rings are stacked. Indeed, a molecular dynamics simulation of the duplex **8** (X = Y = dBP) with the two bipyridyl bases adjacent to each other in the starting conformation already lead to a partially stacked geometry (Figure 2, top) during initial energy minimization. This arrangement remains stable over the whole period of the 200-ps trajectory of the simulation. As can be seen in Figure 2 (bottom), the stacking of the two dBP residues results in an increase in the distance between the two adjacent dG:dC

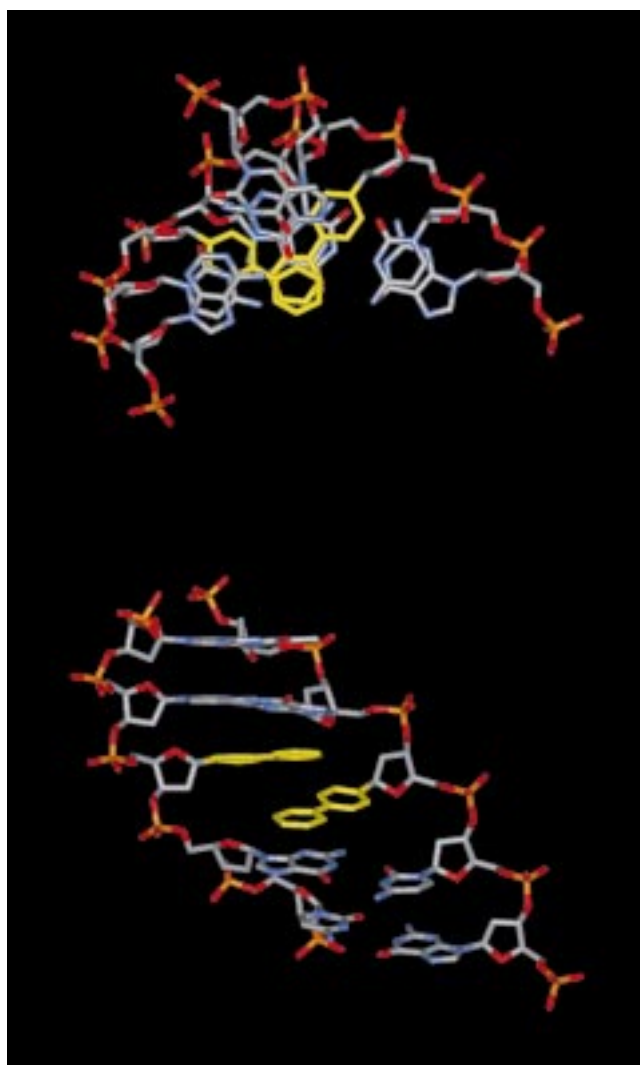


Figure 2. Structure of a section of duplex **8** (X = Y = dBP), which contains the two dBP residues (bases in yellow) as well as the adjacent natural base pairs (two on either side). The structure corresponds to the average of the last 20 ps of an unrestrained molecular dynamics simulation at 300 K on a 200-ps trajectory, using the amber force field incorporated in InsightII/Discover3 V98.0. Instead of explicit solvent molecules, a distance-dependent permittivity of $\epsilon = 4r$ (r = distance) was used as a screening function. Top: view along the helical axis, showing the almost ideal overlap of the distal pyridine rings of dBP; bottom: view from the major groove side which shows the stacked dBP base couple within the base stack of the DNA.

base-pairs, which is permitted by a rearrangement of the local backbone conformation. The overall B conformation of the double helix remains mostly intact, a fact that is fully supported by the circular dichroism (CD) spectra of the duplexes **8** with X:Y = dA:dT and dBP:dBP (Figure 1 b).

The combined experimental and modeling results for the non-hydrogen-bonding dBP-containing oligonucleotide duplex **8** led us to conclude that interstrand stacking of two opposite non-hydrogen-bonded and non-shape-complementary aromatic residues is an integral part of the duplex stabilization. A review of the properties of the recently described duplexes with various hydrophobic base analogues by Romesberg, Schultz and co-workers,^[3, 5, 6] and by Kool and co-workers^[8, 10, 14] is in perfect agreement with the findings

reported herein. The shape-complementary difluorotoluene:4-methylindole and related base couples,^[8, 14] as well as analogous arrangements of substituted phenyl groups with geometries that do not allow interstrand stacking destabilize a DNA duplex.^[5] On the other hand, extended aromatic units, for example, the isocarbostryls (1-hydroxyisoquinolines)^[3, 6] or pyrene,^[10] which have the potential (at least partially) to stack if they occupy opposite positions in a duplex, show equal or higher stability than natural base pairs. The importance of interstrand stacking to duplex stability has also been pointed out recently in the case of the DNA and RNA analogues p-RNA and homo-DNA.^[26] A high-resolution structural analysis of a duplex that contains a dBP base pair is currently in progress.

The reported experimental results provide the basis for the simple rationale that non-hydrogen-bonding non-shape-complementary base-arrangement in a DNA duplex can attain similar or even enhanced stabilities relative to a Watson–Crick base pair only if interstrand base stacking of such opposing units is possible. This rationale may prove useful in further base design for applications in molecular biology or materials science.

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Efficient Photooxidative Degradation of Organic Compounds in the Presence of Iron Tetrasulfophthalocyanine under Visible Light Irradiation**

Xia Tao, Wanhong Ma, Tianyong Zhang, and Jincal Zhao*

The degradation of organic pollutants by photocatalysis^[1] and (photo-)Fenton reactions^[2] has been described extensively. Meunier and co-workers reported an efficient oxidative degradation of trichlorophenol (TCP) in the presence of iron tetrasulfophthalocyanine ([Fe(PcS)]) and H₂O₂ in the dark.^[3] In this process the metal–peroxo species [Fe(OOH)(PcS)] is involved as an active oxygen intermediate. A merit of this system is that [Fe(PcS)] is a readily available biomimetic catalyst that can be fixed onto amberlite and therefore does not enter into the environment and cause additional pollution. It should, however, be noted that the solvent employed contains a large amount of acetonitrile. If water is used as the sole solvent, the conversion rate of TCP is greatly reduced.^[4]

Here we report that when visible light is introduced to an aqueous system containing test compounds, [Fe(PcS)], and

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H_2O_2 , the degradation of organic pollutants is markedly accelerated compared to the reaction carried out in the dark. The visible-light-assisted degradations of organic compounds such as salicylic acid (SA), *p*-hydroxybenzoic acid, Rhodamine B (RhB), Sulfo-Rhodamine B, Crystal Violet, Acridine Orange, and Orange II were examined in an aqueous $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system: All compounds could be degraded rapidly, although these processes are very slow in the dark. The investigation of RhB and SA will be described in detail (see below). The reaction under irradiation with visible light involves the photoassisted generation of $\text{HO}^\bullet$ radicals, which display very high oxidation activity, rather than an iron-peroxo species. This finding provides another approach to the effective treatment of organic pollutants in wastewaters, and also reveals a reaction mechanism different from that taking place in the dark. The latter is also useful for a better understanding of reaction mechanisms in biomimetic photochemistry.

The photodegradation of RhB under various conditions is shown in Figure 1. This compound was scarcely decomposed after 160 min of irradiation with visible light when placed

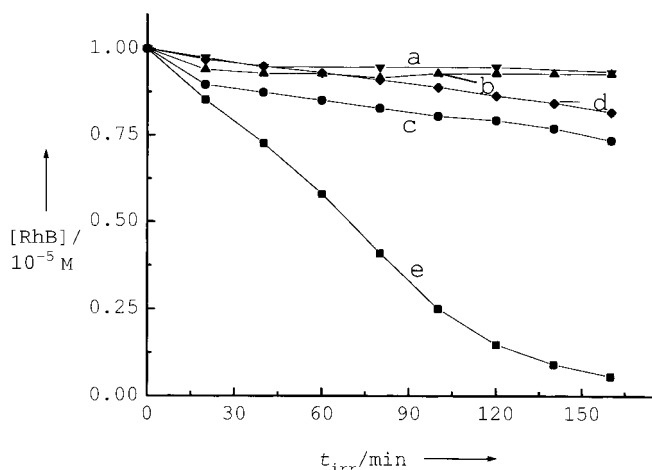


Figure 1. Degradation of RhB (10 μM) under different conditions. a) A solution containing only RhB under irradiation with visible light; b) a solution of RhB and $[\text{Fe}(\text{PcS})]$ (40 μM) under irradiation with visible light; c) a solution of RhB and H_2O_2 (1 mM) under irradiation with visible light; d) RhB in the presence of $[\text{Fe}(\text{PcS})]$ and H_2O_2 in the dark; e) RhB in the presence of $[\text{Fe}(\text{PcS})]$ and H_2O_2 under irradiation with visible light.

alone in a solution (curve a) or together with $[\text{Fe}(\text{PcS})]$ (curve b), and slightly decomposed in the presence of H_2O_2 (curve c). When both $[\text{Fe}(\text{PcS})]$ and H_2O_2 were present, about 20% of RhB disappeared after reaction for 160 min in the dark (curve d). In contrast, approximately 95% of RhB was degraded under otherwise identical conditions as in (d) but under irradiation with visible light (curve e). Evidently, irradiation with visible light significantly accelerated the degradation of RhB in the presence of $[\text{Fe}(\text{PcS})]$ and H_2O_2 . During the photoreaction no degradation of $[\text{Fe}(\text{PcS})]$ was observed by UV/Vis spectroscopy, and no free Fe^{2+} or Fe^{3+} ions were detected, indicating that $[\text{Fe}(\text{PcS})]$ is an efficient photocatalyst to degrade organic pollutants. The removal of total organic carbon (TOC) of RhB in the aqueous RhB/ $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system was about 30% after 8 h of reaction.^[5]

The ESR technique was used to gain insight into the nature of the short-lived radicals formed during the photodegradation. The spectra for the RhB/ $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system under irradiation with visible light ($\lambda > 470 \text{ nm}$) are shown in Figure 2. No signals of significant intensity were observed

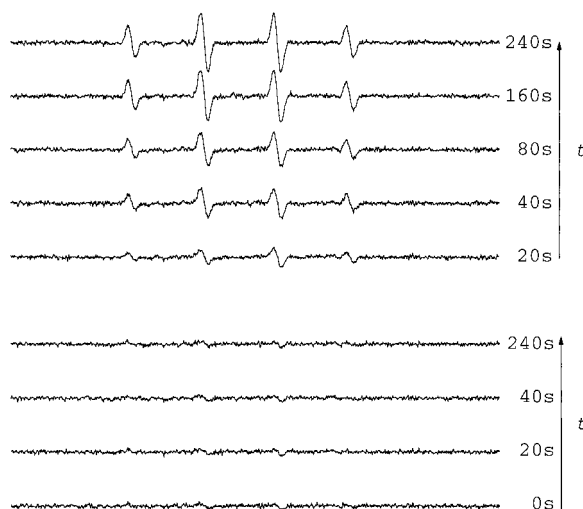


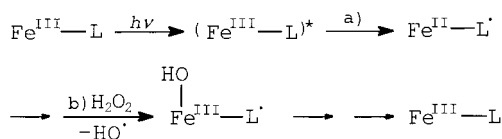
Figure 2. Changes in the ESR spectra of solutions containing RhB (0.1 mM), $[\text{Fe}(\text{PcS})]$ (0.2 mM), and H_2O_2 (0.05 M), measured both in the dark (below) and under irradiation with visible light (above). The 1:2:2:1 quartet is due to the $\text{DMPO} - \cdot\text{OH}$ adducts formed.

when the experiments were run in the dark. This is in good agreement with the results reported by Meunier et al., in which no formation of $\text{HO}^\bullet$ radicals is involved.^[3] However, when the system was irradiated with visible light, signals for $\text{DMPO} - \cdot\text{OH}$ adducts ($\text{DMPO} = 5,5\text{-dimethyl-1-pyrroline-N-oxide}$) with a 1:2:2:1 quartet pattern appeared.^[6, 7] Moreover, these signals were enhanced by further irradiation and then remained unchanged after 4 min. These results provide evidence that irradiation with visible light can greatly enhance the generation of $\text{HO}^\bullet$ radicals, and hence accelerate the degradation of the organic pollutant owing to the high oxidation potential of $\text{HO}^\bullet$ radicals ($E = +2.80 \text{ V}$).^[8, 9] We also carried out ESR experiments with the aqueous SA/ $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system. Although SA has no absorption in the range of visible light, the degradation of SA can be significantly accelerated under irradiation with visible light. The ESR results are similar to those for the RhB system; namely, reactions in the dark gave no ESR signals for $\text{DMPO} - \cdot\text{OH}$, while irradiation with visible light enhanced the intensity of the four characteristic peaks for $\text{DMPO} - \cdot\text{OH}$.

For the RhB/ $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system, we also examined the degradation of RhB with a monochromatic light source ($\lambda = 640 \text{ nm}$, $\Delta\lambda_{1/2} = 10 \text{ nm}$, no light absorption for RhB) basically corresponding to the maximum absorption wavelength of $[\text{Fe}(\text{PcS})]$ ($\lambda_{\text{max}} = 636 \text{ nm}$). Upon excitation of $[\text{Fe}(\text{PcS})]$ by monochromatic light, RhB can also be photodegraded rapidly with a quantum yield of $\phi_{640} = 0.0038$.^[10] In addition, we carried out the photodegradation of RhB in the presence of $[\text{Fe}(\text{PcS})]$ and H_2O_2 under a nitrogen atmosphere. In the present system the effect of oxygen on the degradation of

organic pollutants is minor: Almost the same degradation rate of RhB was observed as that under aerated conditions.

We propose a mechanism, based on the above results, for the photodegradation of organic pollutants in aqueous solutions of $[\text{Fe}^{\text{III}}(\text{PcS})]$ and H_2O_2 (Scheme 1). In this system



Organic pollutants + $\text{HO}^{\cdot} \longrightarrow$ Degraded products

Scheme 1. A possible mechanism for the photooxidation of organic pollutants in the aqueous $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system under irradiation with visible light. See text for details.

$[\text{Fe}^{\text{III}}(\text{PcS})]$ exists mainly in the form of the aqua complex.^[4] Upon irradiation with visible light, the excitation of $[\text{Fe}^{\text{III}}(\text{PcS})]$ can cause an intramolecular electron transfer from the ligand (L) to Fe^{III} , leading to reduction of Fe^{III} to Fe^{II} (step a).^[11] The reduced Fe^{II} complex immediately reacts with H_2O_2 to produce $\text{HO}^{\cdot}$ (step b).^[11d] According to the proposed mechanism, the degradation of organic compounds in the $[\text{Fe}(\text{PcS})]/\text{H}_2\text{O}_2$ system under irradiation with visible light can be well explained without involving high-valence iron-oxo or iron-peroxo complexes.^[3, 12]

Experimental Section

General procedure for the photooxidation reaction: A 500-W halogen lamp was positioned inside a cylindrical Pyrex vessel surrounded by a circulating water jacket (Pyrex) to cool the lamp. The jacket was wrapped with a piece of black paper containing a small window. A cutoff filter was placed on the window (diameter 3 cm) to remove wavelengths less than 470 nm and to ensure irradiation with visible light only. The center distance between the reaction vessel and the light source was 10 cm. A typical reaction mixture for irradiation contained the following initial concentrations: RhB (10 μM) or SA (0.1 mM), $[\text{Fe}(\text{PcS})]$ (40 μM), and H_2O_2 (1 mM). Deionized and doubly distilled water was used throughout this study. The aqueous solution was adjusted to pH 3 with diluted solutions of NaOH or HClO_4 . At the given irradiation time intervals, a sample (3 mL) were taken out and analyzed by UV/Vis spectroscopy with a lambda Bio 20 spectrophotometer (Perkin-Elmer). The ESR spectra were measured on a Bruker EPR 300E spectrometer.

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25 μM $[\text{Fe}(\text{PcS})]$, and 0.05 M H_2O_2 . During the reaction process, no degradation of $[\text{Fe}(\text{PcS})]$ was observed as evidenced by UV/Vis spectroscopy and HPLC analysis, indicating that the TOC removal was caused by the degradation of RhB.

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Branched Star-Type Polysilyllithium Compounds: The Effects of β -Silyl Substitution and of Complexation on Their Molecular Structure**

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Michael Bendikov, Dmitry Bravo-Zhivotovskii,*
Dieter Bläser, and Roland Boese

Dedicated to Professor Hideki Sakurai
on the occasion of his 70th birthday

In contrast to the wealth of information on organolithium reagents,^[1] relatively little is known about the analogous silyllithium compounds, despite their important role in silicon chemistry.^[2] Furthermore, only a limited number of silyl metal reagents have been characterized by X-ray crystallography.^[2, 3] Of special interest are branched “hypersilyl” anions such as $[(\text{Me}_3\text{Si})_3\text{Si}]^-$, which were often used for the synthesis of novel compounds,^[2] for example, by us for the synthesis of stable silenes.^[4] The $[(\text{Me}_3\text{Si})_3\text{Si}]^-$ ion was first prepared in

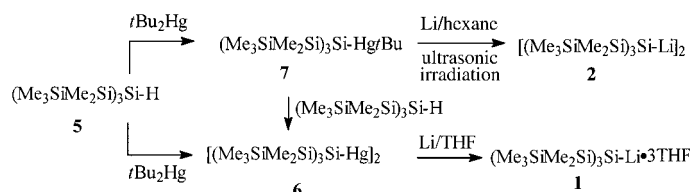
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situ by Gilman and Smith^[5] and later its lithium salt was isolated and characterized as a stable complex with tetrahydrofuran (THF),^[6] with 1,2-dimethoxyethane (DME),^[7a] and with η^5 -2,4,5-tri-*tert*-butyl-1 λ^3 ,3 λ^3 -diphospholide- η^6 -toluene-lithium,^[7b] as well as the noncomplexed dimer.^[8] Except (Me₃Si)₃Si-Li the structures of only two additional branched silyllithium compounds were determined.^[3, 9]

Herein we report on the preparation and X-ray structure determination of the first branched star-type polysilyllithium compound, (Me₃SiMe₂Si)₃Si-Li, in which three silicon atoms are contained in each side chain. Furthermore, it was isolated and characterized by X-ray crystallography both as the THF-complexed monomer (Me₃SiMe₂Si)₃Si-Li · 3 THF (**1**) and as the noncomplexed dimer [(Me₃SiMe₂Si)₃Si-Li]₂ (**2**). Having precise molecular structure information of *both* complexed and noncomplexed forms of the same lithium reagent is quite unique. Except for (Me₃Si)₃Si-Li · 3 THF (**3**)^[6b,c] and [(Me₃Si)₃Si-Li]₂ (**4**)^[8] we were unable to find other examples either of silyl- or alkyl lithium compounds whose noncomplexed *and* complexed form have been structurally characterized. Comparison of the structures of the silanides **1–4** provides a unique opportunity to evaluate the effects of β -Me₃Si substitution and of complexation by THF on the molecular structure and the electronic nature of silyllithium derivatives.

The novel compounds **1** and **2** were synthesized (Scheme 1) by the reaction of (Me₃SiMe₂Si)₃Si-H (**5**) with *t*Bu₂Hg which yields either [(Me₃SiMe₂Si)₃Si-Hg]₂ (**6**) or (Me₃SiMe₂Si)₃Si-Hg*t*Bu (**7**), depending on the ratio of the reagents.^[10]



Scheme 1. Synthesis of the silyllithium compounds **1** and **2**.

Compound **6** reacts with lithium in THF to yield **1**, while **7** reacts with lithium in hexane to give **2** (Scheme 1).^[11] The molecular structures of **1** and **2** were determined by X-ray crystallography^[12] and they are presented in Figures 1 and 2, respectively.

Both **1** and **2** adopt an unusual “back-folded” conformation (Figure 3) in which the β -Si-Si bonds point “inwards”, that is, towards the Si-Li bond (Figures 1 and 2). Thus, the dihedral angle θ ($\theta = \angle$ X-Si1-Si2-Si3; Figure 3) is 46.0° in **1** and 36.0° in **2**. To the best of our knowledge, this is the first report of a “back-folded” conformation of a polysilane—a conformation typical to dendrimers.^[13] Quantum-mechanical density functional calculations at the B3LYP/6-31 + G* level of theory^[14] for (H₃SiH₂Si)₃Si-Li · 3 H₂O, a model of **1** in which all the methyl groups of **1** are substituted by hydrogen atoms and the THF ligands are modeled by water molecules, also adopts a “back-folded” conformation with $\theta = 43.1^\circ$.^[15] Similar “back-folded” conformations are adopted also by (H₃SiH₂Si)₃SiH and other model systems. Thus, the calculations indicate that the “back-folded” conformation is an inherent property of **1**

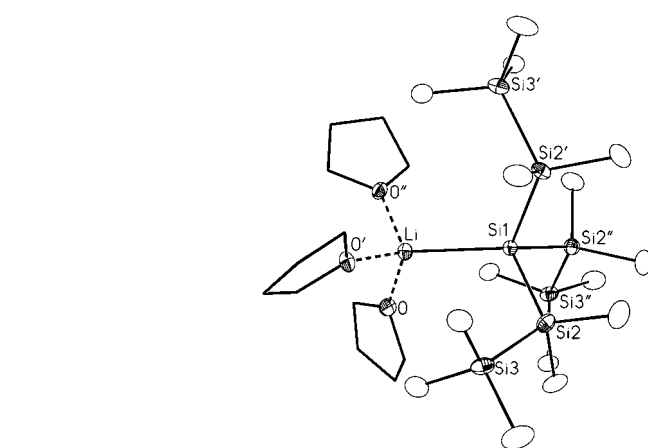


Figure 1. Molecular structure of **1** in the crystal (thermal ellipsoids for carbon (25 %); hydrogen atoms are omitted for clarity, THF carbon atoms are indicated as connective lines). The atoms labeled with ' and ' ' are symmetry equivalent with the operations z, x, y and y, z, x , respectively. Selected bond lengths [Å] and angles [°]: Si1-Li 2.760(11), Si1-Si2 2.363(2), Si2-Si3 2.352(2), Li-O 1.972(6); Li-Si1-Si2 117.74(5), Si2-Si1-Si2' 100.08(6), Si1-Si2-Si3 115.88(7), Si1-Li-O 116.7(3), O-Li-O' 101.3(4).

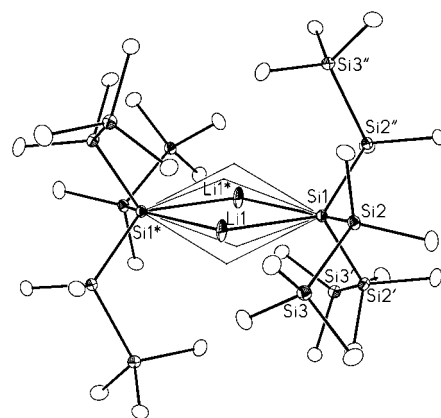


Figure 2. Molecular structure of **2** in the crystal (thermal ellipsoids for carbon (25 %); hydrogen atoms are omitted for clarity, THF carbon atoms are indicated as connective lines). The atoms labeled with ' and ' ' are symmetry equivalent with the operations $-x, x-y, z$ and $y-x, x, -z$, respectively. Si* and Li* are symmetry equivalent by the inversion center at $-x, -y, 1-z$. Only two positions of the six disordered equivalent lithium atoms (in the positions $-x, -y, 1-z$; $-y, x-y, z$; $y, y-x, -z$; $y-x, -x, z$; $x-y, x, -z$) are drawn as shaded ellipsoids, the other four as open circles. Selected bond lengths [Å] and angles [°]: Li1-Si1 2.711(8), Li1-Si1* 2.737(7), Li1...Li1* 2.641(16), Si1-Si2 2.3659(6), Si2-Si3 2.3608(6); Si1-Li1-Si1* 122.0(3), Li1-Si1-Li1* 58.0(3), Li1-Si1-Si2 87.4(2), Li1-Si1-Si2' 133.6(2), Li1-Si1-Si2'' 119.4(2), Li1*-Si1-Si2 143.1(3), Li1*-Si1-Si2' 94.1(2), Si2-Si1-Si2' 102.62(2), Si1-Si2-Si3 112.06(2).

and **2** and not a result of other factors, for example, crystal packing effects.

Comparison of the structures of **1** and **2** with those of the analogous silanides **3**^[6b,c] and **4**,^[8] which have one Me₃Si group less in each branch, reveals the following (Scheme 2): 1) The Si-Li bond lengths in **1** (2.760 Å) and **2** are 0.09–0.14 Å longer than those in **3** (2.669 Å^[6c, 16]) and **4**, respectively. This elongation can be attributed to the

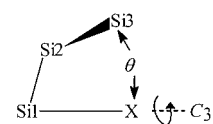
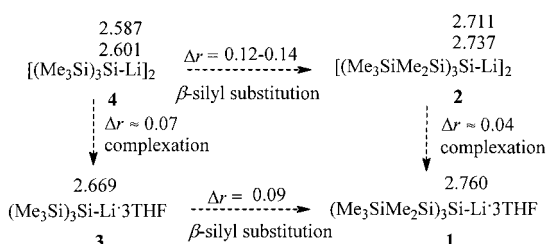


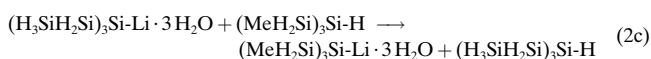
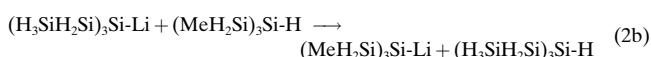
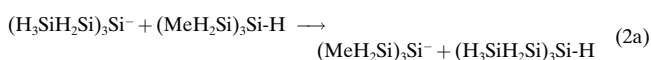
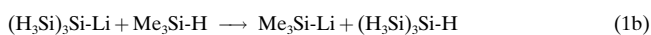
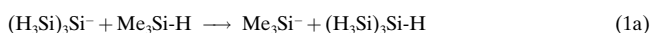
Figure 3. Definition of dihedral angle θ in lithium silanides **1** and **2**: $\theta = \angle$ (X-Si1-Si2-Si3), where the point X is placed along the C₃ axis of the Si₃Si unit.



Scheme 2. Changes in the Si–Li bond length [Å] in compounds **1**–**4**.

effect of β -Me₃Si substitution.^[17] 2) Comparison of the structures of the dimers **2** and **4** with those of the corresponding THF-complexed **1** and **3**, respectively, shows that complexation by THF elongates the Si–Li bond by 0.02–0.08 Å. This elongation is significantly less than the elongation due to β -Me₃Si substitution.^[18]

Calculations (at B3LYP/6-31 + G* + ZPE level of theory) for the model anion [(H₃Si)₃Si][−] show that α -H₃Si substituents strongly stabilize silyl anions, that is, by 43.1 kcal mol^{−1} for three α -H₃Si groups relative to three methyl groups [Eq. (1a)]. In (H₃Si)₃Si–Li the α -silyl effect is smaller, 19.7 kcal mol^{−1} [Eq. (1b)]. β -H₃Si substituents are also stabilizing, but the effect is about three–five times smaller than for α -H₃Si substitution; that is, three β -H₃Si groups stabilize the silyl anion by 12.1 kcal mol^{−1} [Eq. (2a)] and the silyllithium compound by only 3.9 kcal mol^{−1} [Eq. (2b)]. Thus, stabilization by β -H₃Si groups is about three times larger for silyl anions than for the corresponding silyllithium compounds.^[19] The β -H₃Si stabilization of (H₃SiH₂Si)₃Si–Li·3H₂O is 9.0 kcal mol^{−1} [Eq. (2c)], a value intermediate between those for the corresponding silyl anion and the noncomplexed silyllithium compound. The contribution of hyperconjugation (between the anionic silicon center and the adjacent β -Si–Si bonds) to the β -silyl stabilization is small. Thus, the Si2–Si3 bond in [(H₃SiH₂Si)₃Si][−] (2.364 Å) is only slightly longer than that in (H₃SiH₂Si)₃SiH (2.356 Å). Similarly, in **1** the Si2–Si3 bond (2.352 Å) is even shorter than the Si1–Si2 bond (2.363 Å). We conclude that the stabilization of silyl anions by β -H₃Si groups results mainly from charge polarization and not from hyperconjugation.



It is tempting to assign the significant elongation of the Si–Li bond in **1** versus that in **3** and that in **2** versus that in **4**, respectively, to electronic interactions between the Si–Li bond and the β -Si–Si bonds. However, quantum-mechanical calculations show that this is not the case. Thus, the calculated

Si–Li bond lengths (at B3LYP/6-31 + G*) in several relevant model compounds, either complexed or noncomplexed, do not change significantly upon β -H₃Si substitution: $r(\text{Si-Li}) = 2.460$ ((H₃Si)₃Si–Li (**8a**)), 2.459 ((MeH₂Si)₃Si–Li), 2.468 ((H₃SiH₂Si)₃Si–Li (**9a**)), 2.458 ((Me₃Si)₃Si–Li), 2.677 ((H₃Si)₃Si–Li·3H₂O (**8b**)), 2.676 ((MeH₂Si)₃Si–Li·3H₂O), 2.655 Å ((H₃SiH₂Si)₃Si–Li·3H₂O (**9b**)).^[20] These calculations show that the β -H₃Si groups play a minor role in controlling the Si–Li bond length (e.g., compare **9a** with **8a** or **9b** with **8b**). Supporting this conclusion is the fact that (Me₃Si)₃SiMe₂Si–Me₂Si(Me₃Si)₂Si–Li·3THF (**10**), with only one β -Me₃Si substituent, has practically the same Si–Li distance (2.657 Å)^[3] as in **3**. We therefore propose that *steric factors*, present in **1** and **2**, but not in the calculated model systems (where methyl is substituted by hydrogen), are responsible for the significant elongation of the Si–Li bond upon β -Me₃Si substitution (Scheme 2). Furthermore, calculations for **9b** show that elongation of the Si–Li bond from 2.655 to 2.760 Å requires only 0.2 kcal mol^{−1}, indicating that even small steric effects or crystal packing forces may cause significant bond elongation.^[21a] Steric repulsions are evident in the X-ray structures of **1** and **2**, for example, in **2** between the backfolded β -Me₃Si groups (the methyl···methyl distances (C···C 3.861 Å) are somewhat shorter^[22] than the sum of their van der Waals radii (4.00 Å)^[23]) and in **1** between the OCH₂ groups of the THF ligands and the β -Me₃Si groups (C···C 3.926 Å).

Complexation of the model **8a** and **9a** by three water molecules (producing **8b** and **9b**, respectively) elongates significantly the Si–Li bond length of the monomers, that is, by about 0.2 Å,^[20] in contrast to β -H₃Si substitution which has no effect on the Si–Li bond.

What are the effects of β -silyl substitution and complexation by THF on the ionic/covalent character of lithium silanides? The ²⁹Si chemical shift (in toluene) of the formally anionic Si1 atom in **1** is observed at $\delta = -177$, shifted by 12 ppm downfield relative to that of **3** ($\delta = -189$). A similar downfield shift upon β -Me₃Si substitution was observed for Me₃Si(Me₂Si)₂–Li ($\delta = -62.7$) relative to Me₃SiMe₂Si–Li ($\delta = -74.9$)^[11a] and in **10** ($\delta = -182$)^[3] relative to **1**. A downfield shift is usually interpreted as indicating a reduced negative charge. However, as the changes in the chemical shift are relatively small, their interpretation in terms of ionicity is questionable. We have therefore turned to theoretical criteria. In agreement with Klinkhammer^[8] we find that all lithium silanides (e.g., **8** and **9**) have strongly ionic silicon–lithium bonds.

However, according to the calculated natural bond order (NBO) charges^[24a,b] (MP2/6-31 + G**/B3LYP/6-31 + G*) there is very little change in the charge on the lithium atom as a result of β -H₃Si substitution (i.e., 0.80–0.83 electrons in both **8a** and **9a**) or on the Li·3H₂O fragment in **8b** or **9b** (0.89–0.90 electrons). However, β -H₃Si substitution does affect the charge distribution within the silanide anion; that is, the charge on the central silicon atom is reduced by about 0.1 electrons in **9a** and **9b** compared to that in **8a** and **8b**, respectively, due to charge polarization to the β -H₃Si groups. Complexation increases the charge on the Li·3H₂O fragment relative to the charge on lithium in the uncomplexed

monomers (0.90 electrons versus 0.80–0.83 electrons, respectively). Natural resonance theory (NRT) analysis^[24a,c] indicates that the degree of ionicity of the Si–Li bond in **8a** remains essentially unchanged, at 76–81 % ionic character, upon elongation of the Si–Li bond from 2.5 to 2.8 Å, suggesting that the observed elongations of the Si–Li distance in **1** versus those in **3** and in **2** versus those in **4** (Scheme 2) do not indicate a higher ionic character of the Si–Li bonds in **1** and **2** relative to that in **3** and **4**. Our conclusion is that the ionicity of the Si–Li bond does not change significantly by β -H₃Si substitution but that complexation by THF slightly increases its ionicity.

Preliminary experiments show that the dimers **2** and **4** differ in reactivity from THF-complexed lithium silanides **1** and **3**. Further studies aimed at probing the differences in the chemistry of the four closely related silanides **1–4**, as well as attempts to synthesize more complex silanides are in progress.

Experimental Section

All operations were carried out under vacuum using Schlenk techniques. (Me₃SiMe₂Si)₃Si–Li·3 THF (**1**): Compound **6** (7.1 g, 5.7 mmol), which was prepared by reaction of **5** (prepared from HSiCl₃ and Me₃SiMe₂Si–Li in hexane) with tBu₂Hg^[10] was stirred for 48 h with an excess of lithium (7.0 g, 1.0 mol) in THF (in 50 mL). After mercury precipitation was complete the solution was decanted from the metallic residue and evaporated, yielding 4.9 g (7.9 mmol, 69 % yield) of crude **1**. Compound **1** was recrystallized from pentane to give white crystals suitable for an X-ray structure determination.

¹H NMR (C₆D₆): δ = 0.30 (s, 27H; SiMe₃), 0.55 (s, 18H; SiMe₂), 1.33 (m, 12H; CH₂), 3.45 (t, 12H; CH₂O); ¹³C NMR (C₆D₆): δ = 0.02 (SiMe₃), 3.33 (SiMe₂), 25.5 (CH₂), 68.5 (CH₂O); ²⁹Si NMR (C₆D₆): δ = –176.8 (Si–Li), –33.8 (SiMe₂), –15.8 (SiMe₃).

[(Me₃SiMe₂Si)₃Si–Li]₂ (**2**): Compound **7** (6.4 g, 9.4 mmol), which was prepared from **5** (see above)^[10] and tBu₂Hg, was stirred in an ultrasonic bath for 48 h with an excess of Li (7.0 g, 1.0 mol) in hexane (50 mL). After mercury precipitation was complete the solution was decanted from the metallic residue and evaporated yielding 2.4 g (2.8 mmol, 60 % yield) of crude **2**. Compound **2** was recrystallized from pentane to give white crystals suitable for an X-ray structure determination.

¹H NMR (C₆D₆): δ = 0.39 (s, 27H; SiMe₃), 0.55 (s, 18H; SiMe₂); ¹³C NMR (C₆D₆): δ = 0.08 (SiMe₃), 2.95 (SiMe₂); ²⁹Si NMR (C₆D₆): δ = –176.8 (Si–Li), –33.8 (SiMe₂), –15.8 (SiMe₃).

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- [18] In both dimers **2** and **4** the two central silicon and the two lithium atoms form a planar four-membered ring. In **2** the Li...Li distance of 2.641 Å is 0.12 Å shorter than that in **4**. The formally negatively charged Si1 atom is more strongly pyramidalized in **2** than in **4**; that is the Si2–Si1–Si2' bond angles are 102.6° in **2** and 107.5° in **4**. Agostic Li...CH interactions are probably responsible for the relatively short Li...C4'' distance of 2.70 Å in **2**. These Li...C agostic interactions are stronger in **4** ($r(\text{Li}\cdots\text{C}) = 2.49 \text{ Å}$).^[8]
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High Molecular Mass Polyethylene Aqueous Latexes by Catalytic Polymerization**

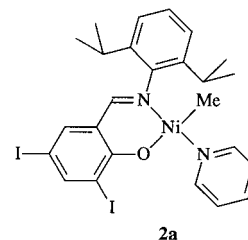
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Emulsion polymerization of olefinic monomers is one of the most important and also versatile polymerization processes.^[1] Polymer latexes are obtained, that is, stable aqueous dispersions of surfactant-stabilized polymer microparticles in the range of 50 to 1000 nm diameter. Numerous applications of latexes (for example, in coatings) involve the formation of films upon evaporation of the dispersing medium, and the

environmental friendliness and nonflammability of water is particularly advantageous. To date, polymer latexes are produced by free-radical processes exclusively.^[2] Transition metal catalyzed coordination polymerization has received comparatively little attention, as the early transition metal catalysts^[3] used commercially for polyolefin production are extremely sensitive to moisture. Carrying out such reactions in water is a highly attractive goal, however, as many polymer microstructures are not available by means other than catalytic polymerization. We^[4] and others^[5] have recently reported the successful polymerization of ethylene in water by neutral nickel(II) complexes.^[6,7] However, dispersions consisting of low molecular mass material with a degree of polymerization of less than 100 (M_n values typically 1000 g mol^{−1}) were obtained. The formation of higher molecular mass polymers (with chain length considerably above the entanglement limit) is a crucial prerequisite to fully exploit the unique property profile of latexes. At the same time, the complex steps of microparticle formation during polymerization must afford stable dispersions of these high molecular mass polymers.

With complexes $[(X^{\wedge}O)Ni^{II}R(L)]$, where X = P (type **1**) or X = N (type **2**, N[^]O = salicylaldimine), materials of relatively similar maximum weight-average molecular mass (M_w) are accessible by traditional ethylene polymerization in apolar organic media.^[8,9] By contrast, in water, the latter offer access to polymers with much higher M_w and M_n values than the low molecular weight materials obtained in water with complexes of type **1**.^[4b] In regard to particle formation, a comparison with free-radical emulsion polymerization is instructive.^[10] In classical emulsion polymerization, water-soluble initiators are used. Chain growth initially affords water-soluble oligomeric radicals, which nucleate particles by collapsing upon themselves after reaching a certain chain length or by entering a surfactant micelle. Similar considerations appear reasonable for the aforementioned emulsion-type catalytic polymerization by a hydrophilically modified water-soluble complex of type **1**, which affords stable latexes of low molecular mass material.^[4] However, a certain water-sensitivity^[4b,9c] of salicylaldimine-substituted complexes (type **2**) can be problematic to an analogous approach.

As a different concept to enable formation of a large number of latex particles during polymerization, a very fine dispersion of the catalyst precursor was achieved in the form of a solution of a lipophilic complex (**2a**) in submicron-size compartments of a hydrocarbon solvent, dispersed in the continuous aqueous phase.^[14] A mixture of water, surfactant, and a solution of the complex in a small amount of hydrocarbon (toluene and a small portion of hexadecane as a hydrophobic additive^[11]) was subjected to high shear, generated either by ultrasound or by means of a modified high-pressure homogenizer. Mini-emulsions consisting of a large number of small hydrophobic droplets (diameter about 100 nm, 10¹⁶–10¹⁷ droplets L^{−1}) containing the catalyst resulted. Exposure of the mini-emulsions to ethylene in a pressure reactor resulted in polymerization to form stable polyethylene latexes



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Table 1. Polymerization conditions^[a] and results.

Entry	<i>n</i> (cat.) [μmol]	Toluene [vol %]	C ₁₆ H ₃₄ [vol %]	Surfactant type ^[b]	<i>c</i> [mmol L ⁻¹]	Emulsification method ^[c]	TON ^[d]	<i>M_n</i> [g mol ⁻¹]	<i>M_w</i> [g mol ⁻¹]	<i>M_w/M_n</i>	<i>d̄</i> ^[e] [nm]
1	25	1	2	SDS	17	US	2515	1.4 × 10 ⁵	3.2 × 10 ⁵	2.3	330
2	23	1	0.3	SDS	35	US	1206	1.2 × 10 ⁵	3.3 × 10 ⁵	2.7	220
3	36	1	0.3	SDS	17	US	1135	8.5 × 10 ⁴	2.6 × 10 ⁵	3.1	260
4	29	1	0.3	SDS	5	US	1230	1.0 × 10 ⁵	4.5 × 10 ⁵	4.5	(485) ^[f]
5	36	1	0.3	Trit	11	US	959	8.9 × 10 ⁴	1.9 × 10 ⁵	2.1	100
6	32	1	0.3	K30	18	US	990	9.0 × 10 ⁴	2.5 × 10 ⁵	2.8	240
7	63	2	1	SDS	17	HP	165	9.5 × 10 ⁴	2.1 × 10 ⁵	2.2	135
8	32	1	0.3	SDS	17	HP	75	2.0 × 10 ⁴	3.4 × 10 ⁴	1.7	90
9	44	4	1	Lut	5	HP	265	1.6 × 10 ⁴	2.4 × 10 ⁴	1.5	250

[a] 30 °C, 45 bar constant ethylene pressure, 2 h reaction time. Total volume of water and organic solvents: 100 mL. [b] SDS = sodium dodecylsulfate, Lut = Lutensol AT80 (C₁₆–C₁₈ fatty alcohol–ethoxylate with an average of 80 ethylene oxide units; BASF), Trit = Triton X-100 (*iso*-octylphenololigoethoxylate), K30: Emulgator K30/40 (Bayer, sodium alkylsulfonates). [c] US = ultrasound, HP = high-pressure homogenizer (for details see Experimental Section). [d] Mol of ethylene per mol of Ni. [e] Average size of latex particles, determined by light scattering. [f] Latex unstable, partial coagulation occurred.

(Table 1). Analysis of the isolated polymer by gel-permeation chromatography (GPC) reveals these dispersions to contain only high molecular mass polyethylene with *M_w* values of up to 4 × 10⁵ g mol⁻¹ and narrow molecular mass distributions. The average latex particle sizes are in the range of 90 to 350 nm, as observed by light scattering measurements.^[12] It should be noted that the polymerization reaction of ethylene reported here differs from typical free-radical polymerization in a preformed mini-emulsion of a liquid monomer. Gaseous ethylene monomer is fed continuously into the reaction mixture after mini-emulsification of the catalyst solution. Thus, polymerization of preformed mini-emulsion droplets of monomer to particles of the same size, as the typical feature of “mini-emulsion polymerization”, does not apply.

The polymer latexes obtained are stable for weeks or longer. As a surfactant, various anionic and nonionic compounds were found to be suited, that is, compatible with the catalyst in the mini-emulsion system employed and capable of forming stable latexes (Table 1). As expected, a decrease in the surfactant concentration under otherwise identical conditions resulted in an increase in the average size of the latex particles (entries 2–4). The lower activities observed on employing the high-pressure homogenizer (entries 7–9) relative to ultrasonification are probably related to deactivation of the catalyst precursor, as the former method required more time for mini-emulsification.

The latex particles were visualized by transmission-electron microscopy (TEM; Figure 1). In comparison to smooth, spherical latex particles of amorphous polystyrene, as a well-studied hydrocarbon polymer prepared by free-radical emulsion polymerization, the ruggedness of the particles shown can be rationalized by their high degree of crystallinity (39 to 51 % by differential scanning calorimetry (DSC); *T_m* = 120–130 °C).^[13]

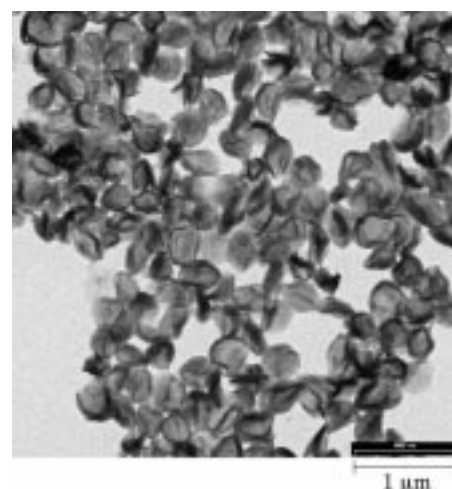


Figure 1. TEM micrograph of latex particles.

measurements on the dispersions were performed on a Malvern particle sizer after dilution. TEM investigations were carried out on a LEO 912 Omega apparatus using an acceleration voltage of 120 kV. The DSC data reported were recorded in the second heating cycle at 10 K min⁻¹.

Polymerization: The catalyst precursor was dissolved in the given amount of toluene and hexadecane (Table 1). The surfactant and about 100 mL of water were added with mechanical stirring. Homogenization was performed under an argon atmosphere by means of a high-pressure homogenizer directly connected to the polymerization reactor (modified version of the EmulsiFlex®-C5 apparatus from Avestin Inc.; 500–1000 bar) or by ultrasound (Bandelin HD2200 with KE76 tip; 2 min at 120 W). The resulting mini-emulsion was transferred to a mechanically stirred 250-mL pressure reactor equipped with a heating/cooling jacket and a constant ethylene pressure (45 bar) was applied. The reaction temperature was controlled (30 °C) by means of a thermocouple dipping into the reaction mixture. After the specified reaction time the ethylene was vented. The resulting latex was filtered through glass wool. For the determination of yields and for further polymer analysis a specified portion was precipitated by pouring into methanol.

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Experimental Section

General: Complex **2a** was prepared as described previously.^[4b] The compound was manipulated by standard Schlenk techniques under argon. Deionized water was degassed prior to use. High-temperature GPC was performed in 1,2,4-trichlorobenzene at 140 °C using a PL-220 instrument equipped with mixed-bed PL columns. The data reported was referenced to polyethylene standards (universal calibration). Dynamic light scattering

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Formal Enantioselective Michael Addition with Umpolung of Reactivity**

Eike Hupe and Paul Knochel*

Dedicated to Professor Dieter Seebach

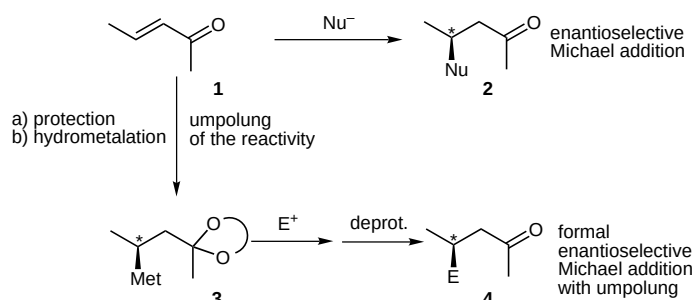
The conjugate addition of nucleophiles to unsaturated carbonyl derivatives is an important reaction,^[1] and its enantioselective variant has received much attention over the last few years.^[2] Several general methods such as copper-catalyzed additions of organolithium or organomagnesium derivatives,^[3] or the addition of diorganozinc compounds^[4] have been described in detail. Recently, a rhodium-catalyzed addition of boronic acids has also been reported.^[5] Although satisfactory results have been obtained in many cases, several classes of organometallic compounds, such as alkynyl,^[6] allylic, and allenyl organometallic compounds still cannot be added enantioselectively. Herein, we report a new synthetic approach to conjugate additions in which an umpolung^[7] of the Michael acceptor reactivity occurs. Whereas the usual reactivity of an enone of type **1** (a³-reactivity)^[7] requires a reaction with a nucleophile (Nu[−]) and leads to the addition product of type **2** (Scheme 1), we envisioned an umpolung of the enone reactivity by successive protection of the carbonyl function and formal hydrometalation, leading to the chiral organometallic species **3**. After the reaction of **3** with an electrophile (E⁺) and deprotection, products of type **4** would be produced. If an asymmetric hydrometalation and a stereoselective reaction with the electrophile (E⁺) is possible, then this would correspond, after deprotection, to a formal enantioselective Michael addition with umpolung of the normal reactivity pattern (Scheme 1).

Recently, we developed a reaction sequence involving an asymmetric hydroboration followed by a boron–zinc ex-

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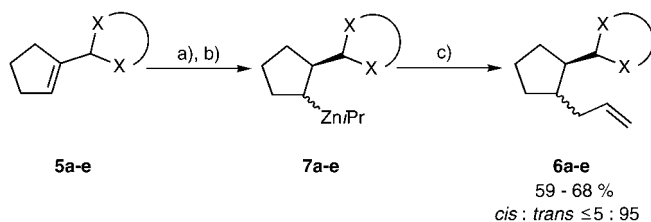
[**] This work was supported by the DFG (Leibniz-Program) and the Fonds der Chemischen Industrie (Kekulé-scholarship to E.H.). We thank D. Renard for preliminary experiments, as well as Chemetall GmbH (Frankfurt), the BASF AG, and the Degussa-Hüls AG (Hanau) for generous gifts of chemicals.

 Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.



Scheme 1. Formal Michael addition with umpolung of reactivity.

change reaction, which allows a general preparation of secondary, optically active organozinc reagents.^[8a,b] Moreover, we showed that their reaction with various electrophiles proceeds with retention of configuration.^[8c] Preliminary experiments on protected enone systems revealed that the nature of the protecting group strongly influences the configurational stability of the resulting diorganozinc species, especially in the case of five-membered systems. Thus, the hydroboration of the 1,3-dioxolane **5a** with Et_2BH followed by treatment with $i\text{Pr}_2\text{Zn}$, $\text{CuCN} \cdot 2\text{LiCl}$, and allylic bromide provides the desired allylated product **6a** in 68% yield (Scheme 2 and Table 1). However, a 47:53 mixture of *cis* and



Scheme 2. Optimization of configurational stability of the zinc intermediate **7a–e** by varying the protecting group. a) Et_2BH (3 equiv, 50 °C, 16 h); b) $i\text{Pr}_2\text{Zn}$ (3 equiv, 25 °C, 5 h); c) $\text{CuCN} \cdot 2\text{LiCl}$ (1 equiv, –78 °C, 0.5 h), then allylic bromide (3 equiv, –78 °C to 25 °C, 10 h).

trans isomers was obtained, showing that an epimerization of the intermediate organozinc reagent **7a** had occurred (Scheme 2 and Table 1). A systematic study revealed that this result can be greatly improved by careful choice of the protecting group. The 1,3-dioxane acetal **5d** gives an improved selectivity, but the best result is obtained with the 1,3-dithiane **5e**, leading to a *cis:trans* selectivity of 5:95.

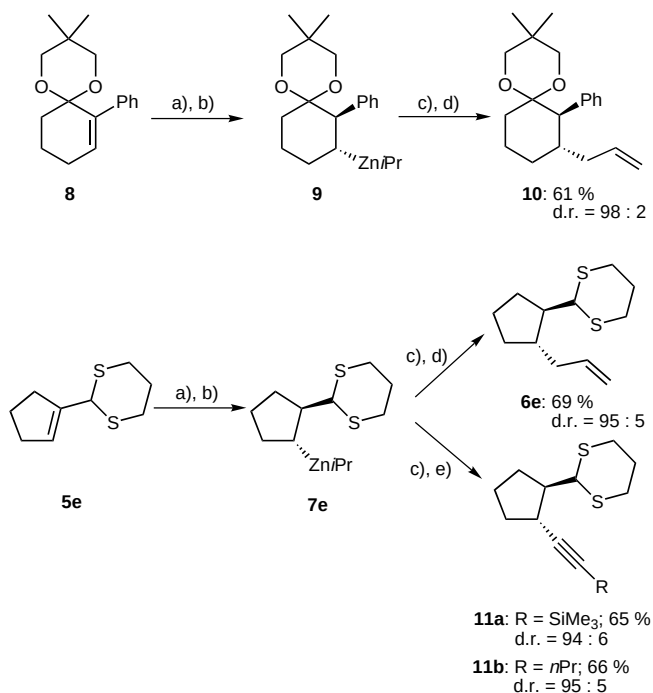
By applying the results of this study to the protected cyclohexenone **8** and to the 1,3-dithiane **5e**, excellent diastereoselectivities (*trans:cis* ratio $\geq 94:6$) can be achieved (Scheme 3). Thus, the allylation of **9**, obtained by the usual sequence from **8**, proceeds with a diastereoselectivity of 98:2 (*trans:cis* ratio) and affords product **10** in 61% yield. Similarly, the alkynylation of the unsaturated dithiane **5e** furnishes the alkynylated product **11b** in 66% yield (d.r. = 95:5).

Our method can also be readily performed with enantio-merically enriched organozinc reagents. Thus, the asymmetric hydroboration of **12** with (–)-isopinocampheylborane ((–)- IpcBH_2)^[9] produces the *trans*-configured, secondary organo-

Table 1. Hydroboration of the unsaturated acetals and thioacetals **5a–e**, followed by boron–zinc exchange and copper-mediated allylation, leading to products **6a–e**.

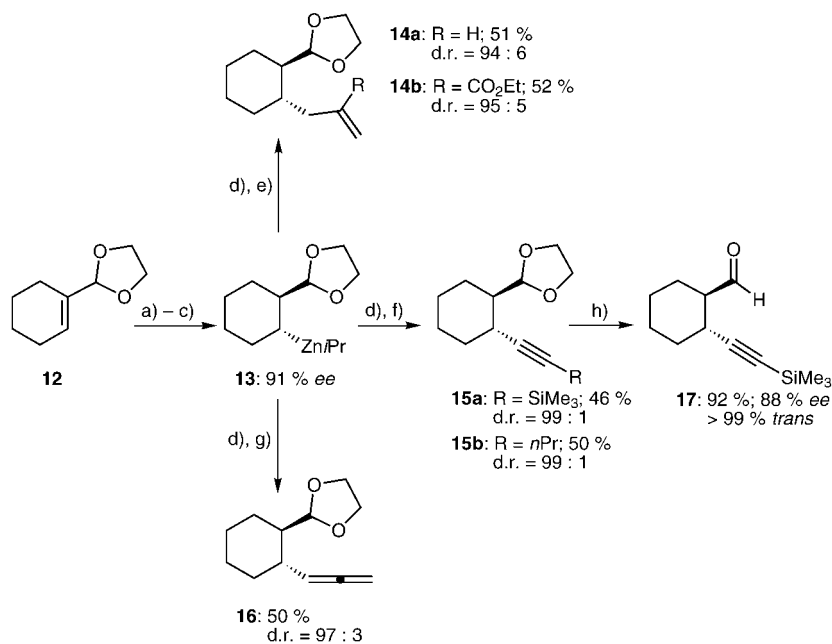
Starting material 5	Product 6	<i>trans:cis</i>	Yield [%] ^[a]
		53:47	68
		60:40	65
		72:28	59
		80:20	62
		95:5	69

[a] Yield of isolated, analytically pure compound.



Scheme 3. Diastereoselective reactions of the zinc reagents **9** and **7e** obtained by sequential hydroboration and boron–zinc exchange. a) Et_2BH (3 equiv, 50 °C, 16 h); b) $i\text{Pr}_2\text{Zn}$ (3 equiv, 25 °C, 5 h); c) $\text{CuCN} \cdot 2\text{LiCl}$ (1 equiv, –78 °C, 0.5 h); d) allylic bromide (3 equiv, –78 °C to 25 °C, 10 h); e) alkynyl bromide (3 equiv, –40 °C, 20 h).

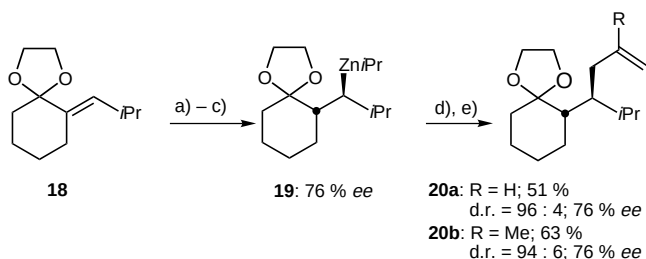
zinc reagent **13** in 91% *ee* after treatment with Et_2BH ^[10] and $i\text{Pr}_2\text{Zn}$ (Scheme 4).^[11] This organozinc compound reacts with allylic, alkynyl, and propargyl bromides, affording the desired



Scheme 4. Enantioselective conjugate functionalization of the unsaturated acetal **12** by an asymmetric hydroboration and boron–zinc exchange sequence. a) (–)-IpcBH₂ (1.1 equiv, –25 °C, 48 h); b) Et₂BH (5 equiv, 50 °C, 16 h); c) *i*Pr₂Zn (5 equiv, 25 °C, 5 h); d) CuCN·2LiCl (1.5 equiv, –78 °C, 30 min); e) allylic bromide (5 equiv, –78 °C to 25 °C, 10 h for **14a** or 5 equiv, –40 °C, 48 h for **14b**); f) alkynyl bromide (5 equiv, –40 °C, 16 h); g) propargyl bromide (5 equiv, –40 °C, 16 h); h) 5 % HCl, 35 °C, 16 h.

allylated products **14a, b**, the alkynylated derivatives **15a, b** and the allenylated compound **16** in satisfactory overall yields (46–52 %).^[12] To demonstrate the validity of the umpolung procedure, we have deprotected the product **15a** by treatment with dilute HCl to obtain the desired *trans*-aldehyde **17** in 92 % yield and 88 % ee (42 % overall yield starting from the unsaturated acetal **12**; Scheme 4).

Finally, we have extended our method to an exocyclic system. Thus, the protected *exo*-alkylidene enone **18** was hydroborated with (–)-IpcBH₂ affording, after further treatment with Et₂BH and *i*Pr₂Zn, the chiral secondary alkylzinc reagent **19** in 76 % ee (Scheme 5).^[11] Following transmetalation with CuCN·2LiCl and allylation, the desired products **20a, b** were obtained with excellent diastereoselectivities (≥94:6), thus allowing the stereocontrol of two contiguous chiral centers.^[13]



Scheme 5. Enantioselective conjugate functionalization of the unsaturated acetal **18** by an asymmetric hydroboration and boron–zinc exchange sequence. a) (–)-IpcBH₂ (1.1 equiv, –25 °C, 48 h); b) Et₂BH (5 equiv, 50 °C, 16 h); c) *i*Pr₂Zn (5 equiv, 25 °C, 5 h); d) CuCN·2LiCl (1.5 equiv, –78 °C, 30 min); e) allylic bromide (5 equiv, –78 °C to 25 °C, 10 h for **20a**, or 5 equiv, –40 °C, 48 h for **20b**).

In summary, we have shown that unsaturated acetals and ketals can be stereoselectively hydroborated and converted by a boron–zinc exchange to a secondary alkylzinc reagent with high retention of configuration. These chiral, functionalized diorganozinc reagents react with retention of configuration with allylic bromides, alkynyl halides, and propargyl bromide, affording the products of formal Michael addition. As such, this new method fully complements the existing, traditional conjugate addition protocols, which often encounter problems in the enantioselective conjugate addition of allylic, alkenyl, and allenyl organometallic species.

Experimental Section

Preparation of 15a: A flame-dried 25 mL flask equipped with a magnetic stirring bar, an argon inlet, and a septum was cooled to –25 °C and charged with freshly prepared (–)-IpcBH₂ (1.1 mL, 1.1 mmol, 1.1 equiv, 1 M solution in THF). The protected olefin **12** (154 mg, 1.0 mmol, 1.0 equiv, 1 M in THF) was added dropwise over a period of 1 h. Stirring at this temperature was continued for 48 h. After pumping off the volatiles (0.1 Torr, 25 °C, 2 h), Et₂BH (0.69 mL, 5.0 mmol, 5 equiv, 7.3 M in Me₂S) was added and the resulting mixture was stirred for 16 h at 50 °C. After pumping off the volatiles (0.1 Torr, 25 °C, 2 h), *i*Pr₂Zn (1.0 mL, 5.0 mmol, 5 equiv, 5.0 M in Et₂O) was added and the mixture was stirred 5 h at 25 °C. According to GC analysis of an oxidized aliquot (3 M NaOH/30 % H₂O₂) the boron–zinc conversion was >85 %. The volatiles were pumped off (0.1 Torr, 25 °C, 0.5 h), and the gray-black residue was diluted with THF (3 mL) and cooled to –78 °C. A freshly prepared solution of CuCN·2LiCl (1.5 mL, 1.5 mmol, 1.5 equiv, 1 M in THF) was slowly added over 40 min by syringe pump and the mixture stirred for 30 min at –78 °C. Then, a solution of 1-bromo-2-trimethylsilylacetylene (885 mg, 5 mmol, 5 equiv) in THF (1 mL) was added slowly (40 min) by syringe pump. After stirring for 30 min at –78 °C, the mixture was allowed to warm to –40 °C and stirred at this temperature for 16 h. The reaction mixture was then poured into a saturated aqueous NH₄Cl solution (150 mL) containing NH_{3(aq)} (2 mL, 30 % in H₂O). After extraction with Et₂O (3 × 100 mL) the combined organic phases were dried over MgSO₄. The solvent was removed and the crude product purified by column chromatography (SiO₂, hexanes/Et₂O 19:1) affording **15a** as a colorless oil (116 mg, 0.46 mmol, 46 %).

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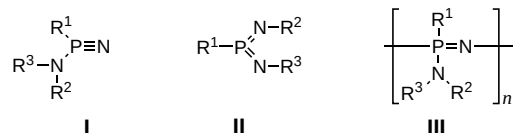
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- [12] The absolute configuration of **14**–**17** was determined by deprotection and reduction of the corresponding *trans*-alcohol, obtained after asymmetric hydroboration and oxidative workup, to *trans*-2-(hydroxymethyl)cyclohexanol. The $[\alpha]_D^{20}$ values were compared with those reported in the literature. See: L. J. Dolby, F. A. Meneghini, T. Koizumi, *J. Org. Chem.* **1968**, 33, 3060.
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Reversible Tautomeric Transformation between a Bis(amino)cyclodiphosph(v)azene and a Bis(imino)cyclodiphosph(v)azane**

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Dedicated to Professor Rolf Appel on the occasion of his 80th birthday

Amino-substituted phosphinonitrenes (**I**) with suitable substitution patterns can be rearranged to tautomeric bis(imino)phosphoranes (**II**).^[1] In the absence of the possibility

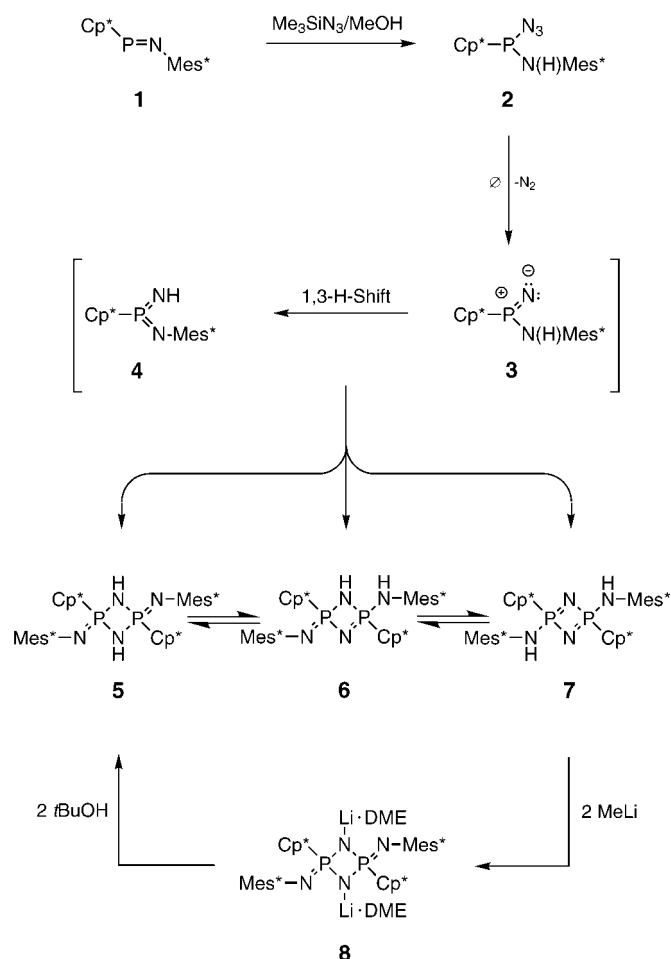


to rearrange, oligo- and polyphosphazenes (**III**) are formed.^[2] Whereas in general the formation of tri- and tetramers appears to be thermodynamically favored,^[2] a sterically overcrowded bisamino-substituted phosphinonitrene (**I**, R¹ = NR²R³, R², R³ = *i*Pr₂N) was noted to undergo instead an unusual cyclodimerization to give a cyclodiphosph(v)azene.^[3] In connection with studies on NH-functionalized bis(imino)phosphoranes and the anions derived therefrom^[4] we also became interested in [2+2] cycloadditions of these species which yielded as yet unknown bis(imino)diphosph(v)azanes. Herein, we report on the synthesis of a cyclodiphosph(v)azane as well as on its reversible conversion into a tautomeric cyclodiphosph(v)azene.

Treatment of the azidophosphane **2**,^[6] which is accessible from reaction of pentamethylcyclopentadienyl-(2,4,6-*tert*-butylphenyl)iminophosphane, Cp*P=NMe₃ (**1**),^[5] with hydrogen azide under dry pyrolysis (pure substance, 130 °C) conditions produced a mixture of the tautomeric four-membered-ring P–N heterocycles **5** and **7** in 1:9 ratio (Scheme 1). Colorless crystals of the bis(amino)cyclodiphosph(v)azene **7** were isolated by crystallization of the mixture from a little toluene.^[7] Probable intermediates during the elimination of nitrogen from **2** are the phosphinonitrene **3** and the bis(imino)phosphorane **4**, which is formed from **3** by subsequent 1,3-H shift.^[8] Metalation of **7** (MeLi, 0 °C) to the dilithiated complex **8**^[9] and subsequent protonation with *t*BuOH gave a quantitative yield of the tautomeric bis(imino)cyclodiphosph(v)azane **5**, which was likewise isolated in crystalline form.^[10]

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Scheme 1. Synthesis of the cyclodiphosph(v)azane **5** and cyclodiphosph(v)azene **7**. Cp* = C₅Me₅, Mes* = *t*Bu₃C₆H₂, DME = 1,2-dimethoxyethane.

The molecular structures^[11] of the cyclodiphosph(v)azane **5** and the tautomeric cyclodiphosph(v)azene **7** are shown in Figures 1 and 2, respectively. A common feature of both molecules is the planar P₂N₂ ring, which both display C_i symmetry and exhibit a rhombic distortion; the latter is more pronounced in **5** (bond angles: **5**: N-P-N 85.0(1), P-N-P 95.0(1)°; **7**: N-P-N 91.5(1), P-N-P 88.5(1)°). The arene moieties in the exocyclic amino (**7**) and imino groups (**5**) are found in *endo*, and the pentamethylcyclopentadienyl substituents in *exo* positions with respect to the central ring; regardless of the protonation, the P-N-C angles remain essentially unchanged (**7**: 130.99(12)°, **5**: 133.0(2)°). Thus, all four substituents adopt a “paddle-wheel” conformation which can be interpreted to arise as a consequence of the minimization of steric interactions. As expected, **5** displays substantial differences between endocyclic (P1–N1 167.2(3), P1–N1a 166.7(3) pm) and exocyclic P–N distances (P1–N2 156.5(2) pm), which lie in the expected range for compounds with the same molecular skeleton.^[13] In contrast, the tautomeric cyclodiphosph(v)azene **7** exhibits an equalization of all P–N distances (P1–N1 165.87(15), P1–N1a 165.94(15), P1–N2 162.56(14) pm). A similar finding was noted for the cyclodiphosph(v)azene [(iPr₂N)₂PN]₂^[3] and is in agreement with the results of quantum-chemical computations which suggest that the bonding situation in cyclodiphosph(v)azenes

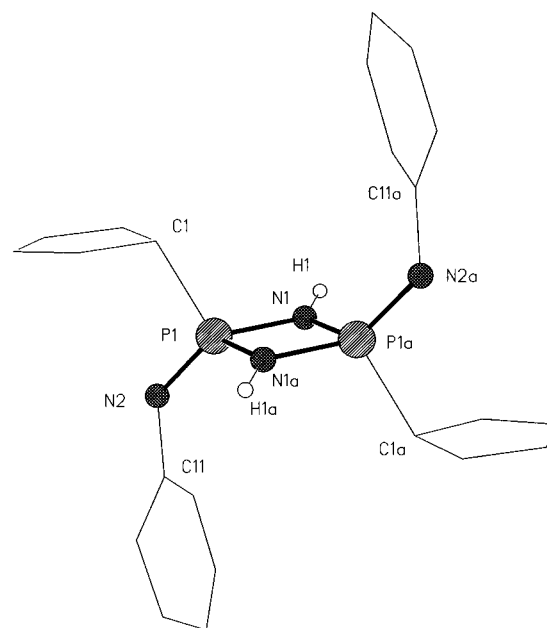


Figure 1. Molecular structure of **5** in the crystal (without hydrogen atoms (apart from H1 and H1a) and peripheral groups). Selected bond lengths [pm] and angles [°]: P1–N1 167.2(3), P1–N2 156.5(2), P1–N1a 166.7(3), P1–C1 185.4(3), N1–H1 85(2), N2–C11 142.6(3); N2–P1–N1 122.04(13), N2–P1–N1a 121.54(13), N1–P1–N1a 84.98(15), N2–P1–C1 105.66(13), N1–P1–C1 109.99(13), N1a–P1–C1 111.54(14), P1–N1–P1a 95.02(15), P1–N1–H1 133(2), C11–N2–P1 133.0(2), P1a–N1–H1 128(2).

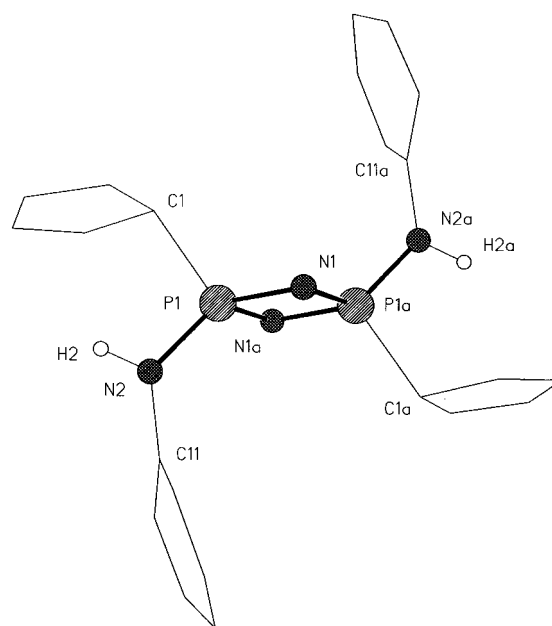


Figure 2. Molecular structure of **7** in the crystal (without hydrogen atoms (apart from H2 and H2a) and peripheral groups). Selected bond lengths [pm] and angles [°]: P1–N1 165.87(15), P1–N2 162.56(14), P1–N1a 165.94(15), P1–C1 186.27 (17), N2–H2 87(2), N2–C11 143.8(2); N2–P1–N1 119.09(8), N2–P1–N1a 119.34(8), N1–P1–N1a 91.52 (8), N2–P1–C1 102.20(7), N1–P1–C1 113.02(8), N1a–P1–C1 112.16(8), P1–N1–P1a 88.48(8), C11–N2–P1 130.99(12), P1–N2–H2 110(1), C11–N2–H2 119(1).

can be described in terms of a quadrupolar resonance structure.^[14]

The reversible interconversion between **5** and **7** in solution was confirmed by temperature-dependent ¹H and ³¹P NMR

studies. The results of these experiments showed that both tautomers exist in dynamic equilibrium with a further species which was formulated on the basis of the available NMR data as the 1,2-dihydro-1,3,2λ⁵,4λ⁵-diazadiphosphete **6** (Scheme 1).^[15] The adjustment of the position of the equilibrium is concentration independent and requires several hours at ambient temperature or a few minutes at 80–120 °C; all attempts to speed up the reaction by addition of acids (imidazole, tertiary ammonium salts) or bases (4-dimethylaminopyridine) were unsuccessful. Direct proof for the reversible interconversion of **6** and **7** was obtained from appropriate correlation signals in 2D-³¹P{¹H}EXSY-NMR spectra recorded at 120 °C. The absence of cross peaks connecting the resonance signals of **7** or **6**, respectively, with those of the cyclodiphosph(v)azane **5** suggested this reaction step to occur more slowly; nonetheless, the interconversion was unequivocally proven by the reversible changes in signal intensities following a temperature change. Evaluation of the temperature dependence of the overall equilibrium constant $K = c(\mathbf{5})/c(\mathbf{7})$, which could be computed from the observed intensities of the signals of **5** and **7** at different temperatures, yielded values of $\Delta H^0 = -13.4(9) \text{ kJ mol}^{-1}$ and $\Delta S^0 = 26.0(25) \text{ J mol}^{-1} \text{ K}^{-1}$ for the tautomeric equilibrium $\mathbf{5} \rightleftharpoons \mathbf{7}$.

Taking into account all results obtained from the NMR investigations, one may conclude that the tautomerization of the cyclodiphosph(v)azene **7** can be mechanistically described in terms of an intramolecular reaction and proceeds through two subsequent 1,3-hydrogen shifts, for which the second step (transformation $\mathbf{6} \rightarrow \mathbf{5}$) is rate-determining. The cyclodiphosph(v)azene **7** is both enthalpically and entropically favored with respect to the cyclodiphosph(v)azane **5**. In the light of these findings, the formation of the thermodynamically less favorable tautomer **5** during the protonation of the dilithiated salt **8** is considered to be the result of a kinetically controlled reaction.

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- [6] **Caution:** Hydrogen azide must be handled exclusively in ethereal solution. For the generation of such solutions, azidotrimethylsilane (0.96 g, 8.4 mmol) was dissolved in anhydrous THF (4 mL), and ethanol (0.39 g, 8.4 mmol) was added with stirring. The solution is immediately ready for use. **2:** Compound **1** (3.1 g, 7.29 mmol) was dissolved in THF (10 mL), and a stoichiometric amount of a solution of hydrogen azide (prepared as described above) was added. Completion of the reaction was indicated by a change of color to yellow. Volatile components were removed by evaporation under vacuum, and **2** was isolated after crystallization of the residue at -30 °C from *n*-hexane. Yield: 1.61 g (47.2 %). ³¹P{¹H} NMR (121.5 MHz, C₆D₆, 30 °C, H₃PO₄): $\delta = 126.2$ (s); ¹H NMR (300 MHz, C₆D₆, 30 °C, TMS): $\delta = 1.36$ (dd, ³J(P,H) = 14.6, ⁵J(H,H) = 0.9 Hz, 3H; PCCCH₃), 1.42 (s, 9H; *p*-*t*Bu), 1.61 (s, 18H; *o*-*t*Bu), 1.87 (s, 3H; PCCCH₃), 2.04 (s, 3H; PCCCH₃), 2.14 (s, 3H; PCCCH₃), 4.91 (d, ²J(P,H) = 10.5 Hz, 1H; NH), 7.54 (s, 2H, Aryl-H).

- [7] **Caution:** Thermolysis of the azidophosphane **2** was carried out with special precautions in a fume cupboard using a plexiglass safety shield. **5:** Azidophosphane **2** (1.6 g, 3.4 mmol) was heated in vacuum to 130 °C (oil bath temperature), and this temperature was maintained for about 15 min. After the mixture had been cooled to ambient temperature, the residue was dissolved in a small amount of toluene. Compound **5** crystallized over a period of several days. Yield: 0.4 g (26.5 %). ³¹P{¹H} NMR (121.5 MHz, CDCl₃, 30 °C, H₃PO₄): $\delta = 49.1$ (s); ¹H NMR (300 MHz, CDCl₃, 30 °C, TMS): $\delta = 0.16$ (m, ³J(P,H) + ⁵J(P,H) = 20.6 Hz, 3H; PCCCH₃), 1.21 (s, 9H; *p*-*t*Bu), 1.43 (brs, 18H; *o*-*t*Bu), 1.69 (brs, 6H; Cp*-CH₃), 1.88 (s, 6H; Cp*-CH₃), 3.72 (m, ²J(P,H) + ⁴J(P,H) = 13.9 Hz, 2H; NH); 7.21 (s, 1H; Aryl-H); MS (16 eV): *m/z* (%): 880 (8) [M^+], 689 (5) [$M^+ - t\text{Bu} - \text{Cp}^*$], 135 (100) [Cp^{*+}].
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- [9] The existence of **7** was proven on the grounds of NMR spectroscopic evidence and a crystal structure analysis of a cyclodiphosph(v)azane with a similar substitution pattern: J. Tirré, M. Nieger, A. V. Ruban, E. Niecke, unpublished results.
- [10] To a cooled (0 °C) suspension of the cyclodiphosph(v)azene **7** (250 mg, 0.28 mmol) in DME (2 mL) was added a solution of methylolithium (0.57 mmol, 0.35 mL of a 1.6 M solution in hexane). The formed lithium salt was quenched after 12 h by addition of *tert*-butyl alcohol (0.05 g in 2 mL DME), and the product **5** was crystallized from DME. Yield: 235 mg (94 %). ³¹P{¹H} NMR (121.5 MHz, CDCl₃, 30 °C, H₃PO₄): $\delta = -15.3$ (s); ¹H NMR (300 MHz, CDCl₃, 30 °C, TMS): $\delta = 0.38$ (m, ³J(P,H) + ⁵J(P,H) = 25.5 Hz, 3H; PCCCH₃), 1.18 (s, 9H; *p*-*t*Bu), 1.31 (brs, 18H; *o*-*t*Bu), 1.53 (brs, 6H; Cp*-CH₃), 1.79 (s, 6H; Cp*-CH₃), 3.98 (t, ²J(P,H) = 7.6 Hz, 2H; NH), 7.00 (d, ⁵J(P,H) = 2.6 Hz, 2H; Aryl-H); MS (16 eV): *m/z* (%): 880 (8) [M^+], 747 (2) [$M^+ - \text{Cp}^*$], 486 (25) [$M^+ - \text{Mes}^* - \text{Cp}^*$], 135 (100) [Cp^{*+}]. The adjustment of the equilibrium proceeds over several days at 25 °C in DME.
- [11] Crystal data of **5**: C₅₆H₉₀N₄P₂, colorless crystals, crystal dimensions 0.10 × 0.05 × 0.05 mm; $M_r = 881.26$; triclinic, space group $P\bar{1}$ (no. 2), $a = 9.8354(11)$, $b = 12.2683(11)$, $c = 12.9324(14)$ Å, $\alpha = 64.466(6)$, $\beta = 83.540(5)$, $\gamma = 73.735(6)^\circ$, $V = 1351.6(2)$ Å³, $Z = 1$, $\mu(\text{MoK}\alpha) = 0.12 \text{ mm}^{-1}$, $T = 123(2) \text{ K}$, $F(000) = 484$. Of 10923 reflections which were collected on a Nonius Kappa-CCD diffractometer using MoK α radiation up to $2\theta_{\text{max}} = 50^\circ$, 4734 were independent and used in all further calculations. The structure was solved by direct methods and refined anisotropically against F^2 ; hydrogen atoms were refined by using a riding model (programs SHELXS-97^[12a] and SHELXL-97^[12b]). The final $wR2(F^2)$ was 0.162 and the conventional $R(F) = 0.068$ for 288 parameters and 1 restraint. Crystal data of **7**: C₅₆H₉₀N₄P₂, colorless crystals, crystal dimensions 0.35 × 0.35 × 0.30 mm; $M_r = 881.26$; triclinic, space group $P\bar{1}$ (no. 2), $a = 10.0227(2)$, $b = 12.3369(3)$, $c = 12.8896(3)$ Å, $\alpha = 63.980(2)$, $\beta = 83.314(2)$, $\gamma = 72.876(2)^\circ$, $V = 1368.60(5)$ Å³, $Z = 1$, $\mu(\text{MoK}\alpha) = 0.12 \text{ mm}^{-1}$, $T = 173(2) \text{ K}$, $F(000) = 484$. Of 21195 reflections which were collected on a Nonius Kappa-CCD diffractometer using MoK α radiation up to $2\theta_{\text{max}} = 56.5^\circ$, 5034 were independent and used in all further calculations. The structure was solved by direct methods and refined anisotropically against F^2 ; hydrogen atoms were refined by using a riding model (programs SHELXS-97^[12a] and SHELXL-97^[12b]). The final $wR2(F^2)$ was 0.123 and the conventional $R(F) = 0.045$ for 326 parameters with 142 restraints. The *p*-*tert*-butyl group in one of the Mes* substituents is disordered. 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-160512 (**5**) and CCDC-160511 (**7**). 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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- [15] Compound **6**: $^{31}\text{P}\{^1\text{H}\}$ NMR (121.5 MHz, $[\text{D}_8]\text{xylene}$, 50 °C, H_3PO_4): $\delta = 36.6$ (d, $^2J(\text{P,P}) = 25.4$ Hz), 0.6 (d, $^2J(\text{P,P}) = 25.4$ Hz). ^1H NMR (300 MHz, $[\text{D}_8]\text{xylene}$, 50 °C, TMS): $\delta = 0.71$ (d, $^3J(\text{P,H}) = 21$ Hz, 3H; PCCH_3), 0.73 (d, $^3J(\text{P,H}) = 23$ Hz, 3H; PCCH_3), 4.12 (d, $^2J(\text{P,H}) = 15.4$ Hz, 1H; NH), 4.50 (br, 2H; NH), 7.18 (d, $^4J(\text{H,H}) = 2.3$ Hz, 1H; Aryl-H), 7.29 (d, $^4J(\text{H,H}) = 2.0$ Hz, 1H; Aryl-H), 7.32 (d, $^4J(\text{H,H}) = 2.0$ Hz, 1H; Aryl-H), 7.44 (d, $^4J(\text{H,H}) = 2.3$ Hz, 1H; Aryl-H). Signal assignments were aided by 2D $^1\text{H}\{^{31}\text{P}\}$ -HMQC spectra; the remaining resonance signals could not be unequivocally assigned.
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Parallel Analysis of the Reaction Products from Combinatorial Catalyst Libraries**

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The combinatorial approach has great potential in many disciplines to optimize systems that have large parameter spaces. Recently, this concept has been introduced to the field of materials science.^[1] The ultimate goal of the combinatorial approach is to efficiently optimize and discover new formulations, be they pharmaceutical products, catalysts, or other materials. Practically, this is accomplished by a systematic and efficient exploration of the parameter space that controls the properties of the final product. The two key components to a successful combinatorial approach are the controlled synthesis of a collection of materials with systematic variations in properties and the subsequent high-throughput analysis of libraries of these materials. Speed, through parallel synthesis and characterization, consequently becomes critical for the success of the combinatorial discovery process. Herein, we report the first analytical technique for truly parallel high-throughput screening of the reaction products from libraries of heterogeneous supported catalysts.

A number of experimental approaches have been reported for screening catalyst libraries. These are based on conventional serial techniques, which have been automated to decrease the screening time. Scanning mass spectrometry is based on rapidly analyzing the gases from one sample in a combinatorial library at a time, in a sequential manner. One approach uses a single probe composed of coaxial gas delivery and gas analysis tubes.^[2] Libraries are analyzed by sequentially placing the tube over each element of the library, feeding reactant gases, and analyzing product gases. This approach is applicable to the initial screening of libraries deposited onto flat, solid substrates due to its gas delivery design. A second approach uses array microreactors with supported catalysts, coupled with capillary microprobe sam-

pling.^[3] Reactants, products, and carrier gas are withdrawn from each microreactor channel using a capillary sampling probe. By repeating this approach for each microreactor, the entire library can be screened. Another experimental method is based on photoionization of reaction products using tunable UV lasers.^[4] The resulting photoions are detected by a microelectrode in close proximity to the sample. One disadvantage of this technique is that a suitable laser frequency for each species of interest must be known and accessible. In general, all of these techniques have the shortcoming that the screening time is proportional to the library size.

In contrast, truly parallel screening techniques gather information simultaneously from all the elements in a library. This category so far only includes heat-sensing techniques. Infrared thermography and thermistor arrays detect heat evolved from active library members and have been used to detect activity for exothermic reactions in combinatorial libraries.^[5] These techniques, however, cannot chemically resolve product composition, which is often the most important issue when studying catalytic reactions, and therefore cannot determine the selectivity of a catalyst. Also, the assumption is made that the exothermicity is derived solely from the desired reaction, and not from any unforeseen side reactions, limiting these techniques to the study of well-known, simple reactions.

FT-IR imaging has the ability to gather chemically sensitive information from all library elements simultaneously. This approach has been demonstrated in recent work, where our group has pioneered infrared spectral imaging for the rapid analysis of reactions on solid bead materials.^[6] IR spectroscopy is a well-established tool for the analysis of the composition of gas mixtures. The lower detection limit depends on several factors, such as the absorptivity of the absorption bands of the specific gas, the path length of the cell, the concentration of the species of interest, the spectral noise level, and the strength and structure of the bands of interfering gases. The true power of FTIR imaging for high-throughput analysis lies in its capability for parallel examination of product streams from multiple reactors. For this purpose, we have developed a novel gas-phase array attached to a multiple sample reactor, which currently allows us to perform parallel screening of the product stream of 16 supported catalyst samples.

To demonstrate the applicability of gas-phase IR imaging to parallel reaction product analysis, we present results, in which the parallel FTIR analysis was used to determine conversion during temperature-programmed complete oxidation of propene. This reaction is important for the automotive three-way catalyst and, in general, hydrocarbon oxidation is mainly catalyzed by platinum group metals.^[7]

The samples examined in this study were commercial catalyst monoliths as well as custom-synthesized supported catalyst powders. Additionally, some channels of the reactor were filled with blank support material. After approximately 0.2 g of each catalyst sample was loaded into the 16-catalyst parallel reactor; all samples were pretreated simultaneously by alternate oxidation and reduction cycles. After the reactant gases were introduced, a temperature ramp of 10 K min⁻¹ was

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established, and absorbance images of the exit stream of all 16 samples were taken every 30 s over a temperature range from 300 K to 670 K. Figure 1 shows a representative FTIR spectrum of the exhaust stream at 300 K. The spectrum is dominated by the absorption bands of propene. This spectrum was taken from one pixel in the detector array using one scan, that is, no coaddition or averaging of spectra was performed. This demonstrates the excellent spectral quality that can be achieved with this instrumental setup.

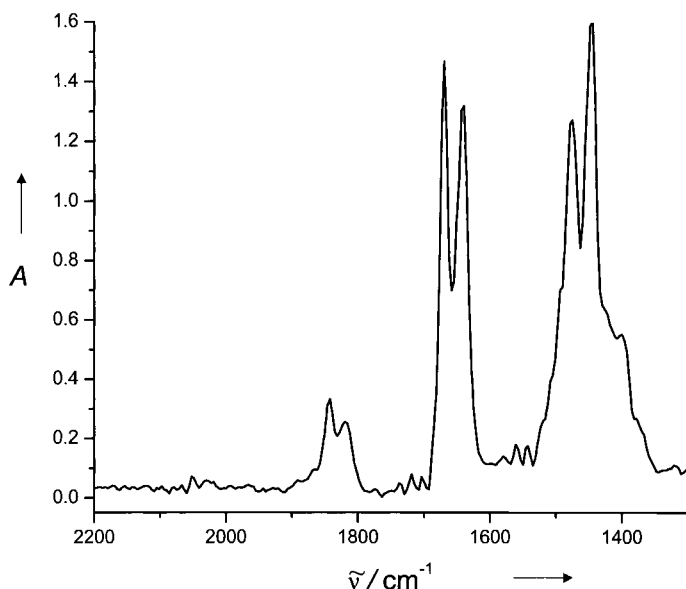


Figure 1. Representative gas-phase spectrum of propylene recorded with the FTIR imaging spectrometer equipped with the gas-phase sampling array.

Figure 2 shows absorbance images for the gas-phase CO_2 vibration band for catalyst temperatures of 440 K and 650 K. Plotting absorbance intensities for a specific reaction product for the entire array reveals the position of the active catalysts. High intensity in the image indicates an active catalyst converting propene to CO_2 . Each pixel in the image contains a full IR spectrum from 2600 to 1300 cm^{-1} . At 440 K, seven catalysts are active for total oxidation of propene, while at

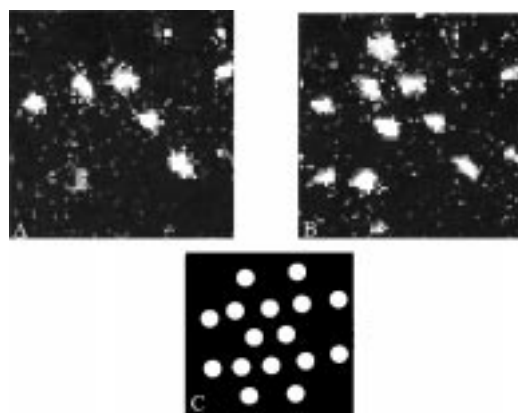


Figure 2. Spectral images of CO_2 concentration (2360 cm^{-1}) measured by FTIR imaging at 440 K (A) and at 650 K (B). Light-off between these two temperatures can be seen for several elements of the array. C) Schematic representation of the location of the individual cells in images A and B.

650 K 12 catalysts produce CO_2 . All of the inactive array elements are those containing the silica and alumina support materials, which are expected to show no reactivity. Notably, one entire data set (i.e., 4096 IR spectra with 8 cm^{-1} spectral resolution) takes less than 20 s to collect and process.

Integration of the IR bands belonging to the desired reaction products from each array element in the image allows quantitative analysis of the product stream. Data for the amount of CO_2 produced as a function of reactor temperature are shown in Figure 3 for seven selected catalysts from the

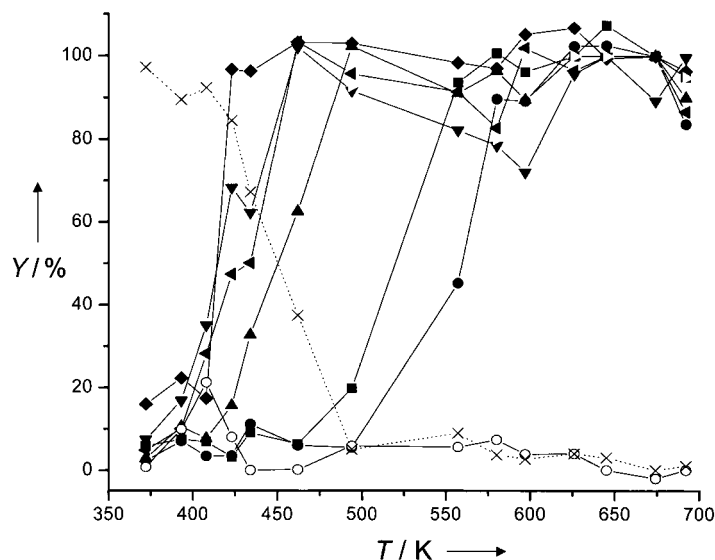


Figure 3. Results for the production of CO_2 during the oxidation of propene for four $\gamma\text{-Al}_2\text{O}_3$ catalysts with 1 wt % Pd (■, 520 K), 1 wt % Pt (●, 560 K), 5 wt % Pt (◆, 450 K), 5 wt % Ru (◇, 420–435 K), a SiO_2 catalyst with 2.67 wt % Pd (▲), pure SiO_2 support (○), and two commercial catalyst samples (◄, 420–435 K). The temperature or temperature range given in parentheses indicates the light-off temperature of the catalyst. (×) propene signal for the 5 wt % Pt on $\gamma\text{-Al}_2\text{O}_3$ catalyst. Y = conversion [%].

array. The pure support shows no measurable reactivity, as expected. The dashed line shows the decrease of propene in the reactor effluent for the 5 wt % Pt on $\gamma\text{-Al}_2\text{O}_3$ catalyst. This line follows very closely the CO_2 production and the material balance closes within an error of less than 5%. The reproducibility of the method is demonstrated by comparing two identical catalysts (commercial samples; Figure 3).

With the temporal resolution of our analytical technique, it becomes possible to monitor the effect of changing control parameters of the reaction (i.e., temperature, gas composition) on a time scale of seconds. Additionally, this allows the high-throughput evaluation of the light-off characteristics of catalysts. For example, it took only around 30 min to assess the light-off characteristics and reactivity of an array of 16 supported catalyst samples over a temperature range of 350 K.

It has been demonstrated that rapid-scan FTIR spectral imaging is the first technique that is capable of monitoring gas-phase reaction products from multiple members of a supported catalyst library in a truly parallel fashion. This experimental methodology is fully scalable, and with the coming availability of larger FPA detectors, this approach will

be capable of screening 100 s to 1000 s of supported catalyst samples on a time scale of seconds. In addition, rapid-scan FTIR imaging is a flexible technique, which can be used for analysis of many combinatorial systems, that is, the technique is independent of the specifics of the reactor setup.

Experimental Section

The optical setup consists of a Nicolet Magna 860 FTIR spectrometer, barium fluoride (BaF₂) optics, a long pass optical filter, a KBr diffuser, and a 64 × 64 pixel mercury cadmium telluride (MCT) focal plane array (FPA) detector. A more detailed description of the rapid-scan imaging setup can be found in reference [8]. The reactor has 16 individual channels that each hold one powdered catalyst sample. The individual gas outlets are connected to the gas sampling array constructed from 16 stainless steel tubes, each with a separate gas inlet and outlet. The ends of the cells are equipped with IR transparent windows sealed by gaskets. The cells are arranged in an array fashion, so that all 16 can be studied simultaneously using our FTIR imaging system. The entire path that the reactant and product gases travel through is composed of stainless steel, teflon, buna rubber, and calcium fluoride, all of which are not catalytically active at the temperatures used in the experiments. Currently, each cell has a path length of 15 cm. However, due to the fact that a parallel IR beam travels through the array, it is possible to extend the cells. This might become necessary, for example, to study gas concentrations below the current detection limit, which was determined to be around 1000 ppm for the case of propene. This may also become necessary when studying mixtures of gases with similar IR signatures, where weak vibrational bands have to be followed to distinguish various components of the gas mixture. Also, due to the inherent nature of IR vibrational spectra, this technique is not suited for the detection of homonuclear diatomic gases, such as O₂ and N₂.

The catalyst samples used were 1.33 % Pt and 2.67 % Pd co-impregnated on silica support, 1.33 % Pd and 2.67 % Pt co-impregnated on silica support, 1 % Pt on γ -Al₂O₃, and 1 % Pd on γ -Al₂O₃ (all synthesized in house), and 5 % Pt on γ -Al₂O₃, (from Alfa Aesar), 5 % Pd on γ -Al₂O₃ (from Alfa Aesar), 5 % Ru on γ -Al₂O₃ (from Alfa Aesar), pure silica support powder, and a commercial automotive monolith catalyst. The synthesized catalysts were prepared by using the incipient wetness technique, calcined in air at 573 K

for 6 h, and reduced at 473 K in 20 % H₂/He for 12 h. All catalysts were pretreated simultaneously in situ by flowing oxygen in nitrogen at 625 K for 90 min. Reaction conditions were established with flow ratios of propene:O₂:N₂ of 0.1:0.57:1 with a total flow of 210 sccm. The reactor was heated to the desired temperature, allowed to equilibrate, and infrared spectral imaging data of the gas-phase array were collected.

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Ferromagnetic Ordering in a Diamond-Like Cyano-Bridged Mn^{II}Ru^{III} Bimetallic Coordination Polymer**

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The field of molecule-based magnetic materials has made significant advances in recent years.^[1] A popular approach to the synthesis of these materials has been to use a paramagnetic [M(CN)₆]ⁿ⁻ (M = Cr^{III}, Mn^{III}, Fe^{III}, or V^{II}) building block in conjunction with another paramagnetic 3d metal ion, and this resulted in the formation of a large number of one-, two-, and three-dimensional polymers that exhibit various magnetic behaviors.^[2] We are interested in extending this approach to 4d and 5d metal ions, since their orbitals are more diffuse, and enhanced magnetic interactions can therefore be expected. In general, however, it is much more difficult to construct polymers from 4d or 5d metal complexes, since they are usually much more substitution-inert than 3d metal complexes. Our strategy is to use a 4d or 5d metal cyanide in conjunction with a 3d metal ion to produce 4d–3d and 5d–3d polymers, and we note that examples of such polymers are limited to Mo–C≡N–Mn^{III} and NBu₄[M^{II}Ru^{III}(ox)₃] (M = Mn, Fe, Cu; ox = oxalato).^[4] We initially planned to use this strategy to prepare 3d–Ru^{III} polymers from [Ru(CN)₆]³⁻, since a number of novel Fe^{III}-containing magnetic materials were prepared from [Fe(CN)₆]³⁻. However, whereas [Fe(CN)₆]³⁻ is robust and readily available, [Ru(CN)₆]³⁻ is very unstable, especially in solvents such as water and alcohols.^[5] We therefore attempted to design alternative stable cyano complexes of ruthenium(III) as building blocks, and here we report on the synthesis and structure of *trans*-Ph₄P[Ru^{III}(acac)₂(CN)₂] (**1**, Hacac = acetylacetonate) and its reaction with Mn²⁺ to produce the novel cyano-bridged Mn^{II}Ru^{III} polymer {Mn[Ru(acac)₂(CN)₂]₂}_n (**2**), which has a diamond-like structure and exhibits ferromagnetic ordering

below *T*_C = 3.6 K. A diamond-like structure was also proposed for Mn^{II}[Mn^{III}(CN)₄], which is antiferromagnetic.^[6]

Reaction of *trans*-Ph₄P[Ru^{III}(acac)₂Cl₂] with KCN in methanol produced **1**. The IR spectrum showed a strong C≡N stretching band at 2099 cm⁻¹. The structure of **1** was determined by X-ray crystallography (Figure 1).^[7] The ruthenium

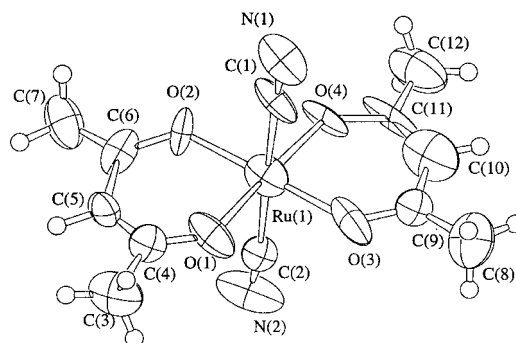


Figure 1. ORTEP plot of the anion of **1**. Selected bond lengths [Å] and angles [°]: Ru(1)–O(1) 2.03(2), Ru(1)–O(2) 1.98(2), Ru(1)–O(3) 1.98(2), Ru(1)–O(4) 2.03(2), Ru(1)–C(1) 2.06(3), Ru(1)–C(2) 2.09(3), N(1)–C(1) 1.15(3), N(2)–C(2) 1.12(3); O(1)–Ru(1)–O(2) 92.1(9), O(1)–Ru(1)–O(3) 87.8(8), O(1)–Ru(1)–O(4) 177.6(10), O(1)–Ru(1)–C(1) 90.3(8), O(1)–Ru(1)–C(2) 89.7(8), C(1)–Ru(1)–C(2) 178(1), Ru(1)–C(1)–N(1) 178(3), Ru(1)–C(2)–N(2) 173(3).

atom is octahedrally coordinated by the four oxygen atoms of the two acac ligands and the carbon atoms of the cyanide ions in a *trans* configuration. The four Ru–O distances are almost identical (1.98–2.03 Å) and are also similar to that of [Ru(acac)₃] (av 2.003 Å).^[8] The two Ru–CN distances are also similar, as are the two C–N distances. The cyclic voltammogram of **1** in CH₃CN (with 0.1 M Bu₄NPF₆) shows two reversible waves at +0.70 and –1.15 V (vs Ag/Ag⁺), which are assigned to the Ru^{IV}/Ru^{III} and the Ru^{III}/Ru^{II} couples, respectively. These electrochemical data indicate that [Ru(acac)₂(CN)₂]⁻ is very stable with respect to oxidation and reduction. Electrospray mass spectrometry (ES-MS) on a methanolic solution of **1** in the anionic mode showed only a peak at *m/z* 352 for [Ru(acac)₂(CN)₂]⁻, and agreement between calculated and experimental isotopic distribution patterns was excellent. All these results suggest that [Ru(acac)₂(CN)₂]⁻ is stable in solution and is therefore a suitable precursor for the construction of ruthenium(III)-containing coordination polymers.

Reaction of **1** with Mn(ClO₄)₂·6H₂O in MeOH produced {Mn[Ru(acac)₂(CN)₂]₂}_n (**2**) as dark purple crystals. The IR spectrum showed a C≡N stretching band at 2125 cm⁻¹, which is at a higher frequency than that of **1**, consistent with coordination of RuC≡N to Mn. The structure of **2** was determined by X-ray crystallography (Figure 2).^[9] Each Mn center is tetrahedrally coordinated by four [Ru^{III}(acac)₂(CN)₂]⁻ ions through the cyano nitrogen atoms to produce a three-dimensional polymer with a diamond-like structure. The bond lengths of each [Ru^{III}(acac)₂(CN)₂]⁻ unit in the polymer (Ru–O 1.995, 2.001, Ru–C 2.052, C–N 1.148 Å) are essentially the same as in **1**.

The temperature dependence of the molar magnetic susceptibility χ_M of **1** and **2** was investigated in the range 2–

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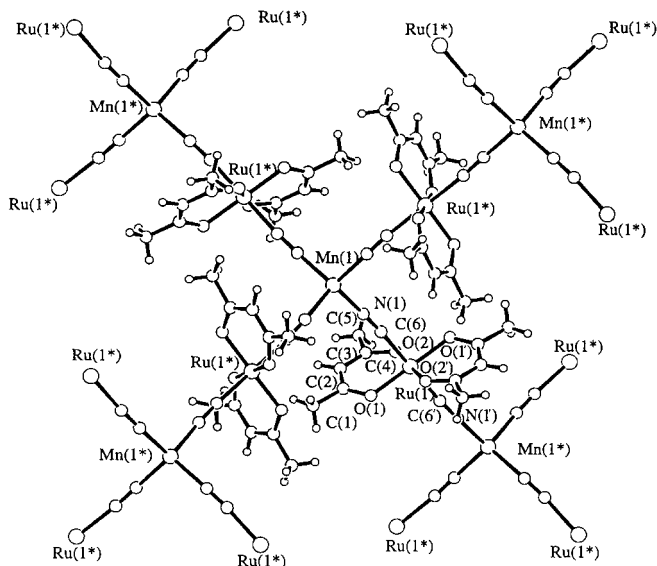


Figure 2. View of **2**. Selected bond lengths [$\text{\AA}$] and angles [$^\circ$]: Ru(1)-O(1) 2.001(3), Ru(1)-O(2) 1.995(3), Ru(1)-C(6) 2.052(4), Mn(1)-N(1) 2.076(3), N(1)-C(6) 1.148(5); O(1)-Ru(1)-O(1') 180.0, O(1)-Ru(1)-O(2) 89.5(2), O(1')-Ru(1)-C(6) 94.6(2), O(1)-Ru(1)-O(2) 90.5(2), O(1)-Ru(1)-C(6) 85.4(2), O(2)-Ru(1)-O(2') 180.0, O(2)-Ru(1)-C(6) 89.9(1), C(6)-Ru(1)-C(6') 180.0, N(1)-Mn(1)-N(1) 103.49(8), N(1)-Mn(1)-N(1) 122.2(2), Mn(1)-N(1)-C(6) 170.8(4), Ru(1)-C(6)-N(1) 175.6(4).

300 K on an Oxford Instruments Maglab2000 magnetometer, and plots of $\chi_M T$ versus T are shown in Figure 3a. At room temperature $\chi_M T$ of **1** is $0.46 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$ ($1.91 \mu_B$), which is comparable to that of $[\text{Ru}(\text{acac})_3]$,^[10] and is consistent with the low-spin configuration (t_{2g}^5)⁵ with $S = 1/2$. The magnetic susceptibility above 10 K obeys the Curie–Weiss law [$\chi_M = C/(1 - \theta)$] with $C = 0.46 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$ and $\theta = -3.3 \text{ K}$. The slight decrease in $\chi_M T$ below 10 K and the negative θ do not necessarily imply antiferromagnetic interaction between Ru^{III} ions, but are more likely due to complicated spin–orbital coupling that combines the effects of ligand-field and thermally populated excited states.^[11]

The $\chi_M T$ value of **2** at room temperature is $5.25 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$, which is slightly larger than the uncoupled, spin-only value of 5.12 for one Mn^{II} ($S = 5/2$) and two Ru^{III} ($S = 1/2$) centers, but is very close to the value of 5.29 calculated from the measured $\chi_M T$ value for Ru^{III} in **1**. The monotonic increase in $\chi_M T$ on cooling indicates the presence of ferromagnetic coupling between Mn^{II} and Ru^{III} via the cyano bridges. The $\chi_M T$ value increases abruptly below 50 K and reaches a maximum ($11.8 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$) at 5.5 K, which is much higher than the value of $7.88 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$ for the ferromagnetic spin state $S_T = 7/2$, assuming $g_T = 2.0$;^[4] this suggests long-range ferromagnetic ordering. The magnetic susceptibility above 10 K obeys the Curie–Weiss law with $C = 5.16 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$ and a positive Weiss constant $\theta = +5.4 \text{ K}$, which also proves the presence of ferromagnetic coupling in **2**. The onset of a long-range magnetic phase transition is evidenced by the low-field temperature dependence of the magnetization (Figure 3b), which increases abruptly below 4 K, characteristic of a long-range magnetic ordering. The ferromagnetic ordering temper-

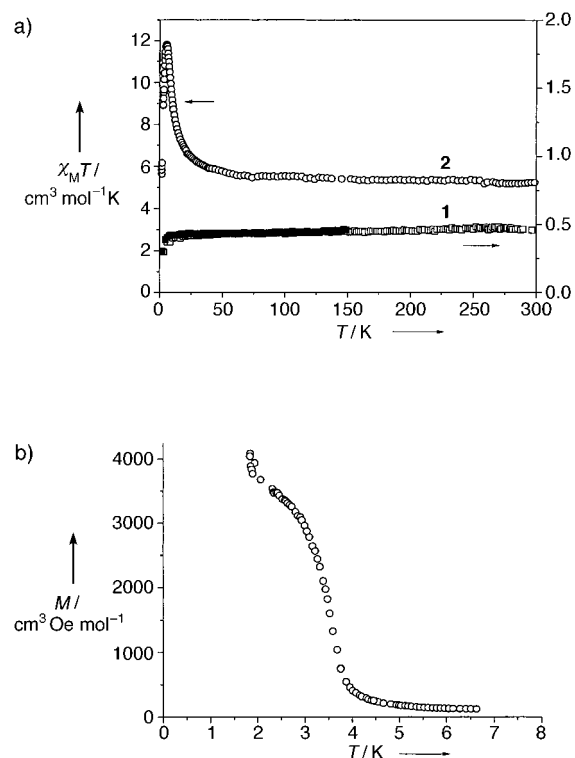


Figure 3. a) Temperature dependence of $\chi_M T$ for **1** (right axis) at 10 kOe ($\blacksquare$) and 50 kOe ($\square$), and for **2** (left axis) at 10 kOe ($\circ$). b) Temperature dependence of magnetization for **2** at 100 Oe.

ature $T_C = 3.6$ was further determined from the maximum of χ'_M as shown in Figure 4, where χ'_M and χ''_M are the in-phase and out-of-phase alternating current (ac) susceptibilities at zero external magnetic field and an oscillating field frequency range of 111–1111 Hz. No frequency dependence was observed, and this rules out the presence of glassy behavior.

The field dependence of the magnetization for **1** and **2** was measured at 2.0 and 1.86 K, respectively. The saturation value for **1** is $1.06 N\mu_B$, consistent with the value for low-spin Ru^{III} ($S = 1/2$). The magnetization of **2** at 1.86 K increases rapidly at low field, as expected for a magnet, and reaches $6.8 N\mu_B$ at

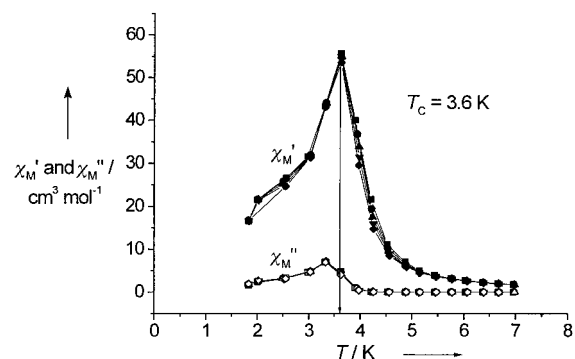


Figure 4. Temperature dependence of ac susceptibility measured at zero external magnetic field and $H_{ac} = 2 \text{ Oe}$ and different frequencies: 111 Hz ($\square$), 199 Hz ($\circ$), 355 Hz ($\triangle$), 633 Hz (∇), 1111 Hz ($\diamond$); in-phase susceptibility χ'_M (filled symbols) and out-of-phase susceptibility χ''_M (open symbols). Further magnetic measurements can be found in the Supporting Information.

60 kOe, which is very close to the expected value of $7.0 N\mu_B$ for a parallel alignment of the magnetic moments of two Ru^{III} and one Mn^{II} ion. Furthermore, a characteristic hysteresis loop is observed at 1.85 K but with negligible remanent magnetization ($0.06 N\mu_B$) and coercive field (6 Oe). Although ferromagnetic $\text{Ru}^{\text{III}}-\text{Mn}^{\text{II}}$ interaction was also observed in two-dimensional $(\text{NBu}_4)[\text{Mn}^{\text{II}}\text{Ru}^{\text{III}}(\text{ox})_3]$ via the oxalato bridges, no long-range ordering occurs down to 2 K.^[4] We are currently investigating related 3d–4d and 4d–5d systems in order to understand the origin of the ferromagnetic behavior in **2**.

Experimental Section

1: *trans*- $\text{Ph}_4\text{P}[\text{Ru}^{\text{III}}(\text{acac})_2\text{Cl}_2]$ ^[12] (355 mg, 0.5 mmol) and KCN (325 mg, 5.0 mmol) were heated in refluxing methanol (30 mL) for 24 h. The solution was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (10 mL). After filtration, the product was precipitated as a dark purple solid by adding diethyl ether. Yield: 225 mg (65 %). Crystals suitable for X-ray crystallography were obtained by slow diffusion of diethyl ether into a solution of **1** in CH_2Cl_2 . IR (KBr): $\tilde{\nu}_{\text{CN}} = 2099 \text{ cm}^{-1}$; UV/Vis (CH_3CN): λ_{max} (lg ϵ) = 349 (3.76), 407 sh (3.16), 540 (3.22); elemental analysis (%) calcd for $\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_4\text{RuP}$: C 62.60, H 4.96, N 4.06; found: C 62.96, H 5.05, N 4.21.

2: A mixture of $\text{MnClO}_4 \cdot 6\text{H}_2\text{O}$ (36 mg, 0.1 mmol) and **1** (69 mg, 0.1 mmol) was stirred in methanol (30 mL) at room temperature. The solution was filtered after 3 h, and slow evaporation of the filtrate afforded dark purple crystals. Yield: 34 mg (45 %). IR (KBr): $\tilde{\nu}_{\text{CN}} = 2125 \text{ cm}^{-1}$; elemental analysis (%) calcd for $\text{Ru}_2\text{MnN}_4\text{C}_{24}\text{O}_8\text{H}_{28}$: C 38.05, H 3.73, N 7.40; found: C 38.24, H 3.82, N 7.56.

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- [7] a) Crystal data for **1** ($\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_4\text{RuP}$): $M_r = 690.72$, dark purple prisms, monoclinic, space group $P2_1/c$ (no. 14), $a = 7.727(8)$, $b = 27.541(8)$, $c = 15.645(9)$ Å, $\beta = 103.13(8)^\circ$, $V = 3242(3)$ Å³, $F(000) = 1420.00$, $Z = 4$, $\rho_{\text{calcd}} = 1.415 \text{ g cm}^{-3}$, $\mu(\text{MoK}\alpha) = 5.74 \text{ cm}^{-1}$. Data were measured on a Rigaku AFC7R diffractometer with graphite-monochromatized $\text{MoK}\alpha$ radiation and a 12-kW rotating anode generator at 28.0 °C. Of the 3670 reflections measured, 3298 were unique ($R_{\text{int}} = 0.053$), and 1403 with $I > 2\sigma(I)$ were observed. Data were corrected for Lorentzian and polarization effects. The structure was solved by direct methods (SHELXS86), and the full-matrix least-squares refinement converged to $R = 0.094$ and $R_w = 0.077$ with a GOF of 3.91. 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-161807 (**1**) and -161808 (**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).
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- [9] Crystal data for **2** ($\text{Ru}_2\text{MnN}_4\text{C}_{24}\text{O}_8\text{H}_{28}$): $M_r = 757.59$, dark purple blocks, tetragonal, space group $P4_2/n$ (no. 86), $a = 13.6900(7)$, $c = 8.1530(5)$ Å, $V = 1528.0(1)$ Å³, $F(000) = 747.00$, $Z = 2$, $\rho_{\text{calcd}} = 1.646 \text{ g cm}^{-3}$, $\mu(\text{MoK}\alpha) = 14.31 \text{ cm}^{-1}$. Data were collected at 25.0 °C. Of the 9457 reflection measured, 1862 were unique ($R_{\text{int}} = 0.034$), and 1091 with $I > 1.5\sigma(I)$ were observed. Data were corrected for Lorentzian and polarization effects. The structure was solved by direct methods (SIR92), and the full-matrix least-squares refinement converged to $R = 0.036$ and $R_w = 0.021$ with a GOF of 1.18.^[7b]
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A Silyl Cation with a Three-Center Si-H-Si Bond**

Thomas Müller*

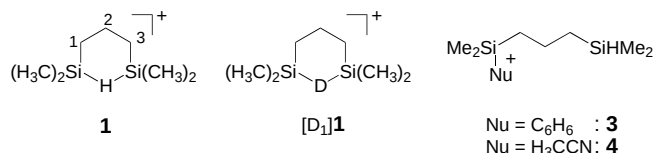
Three-center, two-electron bonds involving hydrogen as a bridging atom are a common feature in the chemistry of early main group metals. Several hydrido-bridged carbocations were identified by low-temperature NMR spectroscopy.^[1] It was predicted that the isoelectronic silylium ions will even interact with methane,^[2] and it was suggested that such interactions between a positively charged silicon atom and appropriately arranged alkyl groups on its substituents can be

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

used to stabilize a silylium ion by intramolecular solvation.^[3] More suitable for such interactions than C–H bonds are the Si–H bonds of silanes. Due to the higher lying σ -bonding orbital, the interaction with the empty $3p(\text{Si})$ orbital is more favorable. Recently, Wrackmeyer et al. gave convincing NMR evidence for the presence of an Si–H–B bridge in silylated vinylboranes.^[4] Here we report the synthesis of silyl cation **1** having a symmetrical Si–H–Si bridge, a previously unknown structural motif in organosilicon chemistry.^[5]



The silyl cation **1** is formed by a hydride transfer^[6] from 2,6-dimethyl-2,6-disilaheptane (**2**) to trityl tetrakis(pentafluorophenyl)borate (TPFPB) in [D₆]benzene at room temperature. The orange-red color of the trityl salt instantaneously disappears, and the reaction mixture separates into two layers. The upper layer containing triphenylmethane was separated from the reaction mixture. ¹H, ¹³C, ²⁹Si, and ¹⁹F NMR spectra of the lower layer confirm the exclusive formation of the symmetrical cation **1**.^[7]

The unusually shielded ¹H NMR signal for the SiH group in **1** ($\delta(^1\text{H}^{\text{br}}) = 1.47$, $\Delta\delta = -2.43$ relative to **2**) is indicative of the hydrogen-bridged structure.^[8] The ¹³C{¹H} NMR spectrum shows, besides the signals for the anion, only three signals corresponding to C² ($\delta = 18.1$), C^{1/3} ($\delta = 14.9$), and the methyl groups ($\delta = -4.0$; see Table 1 and Supporting Information). The single, deshielded ²⁹Si NMR signal at $\delta = 76.7$ ^[8] splits in the ¹H-coupled spectrum into a broad doublet with an unusually small coupling constant ($^1J_{\text{Si,H}} = 39$ Hz, Figure 1 a, b). The ²⁹Si signal of the deuterated cation **[D₁]**1****^[9] is split into a triplet with $^1J_{\text{Si,D}} = 6.3$ Hz (Figure 1 c), as expected for the gyromagnetic ratio $\gamma_{\text{H}}/\gamma_{\text{D}}$.^[10] In arene solvents, the ²⁹Si NMR signal of **1** shows only minor solvent dependence ($\delta(^{29}\text{Si}) = 77.1$ in [D₈]toluene). This indicates only small interactions between the silyl cation and the solvent; in particular, the formation of a silylated arenium ion **3** can be excluded, since for trialkylsilyl-substituted arenium ions solvent effects of $\Delta\delta = 10$ or more are reported.^[6a, 11, 12] In contrast, when acetonitrile is added to a solution of **1** in

Table 1. Measured and calculated (in parenthesis) chemical shifts of silyl cations **1** and **5b**; $|^1J(\text{Si,H})|$ values in square brackets.

Compound	Si	C ¹	C ²	C ³	H ^{br}
1 ^[a]	76.7 [39 Hz]	14.9	18.1	14.9	1.47
1 ^[b]	77.0	15.2	18.5	15.2	1.40
1 ^[c]	(88.0)	(18.6)	(22.9)	(18.6)	
5b ^[c]	(347.9, -13.3)	(36.4)	(16.0)	(25.3)	
1 ^[d]	(96.5)	(20.7)	(25.7)	(20.7)	
1 ^[e]	(93.4 [-43.8 Hz ^[g]])	(18.3)	(23.4)	(18.3)	
1 ^[f]	(87.4)				

[a] In [D₆]benzene at 300 K. [b] In [D₈]toluene at 300 K. [c] At the GIAO/MPW1PW91/6-311G(3d,p)//MP2/6-311G(d,p) level, $\sigma(^{13}\text{C}, \text{TMS}) = 187.51$, $\sigma(^{29}\text{Si}, \text{TMS}) = 339.97$.^[23, 24] [d] At the SOS/DFPT/IGLO/PW91/BasisIII//B3LYP/6-311G(d,p) level, $\sigma(^{13}\text{C}, \text{TMS}) = 183.6$, $\sigma(^{29}\text{Si}, \text{TMS}) = 346.7$.^[25, 26] [e] At the GIAO/MPW1PW91/6-311G(3d,p)//B3LYP/6-311G(d,p) level, $\sigma(^{13}\text{C}, \text{TMS}) = 186.90$, $\sigma(^{29}\text{Si}, \text{TMS}) = 338.76$.^[23, 24] [f] At the GIAO/MP2/6-311G(2df,p)(Si), 6-311G(d)(C,H)//MP2/6-311G(d,p) level, $\sigma(^{29}\text{Si}, \text{TMS}) = 370.61$.^[23, 27] [g] $^1J(\text{Si,H})$ calculated by the SOS/DFPT/IGLO method utilizing the Perdew–Wang exchange with Perdew correlation functional and the basis set III on geometries optimized at the B3LYP/6-311G(d,p) level.^[25, 26]

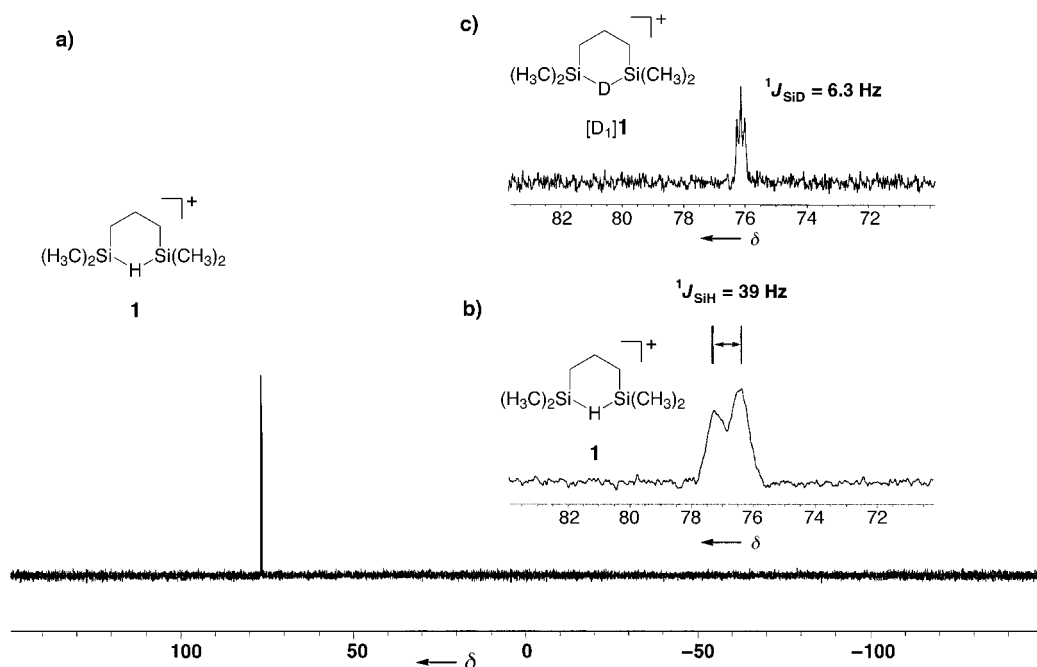
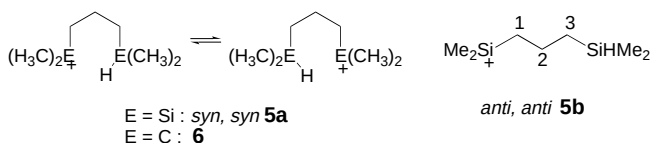


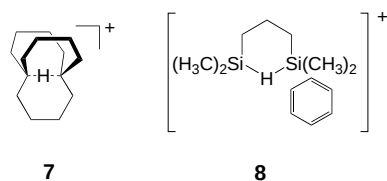
Figure 1. a) ²⁹Si{¹H} INEPT NMR spectrum (79.5 MHz) of **1** at 300 K. b) ²⁹Si INEPT+ NMR spectrum (49.5 MHz) of **1** at 300 K. c) ²⁹Si{¹H} INEPT NMR spectrum (49.5 MHz) of **[D₁]**1**** at 300 K.

[D₆]benzene, the silylated nitrilium ion **4** is formed, as shown by two ²⁹Si NMR signals at $\delta = 32.3$ and -14.2 (d, $^1J_{\text{Si,H}} = 180.6$ Hz).^[13]

The number of observed NMR signals is in agreement with the symmetrical structure **1**, but a fast degenerate 1,5-H shift which interconverts two open silylium ions **5** can not be a priori excluded. The carbocation **6** was shown to be a rapidly equilibrating system with a barrier of about 5 kcal mol⁻¹ for



the 1,5-H shift, and the symmetry reflected in the NMR spectra at higher temperatures is therefore time-averaged.^[1i, 14] Low-temperature NMR studies at -40°C with **1** in $[\text{D}_8]\text{toluene}$ showed no kinetic line broadening of the ^{29}Si NMR signal,^[15a] and this suggests a barrier of less than 8.5 kcal mol^{-1} for a conceivable degenerate 1,5-H shift in **5**.^[15b] An analysis of the NMR data^[16, 17] and the use of deuterium isotope effects on the NMR chemical shift,^[14b, 18, 19] however, provide conclusive arguments for the static structure **1** and against an equilibrating system **5**: 1) The time-averaged ^{29}Si NMR signal for equilibrating **5** is expected to be at about $\delta = 168$ (average of 348 and -13 , calculated for **5b**; Table 1).^[16] 2) The expected value of the time-averaged coupling constant $^1J_{\text{Si,H}}$ for **5** is about 90 Hz (average of 180 (RSiHMe_2) and 0 Hz (RSiMe_2^+)).^[16a] 3) The hexadeuterated cation $[(\text{H}_3\text{C})_2\text{Si}(\text{CH}_2)_3\text{SiH}(\text{CD}_3)_2]^+$ (**[D₆]****1**) exhibits two ^{29}Si NMR signals ($\delta = 77.01, 76.78$), that is, the intrinsic isotope effect on the chemical shift is $\Delta\delta^{\text{int}} = -0.23$ and it is constant over the whole accessible temperature range. This is consistent only with a single minimum potential (i.e., structure **1**).^[14b, 18] 4) When a 1:1 mixture of cations **1** and **[D₁]****1** is generated, a ^2H NMR signal at 1.77 is recorded for the bridging deuterium atom in **[D₁]****1**; hence, the primary deuterium isotope effect on the chemical shift is $\Delta\delta(^1\text{H}, ^2\text{H}) = -0.30$.^[19a] Negative $\Delta\delta(^1\text{H}, ^2\text{H})$ values indicate a single-minimum potential^[19b] and are found for symmetrically hydrogen-bridged carbocations. For example, the tricyclic cation **7** has $\Delta\delta(^1\text{H}, ^2\text{H}) = -0.10$.^[1k]



The experimental characterization of **1** is strongly corroborated by quantum mechanical calculations^[20] at the hybrid density functional^[21] B3LYP/6-311G(d,p)+ZPVE level of theory. At this level, **1** is more stable than the *anti*, *anti* conformer **5b** by $24.0\text{ kcal mol}^{-1}$. The *syn*, *syn* conformer **5a** is not a stationary point on the potential energy surface but collapses without a barrier to the symmetrical, cyclic isomer **1**. The calculated dipole moment of **1** is rather small due to its symmetrical structure; in contrast, silylium ions like **5b** have dipole moments more than an order of magnitude larger ($\mu(\mathbf{1}) = 0.61$, $\mu(\mathbf{5b}) = 10.4$ Debye, at the B3LYP/6-311G(d,p) level). Consequently, solvation has a considerable effect on the relative energy of both isomers, but it does not change the relative stability order. Thus, according to SCRF theory^[22] at the B3LYP/6-311G(d,p) level, the energy difference for **1** and **5b** decreases to $15.7\text{ kcal mol}^{-1}$, whereby cyclic **1** is more stable.

The association energy E_A between **1** and benzene, as calculated for the complex **8**, is relatively small ($E_A = 3.7\text{ kcal mol}^{-1}$). Therefore, it is unlikely that this complex exists at ambient temperature, and **1** is best described as a free silyl cation with no direct coordination to the solvent, in

agreement with the negligible solvent effects on $\delta(^{29}\text{Si})$ in aromatic hydrocarbons.

Calculations of NMR data^[23–27] at several levels of theory (see Table 1) predict $\delta(^{29}\text{Si}) = 87–96$ for **1**, near to the observed value. In addition, density functional calculation of $^1J_{\text{Si,H}}$ at the SOS/IGLO/DFPT/PP/BasisIII level^[25, 26] predict for **1** a small $|^1J_{\text{Si,H}}|$ value of 43.8 Hz, in agreement with the experiment.

The calculated structure of **1** (Figure 2a) clearly reveals the existence of the three-center Si-H-Si bond. Compound **1** adopts a regular chair conformation with long Si-H bonds

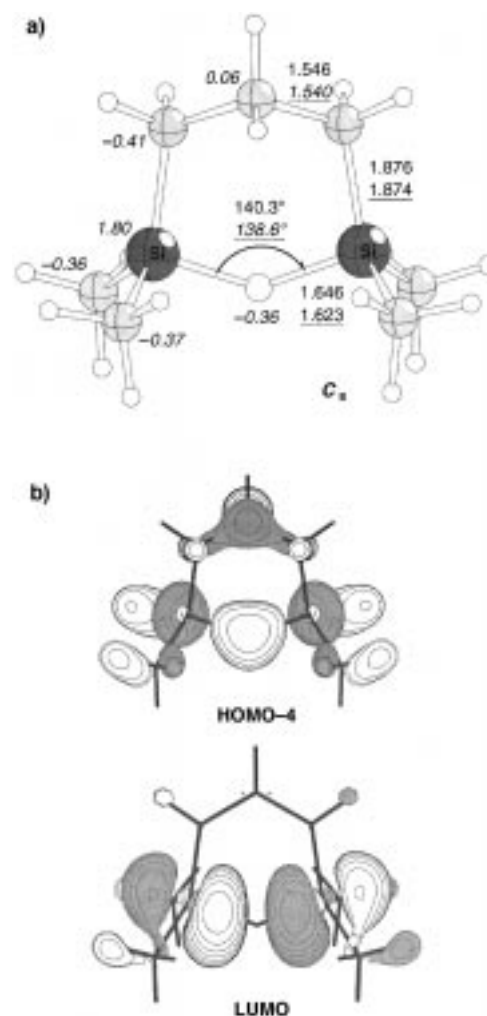


Figure 2. a) Calculated structure (at B3LYP/6-311G(d,p) and MP2/6-311G(d,p) (underlined) levels) and charge distribution (italics; NBO analysis^[28] at B3LYP/6-311G(d,p)/B3LYP/6-311G(d,p) level) of **1**. b) Orbital plots of the occupied MO (HOMO – 4) and of the nonbonding MO (LUMO) of the Si-H-Si bond.^[29]

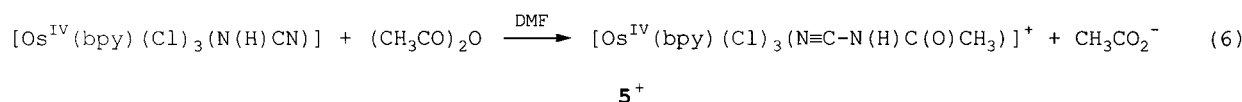
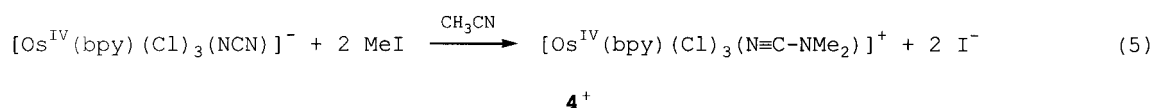
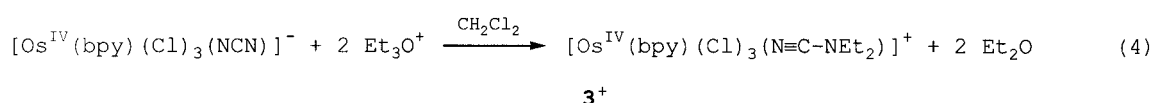
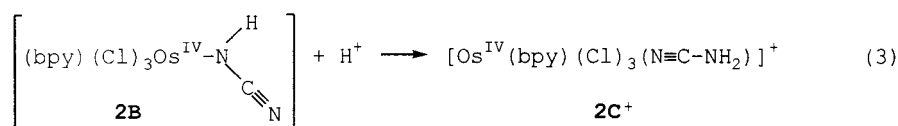
($1.646\text{ \AA}$), and the positive charge is distributed over the two silicon atoms, while negative charge is accumulated at the bridging hydrogen atom (see Figure 2a), as expected for a three-center, two-electron bond with high electron density on the $\mu\text{-H}$ atom in the bonding orbital, and a LUMO which is centered predominantly on the two silicon atoms (Figure 2b).

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Direct evidence for monoprotonation at N_α comes from the IR spectra. In **2A**[−] and its ¹⁵N analogue, [Os^{IV}(bpy)(Cl)₃(¹⁵NCN)][−] (**2A**[−]), prepared from the ¹⁵N-nitrido complex precursor as in [Eq. (1b)], the IR band assigned as $\nu(\text{C}\equiv\text{N}_\beta)$ is at 1975 cm^{−1} for **2A**[−], and $\nu(\text{C}\equiv\text{N}_\beta)$ at 1977 cm^{−1} for **2A**[−]. Upon protonation, $\nu(^{14}\text{N}_\alpha\text{--H}) = 3230$, $\nu(\text{C}\equiv\text{N}_\beta) = 2187$ cm^{−1} for **2B** and $\nu(^{15}\text{N}_\alpha\text{--H}) = 3213$, $\nu(\text{C}\equiv\text{N}_\beta) = 2181$ cm^{−1} for **2B**⁺. Based on ¹H NMR spectra, **2A**[−] contains a paramagnetic d⁴ Os^{IV} center which results in a highly contact-shifted spectrum; **2B** is diamagnetic.

In the presence of a second equivalent of HPF₆ in CH₃CN, a second protonation occurs to give the Os^{IV}–cyanamido complex, [Os^{IV}(bpy)(Cl)₃(NCNH₂)]⁺ (**2C**⁺) [Eq. (3)], which



was isolated as the PF₆[−] salt. Diprotonation at N_β is supported by IR spectra which show $\nu(^{14}\text{N}_\alpha\equiv\text{C}) = 2280$ cm^{−1} for **2C**⁺ and $\nu(^{15}\text{N}_\alpha\equiv\text{C}) = 2249$ cm^{−1} for **2C**⁺; the bands for $\nu(^{14}\text{N}_\beta\text{--H})$ are 3332 ± 4 and 3200 ± 3 cm^{−1} for **2C**⁺ and **2C**⁺, respectively.

Dialkylation of **2A**[−] to give the corresponding Os^{IV}–dialkylcyanamido complexes, [Os^{IV}(bpy)(Cl)₃(NCNR₂)]⁺ (**3**⁺, R = Et, BF₄[−] salt and **4**⁺, R = Me, PF₆[−] salt), occurs under N₂ in reactions with triethyloxonium tetrafluoroborate ([Et₃O]⁺BF₄[−]) in CH₂Cl₂ and iodomethane (MeI) in CH₃CN, respectively [Eqs. (4) and (5)].

Acylation^[1d] of **2B** by acetic anhydride ((MeCO)₂O) in *N,N*-dimethylformamide (DMF) also occurs at N_β to give the Os^{IV}–acetylcyanamido complex, [Os^{IV}(bpy)(Cl)₃(NCN(H)C(O)Me)]⁺ (**5**⁺) [Eq. (6)], which was isolated as the PF₆[−] salt.

In all three cases, the differences in $\nu(\text{N}_\alpha\equiv\text{C})$ between the ¹⁴N_α and ¹⁵N_α forms are consistent with dialkyl or acyl addition at N_β , that is, **3**⁺: $\nu(^{14}\text{N}_\alpha\equiv\text{C}) = 2206$ and $\nu(^{15}\text{N}_\alpha\equiv\text{C}) = 2176$ cm^{−1}, **4**⁺: $\nu(^{14}\text{N}_\alpha\equiv\text{C}) = 2202$ and $\nu(^{15}\text{N}_\alpha\equiv\text{C}) = 2173$ cm^{−1}, and **5**⁺: $\nu(^{14}\text{N}_\alpha\equiv\text{C}) = 2208$ and $\nu(^{15}\text{N}_\alpha\equiv\text{C}) = 2179$ cm^{−1}.

There is also an extensive electron-transfer chemistry for the cyanoimido complexes reminiscent of the analogous hydrazido complexes. The electron transfer is based on electron occupation and deoccupation of levels largely localized in the Os–hydrazido π -bonding framework.^[4, 8] Cyclic voltammetry of the new compounds shows a series of reversible redox waves arising from the multiple oxidation/reduction of the osmium center (Table 1).

Our results provide a novel approach to the preparation of the NCN^{2−} ligand and its subsequent derivatization after

Table 1. Redox potentials for the new osmium compounds.^[a]

Compound	$E_{1/2}(\text{Os}^{\text{VI}}/\text{Os}^{\text{V}})$	$E_{1/2}(\text{Os}^{\text{V}}/\text{Os}^{\text{IV}})$	$E_{1/2}(\text{Os}^{\text{IV}}/\text{Os}^{\text{III}})$	$E_{1/2}(\text{Os}^{\text{III}}/\text{Os}^{\text{II}})$
1A	1.72	0.90	−0.40	−1.30
2A [−]	1.87	1.19	0.67	−0.90
3 ⁺			0.79	−0.63
4 ⁺			0.74	−0.58
5 ⁺			0.84	−0.55

[a] Potentials are given in Volts and measured against the sodium saturated calomel electrode (SSCE) in 0.1M TBAH/CH₃CN, (TBAH = tetrabutylammonium hexafluorophosphate). All processes are reversible.

coordination. The stepwise assembly of the dialkylated ligands provides a potentially general route to substituted cyanamido complexes that cannot be prepared directly because of polymerization of the preformed cyanamido organic precursors. Finally, the existence of multiple oxidation states and

the acid–base behavior of the NCN^{2−} ligand in the Os^{IV} complex may point to oxo-like reactivity based on proton-coupled electron transfer or atom transfer involving couples such as [Os^{VI}(bpy)(Cl)₃(NCN)]^{+/}[Os^V(bpy)(Cl)₃(N(H)CN)]^{+/} or [Os^{VI}(bpy)(Cl)₃(NCN)]^{+/}[Os^{IV}(bpy)(Cl)₃(NCNH₂)]^{+/}.

For full details on the preparation and characterization of all the new compounds see the Supporting Information.

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Ferromagnetic Coupling through Spin Polarization in a Dinuclear Copper(II) Metallacyclophane**

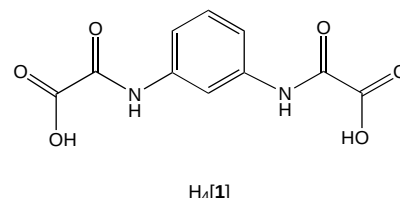
Isabel Fernández, Rafael Ruiz,* Juan Faus, Miguel Julve, Francesc Lloret,* Joan Cano, Xavier Ottenwaelder, Yves Journaux,* and M. Carmen Muñoz

In memory of Olivier Kahn

The design and synthesis of polynuclear coordination compounds with predictable magnetic properties have always interested inorganic chemists working in the field of magnetochemistry.^[1, 2] In particular, the preparation of ferromagnetically coupled dinuclear complexes appears intimately linked to the history of this relatively young discipline, namely molecular magnetism. Of the theoretical strategies to achieve a ferromagnetic spin alignment between two metal centers, those based either on orbital symmetry or spin polarization

effects are the easiest ones to be managed by the synthetic chemist.^[3] The second approach is particularly appealing and, however, has received more limited attention for metal complexes,^[4] when compared with the extensive work concerning high-spin organic molecules (polyradicals).^[5]

In our research into ligand design as a means to control the electronic and magnetic properties of polynuclear complexes,^[6, 7] we prepared the new ligand H₄[**1**], the parent acid



of *N,N'*-1,3-phenylenebis(oxamate). This ligand cannot act in a tetradentate manner towards a Cu^{II} center to give a monomeric four-coordinate copper(II) species, unlike the related ligand *N,N'*-1,2-phenylenebis(oxamate). Instead, it can self-assemble with Cu^{II} ions in a 2:2 ratio to give a dimeric copper(II) complex, [Cu₂(η²:η²-**1**)₂]⁴⁻, where the two ligands adopt the bisbidentate coordination mode, each donating one of its oxamate donor sets to each copper. In this case, two bis(oxamate)copper(II) spin-bearing residues are bridged by two *m*-phenylene moieties, which have already proven to be very effective ferromagnetic spin-coupling units in purely organic π-conjugated polyradicals.^[8] Here, we report the synthesis, crystal structure, and magnetic properties of the sodium salt of this novel oxamatocopper(II) complex of formula Na₄[Cu₂(**1**)₂] · 10H₂O (**2**).

The structure of **2** consists of noncentrosymmetric dinuclear copper(II) complex ions, [Cu₂(η²:η²-**1**)₂]⁴⁻, sodium cations, and both coordinated and noncoordinated water molecules (Figure 1). Actually, all twelve oxygen atoms from the four oxamate groups of the dicopper anion are bound to the sodium atoms, affording an intricate two-dimensional structure. The intramolecular copper–copper separation, Cu(1)⋯Cu(2), is 6.822(2) Å, while the shortest intermolecular ones, Cu(1)⋯Cu(1'') and Cu(2)⋯Cu(2'), are 6.335(3) and 6.999(3) Å, respectively.

The [Cu₂(η²:η²-**1**)₂]⁴⁻ unit is a metallamacrocycle composed of two bisbidentate oxamate ligands and two copper(II) cations. Moreover, owing to the almost parallel arrangement of the two benzene planes (dihedral angle of only 7.6(8)° and average interplanar separation of 3.4(1) Å), this dinuclear metallamacrocycle is of the cyclophane type (Figure 2a). In fact, a rather unusual near to perfect face-to-face π–π alignment of the two aromatic rings exists, such that a ring carbon atom lies just over the equivalent atom of the other ring (Figure 2b) with strong carbon–carbon contacts (C–C distances in the range 3.20(1)–3.57(2) Å). Hence, the resulting twelve-membered metallaza-linked [3.3]metacyclophane ring system (internal cavity of ca. 3.4 × 6.8 Å) has an approximate C_{2v} symmetry, with the copper basal planes being almost perpendicular to the benzene planes (dihedral angles in the range 72.1(3)–82.0(3)°). This imposes a non-

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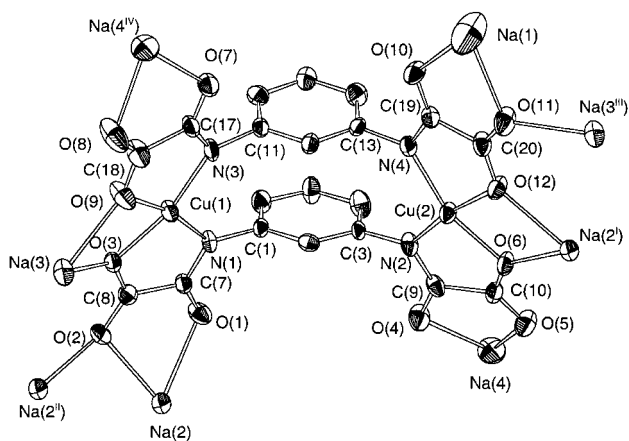


Figure 1. Perspective view of the asymmetric unit of **2**. Thermal ellipsoids are set at the 30% probability level (all hydrogen atoms and the water oxygen atoms have been omitted for clarity). Selected bond lengths [Å] and angles [°]: Cu(1)–N(1) 1.954(5), Cu(1)–N(3) 1.925(5), Cu(1)–O(3) 1.928(4), Cu(1)–O(9) 1.963(5), Cu(2)–N(2) 1.969(5), Cu(2)–N(4) 1.952(5), Cu(2)–O(6) 1.956(4), Cu(2)–O(12) 1.995(4); N(1)–Cu(1)–N(3) 107.3(2), N(1)–Cu(1)–O(3) 84.4(2), N(1)–Cu(1)–O(9) 168.2(2), N(3)–Cu(1)–O(3) 166.3(2), N(3)–Cu(1)–O(9) 83.6(2), O(3)–Cu(1)–O(9) 85.4(2), N(2)–Cu(2)–N(4) 106.2(2), N(2)–Cu(2)–O(6) 83.3(2), N(2)–Cu(2)–O(12) 170.7(2), N(4)–Cu(2)–O(6) 162.4(2), N(4)–Cu(2)–O(12) 82.5(2), O(6)–Cu(2)–O(12) 87.4(2) (I = $-x, 1-y, 1-z$; II = $1-x, -y, 1-z$; III = $x-1, 1+y, z$; IV = $x, y-1, 1+z$).

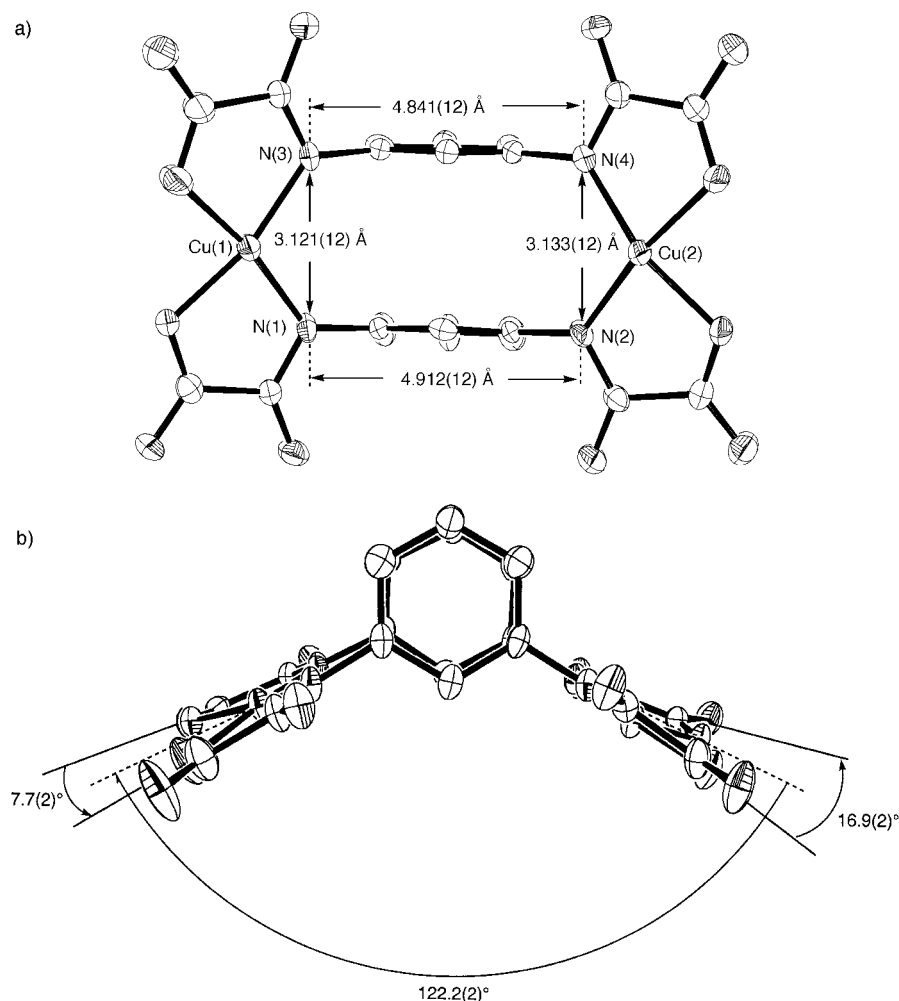


Figure 2. a) Side and b) top views of the $[\text{Cu}_2(\eta^2\text{-}\eta^2\text{-1})_2]^{4-}$ ion of **2** with relevant dimensions for the metallamacrocyclic moiety.

planar conformation for the N,N' -1,3-phenylenebis(oxamate) ligands, which is the only one compatible with the square-planar environment at the metal ions. The severe distortion from planarity of these ligands is accommodated by a considerable twist of the C–N bonds between the benzene carbon and the amide nitrogen atoms (torsion angles in the range $73.6(7)–106.3(7)^\circ$). However, the angles at the N atoms ($112.4(4)–132.1(4)^\circ$) remain close to the value expected for trigonal rather than tetrahedral hybridization.

The magnetic behavior of **2** is shown in Figure 3a as a $\chi_M T$ versus T plot, χ_M being the molar magnetic susceptibility per two copper(II) ions and T the temperature. At room temperature, $\chi_M T$ is equal to $0.80 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$, a value which is close to that expected for two magnetically noninteracting Cu^{II} ions. Upon cooling, $\chi_M T$ continuously increases until the temperature is around 5.0 K, and then remains constant between 5.0 and 1.8 K, with $\chi_M T = 1.08 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$ (insert of Figure 3a). This behavior is characteristic of ferromagnetic coupling within the dinuclear copper(II) metallacyclophane entity in **2**. Moreover, the presence of a plateau below 5.0 K indicates that the intermolecular interactions through the sodium ions and the zero-field splitting (ZFS) of the ground triplet spin state are both negligible. Indeed, this corresponds to the temperature range where only the $S=1$ ground state is

thermally populated, with $\chi_M T = 2N\beta^2 g^2 / 3k$ and $g = 2.08$ (N = Avogadro's number, β = Bohr magneton, k = Boltzmann's constant, and g is the Zeeman factor). The magnetization data for **2**, at $T = 2.0 \text{ K}$, are shown in Figure 3b as an M versus H plot, M being the molar magnetization per two copper(II) ions and H the applied magnetic field. As expected, the isothermal curve is well matched by the Brillouin function for an isolated triplet spin state with $g = 2.08$ and no ZFS (solid line in Figure 3b).

The least-squares fit of the susceptibility data of **2** to the Bleaney–Bowers equation gives $J = +16.8 \text{ cm}^{-1}$ and $R = 7 \times 10^{-6}$ (J is the exchange coupling parameter in the isotropic spin Hamiltonian $\mathcal{H} = -JS_1S_2$, with $S_1 = S_2 = 1/2$, and R is the agreement factor defined as $R = \sum[(\chi_M T)_{\text{exp}} - (\chi_M T)_{\text{calcd}}]^2 / \sum[(\chi_M T)_{\text{exp}}]^2$). The theoretical curve (solid line in Figure 3a) closely follows the experimental data in the whole temperature range. This exceptionally strong ferromagnetic coupling between two copper(II) ions separated by 6.8 Å cannot be explained as resulting from spin delocalization only. In this compound, the point group symmetry of the dimeric copper(II) unit $[\text{Cu}_2(\eta^2\text{-}\eta^2\text{-1})_2]^{4-}$ is very close to C_{2v} . Then, the orbitals describing the unpaired electrons (the so-

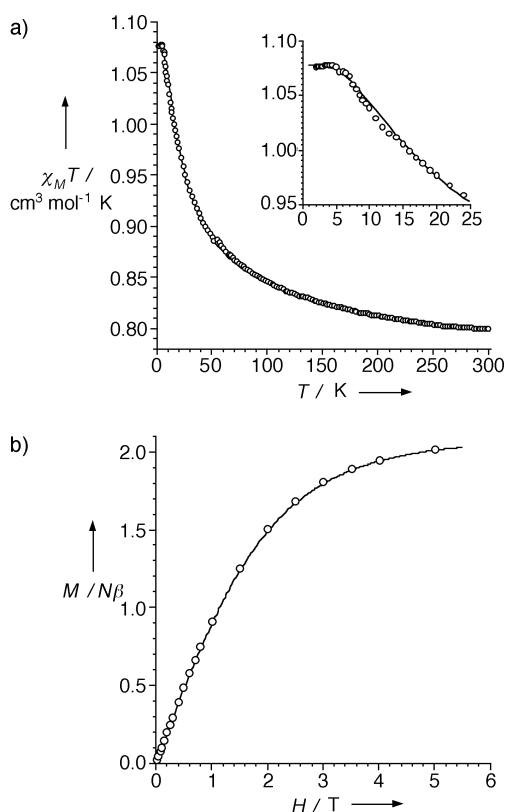


Figure 3. a) Thermal dependence of $\chi_M T$ and b) field dependence of M at 2.0 K for **2**: (○) experimental data; (—) best fit. The insert shows the thermal dependence of $\chi_M T$ in the low-temperature range.

called “magnetic orbitals”) and centered on each Cu^{II} ion (built from each of the metallic d_{xy} orbitals pointing along the Cu–N and Cu–O bonds) have the same symmetry (see below). A certainly weak although non-negligible antiferromagnetic interaction should arise because of the nonzero overlap between these two nonorthogonal orbitals. Hence, the experimentally observed large ferromagnetic interaction must be a result of spin polarization effects leading to the alternation of the spin density at the double *m*-phenylenediamide bridging framework, as expected for a bridging ligand designed with this peculiar topology. To give a clear-cut answer to the spin delocalization versus the spin polarization mechanism for the propagation of the exchange interaction in **2** we have carried out density functional theory (DFT) calculations.^[9]

The DFT calculations performed on the dinuclear copper(II) anionic species $[\text{Cu}_2(\eta^2\text{:}\eta^2\text{-}\mathbf{1})_2]^{4-}$ indicate a ground triplet spin state lying well below the excited singlet spin state. The calculated value of the singlet–triplet energy gap ($\Delta E_{\text{ST}} = J$) is 14.2 cm^{-1} , in very good quantitative agreement with the experimental value obtained from the fit of the susceptibility data. To qualitatively understand this result, we can now turn our attention to the nature of the singly occupied molecular orbitals (SOMOs) depicted in Figure 4a. As expected, the two SOMOs, b_1 and a_2 , are nearly degenerate, with a small energy gap of 1036 cm^{-1} . They are composed by the in-phase and out-of-phase combinations, respectively, of the d_{xy} metallic orbitals of copper mixed with two π -type nonbonding molecular orbitals of *m*-phenylenediamide of

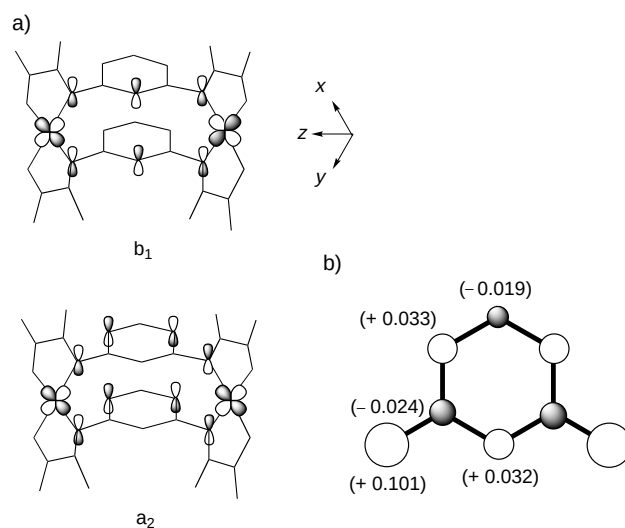


Figure 4. Schematic representation of a) the two SOMOs and b) the spin-density distribution on the bridge region for **2** on the basis of DFT calculations. Empty and full contours represent positive and negative spin densities, respectively. Calculated average atomic spin densities are given in parentheses.

appropriate symmetry. Hence, the exchange interaction is transmitted through the delocalized π system of the bridging ligands.

More importantly, an inspection of the atomic spin densities in the triplet ground state, as obtained by a Mulliken population analysis^[13] (Figure 4b), provides a precise orbital picture of the exchange interaction in **2** and, concomitantly, furnishes the ultimate reason for the ferromagnetic coupling observed in this compound. The spin densities at the amide nitrogen and carboxylate oxygen atoms have the same sign as in the copper atom, indicating spin delocalization toward the donor atoms. Moreover, the delocalization of the metal spin density (average value of $+0.561$) toward the bridging amide N atoms is important (average value of $+0.101$), mainly because of the substantial covalency of the Cu–N bond resulting from the strong basicity of the amide donor group. On the other hand, the sign alternation of the spin density at the carbon atoms of the bridging benzenes agrees with spin polarization by the amide nitrogen donor atoms. This leads to significant negative spin densities at the carbon ring atoms to which they are directly attached (average value of -0.024). Given the peculiar topology (*meta* substitution pattern) of these bridges, the spin densities at both copper atoms have the same sign, and then a net ferromagnetic exchange interaction results.

In summary, complex **2**, obtained from the self-assembly of the novel benzene-substituted bis(oxamate) ligand **1** by copper(II) ions, constitutes a rare example of paramagnetic dinuclear metallacycle.^[14, 15] This unique compound is, to our knowledge, the first dinuclear copper(II) complex for which the ferromagnetic spin coupling between the two Cu^{II} centers arises from spin polarization effects.^[16] We are currently investigating the ability of this novel dinuclear ferromagnetic synthon to bind paramagnetic species, through the substitution of Na^{I} cations by first row transition and rare earth metal cations.^[17] A wide variety of discrete high nuclearity com-

plexes and extended networks can be envisaged to elaborate new supramolecular magnetic materials (high-spin molecules and molecular-based magnets).

Experimental Section

H₄[1]: The proligand was prepared in a standard manner^[18] from the condensation of 1,3-phenylenediamine and ethyl oxalyl chloride in THF, and was isolated as the diethyl ester derivative (90%). Elemental analysis calcd (%) for C₁₄H₁₆N₂O₆ (308): C 54.55, H 5.19, N 9.09; found: C 54.57, H 5.18, N 9.04; ¹H NMR ([D₆]DMSO): δ = 1.32 (t, 6H; 2CH₃), 4.31 (q, 4H; 2CH₂O), 7.34 (dt, 1H; 5-H of C₆H₄N₂), 7.50 (dd, 2H; 4-H and 6-H of C₆H₄N₂), 8.22 (t, 1H; 2-H of C₆H₄N₂), 10.83 (s, 2H; 2NH); ¹³C NMR ([D₆]DMSO): δ = 14.17 (2CH₃), 62.71 (2CH₂O), 113.25 (2-C of C₆H₄N₂), 117.45 (4-C and 6-C of C₆H₄N₂), 129.34 (5-C of C₆H₄N₂), 138.08 (1-C and 3-C of C₆H₄N₂), 156.13 (2-C(O)NH), 161.02 (2CO₂); IR (KBr): ν̄ = 3349 (N–H), 2979, 2943 (C–H), 1725, 1715, 1698 cm^{−1} (C=O); UV/Vis (THF): λ_{max} (ε) = 335 nm (800).

Complex **2** was obtained following a reported procedure^[18] by reaction of the diethyl ester derivative of the ligand with Cu²⁺ ions in basic aqueous media, and isolated as its octahydrate sodium salt (85%). Elemental analysis calcd (%) for C₂₀H₂₄Cu₂N₄Na₄O₂₀ (859): C 27.94, H 2.79, N 6.52; found: C 27.77, H 2.62, N 6.40; IR (KBr): ν̄ = 3423 (O–H), 1659, 1597 cm^{−1} (C=O); UV/Vis (H₂O): λ_{max} (ε) = 645 (150), 380sh (1600), 345 nm (2650). Crystals of **2**, well-formed small dark green prisms, suitable for X-ray diffraction were obtained by slow vapor diffusion of methanol into concentrated aqueous solutions of **2** at 5 °C.

Crystal data for **2**: C₂₀H₂₄Cu₂N₄Na₄O₂₂, *M_r* = 895.50, triclinic, space group P1̄, *a* = 11.1489(15), *b* = 12.8545(9), *c* = 13.3454(19) Å, α = 63.855(8), β = 78.305(12), γ = 68.695(10)°, *V* = 1597.5(3) Å³, *T* = 293 K, *Z* = 2, ρ_{calcd} = 1.862 g cm^{−3}, μ(MoKα) = 1.485 mm^{−1}. Lorentz and polarization effects and absorption correction. 5579 unique reflections, and 3616 observed with *I* > 2σ(*I*). The structure was solved by direct methods using SHELXS 97, and refined by the full-matrix least-squares method on *F*² using SHELXL 97. The hydrogen atoms from the organic ligand were located from a difference synthesis and refined with an overall isotropic thermal parameter, while those from the water molecules were not found or calculated. Refinement of 471 variables with anisotropic thermal parameters for all non-hydrogen atoms gave *R* = 0.0503 and *R_w* = 0.112, with *S* = 0.97. 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-157314. 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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Conjugated Complexes Composed of Quinonediimine and Palladium: Controlled Formation of a Conjugated Trimetallic Macrocycle**

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π-Conjugated polymers have attracted much attention for application as electrical materials owing to their electrical properties.^[1] The properties of π-conjugated polymers are considered to be modified drastically by the incorporation of metal atoms.^[2, 3] Conjugated polymeric complexes in which transition metals are incorporated in the main chain represent one approach.^[3] Most of the interest in these conjugated polymeric complexes has been concerned with the nature of the interactions between metal centers through a π-conjugated chain. Transition metal directed assembly is regarded as

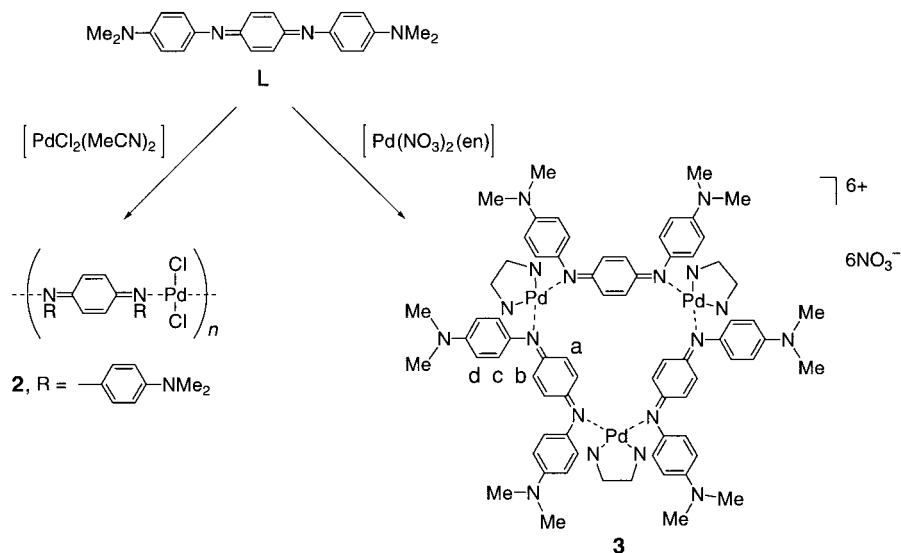
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a useful approach to an architecturally controlled formation of conjugated polymeric or macrocyclic complexes with π -conjugated ligands.^[4] We have already demonstrated that the redox-active π -conjugated ligand *N,N'*-bis(4-dimethylamino-phenyl)-1,4-benzoquinonediimine (L) serves as a bridging ligand to afford the conjugated homobimetallic palladium(II) complex [L'PdLPdL'] (**1**) with the palladium(II) complex bearing the *N*-heterocyclic tridentate podand ligand *N,N'*-bis(2-phenylethyl)-2,6-pyridinedicarboxamide (L').^[5] Complex **1** is present as a mixture of *syn* and *anti* isomers in dichloromethane. An array of L with a palladium complex having two coordination sites was expected to give a conjugated polymeric or macrocyclic complex. In fact, control of the coordination mode of the quinonediimine moiety of L allowed the formation of a conjugated trimetallic macrocycle.

Treatment of L with [PdCl₂(MeCN)₂] in acetonitrile led to the formation of the conjugated polymeric complex **2**, in which palladium centers are incorporated in the main chain (Scheme 1). The electronic spectrum of **2** in DMSO exhibited



Scheme 1. Reaction of L with [PdCl₂(MeCN)₂] and [Pd(NO₃)₂(en)].

a strong, broad absorption around 600–1000 nm, probably assignable to a low-energy charge-transfer transition with significant contribution from palladium; this indicates the coordination of the quinonediimine nitrogen atoms to palladium centers.^[5] The broad absorption band supports the formation of the conjugated polymeric complex **2**.^[6] In **2**, both *syn* and *anti* configuration of the quinonediimine moieties appear to be present.

To regulate the coordination mode of the quinonediimine moiety, a metal-directed strategy for the construction of metallomacrocycles was embarked upon by using [Pd(NO₃)₂(en)] which has *cis* binding sites as a “metal clip”. The conjugated trimetallic macrocycle [{Pd(en)(L)}₃](NO₃)₆ (**3**) was obtained quantitatively by treating L with an equimolar amount of [Pd(NO₃)₂(en)] (Scheme 1). The formation of the trimetallic macrocycle **3** was elucidated from spectral data. Only one well-resolved signal for each type of proton was observed in the downfield region of the ¹H NMR

spectrum (Figure 1); this indicates the formation of a symmetrical macrocycle. Furthermore, the H_a and H_b protons of the quinonediimine moiety were observed as singlet, which suggests the *syn* configuration. No other products were

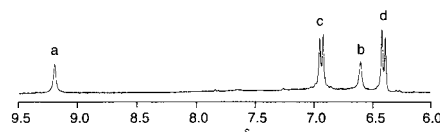


Figure 1. Downfield region of the ¹H NMR spectrum of **3**. For details see text.

detected. The ESI-MS spectrum also supported the formation of **3** (*m/z* 255.3 [M – 6NO₃]⁶⁺, 318.4 [M – 5NO₃]⁵⁺, 414.0 [M – 4NO₃]⁴⁺, 573.9 [M – 3NO₃]³⁺, 890.9 [M – 2NO₃]²⁺; Figure 2). The electronic spectrum of **3** in MeOH exhibited a strong, broad absorption band around 800 nm (Figure 3), probably assignable to a low-energy charge-transfer transition

with significant contribution from palladium owing to the coordination of the quinonediimine nitrogen atoms to palladium centers.

Further structural information on **3** was obtained by X-ray crystallography.^[7] The solid-state molecular structure of **3** confirmed a trimetallic macrocyclic skeleton and the coordination of both quinonediimine nitrogen atoms to the palladium centers in the *syn* configuration with a Pd–Pd distance of 7.68 Å (Figure 4). The molecule sits on an inversion center. The most noteworthy structural feature is that the quinonediimine planes are inclined at about 28° to the plane defined by the six nitrogen atoms of the quinonediimine moieties. An open cavity with different faces is formed, which is

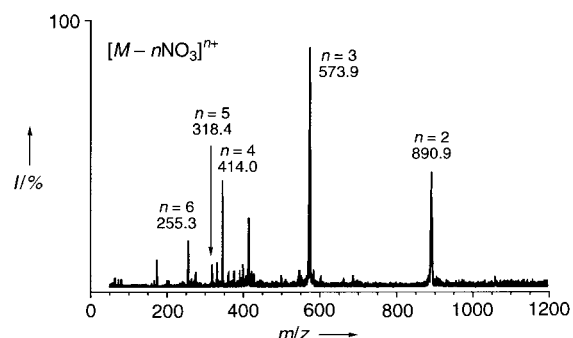


Figure 2. ESI-MS spectrum of **3**. I = intensity.

reminiscent of the cone conformation of calixarenes. This inclination of the quinonediimine planes is probably due to the coordination of the nitrogen atoms to the palladium centers, which results in ideal bite angles at Pd (N_{qd}–Pd–N_{qd} 91.2, 91.3°). The top and bottom sizes of the cavity, defined as

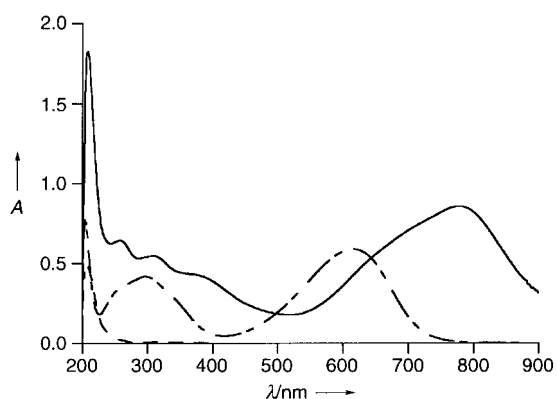


Figure 3. Electronic spectra of $[\text{Pd}(\text{NO}_3)_2(\text{en})]$ (----), **L** (— · —), and **3** (—); $2.0 \times 10^{-5} \text{ M}$ in MeOH. A = absorbance.

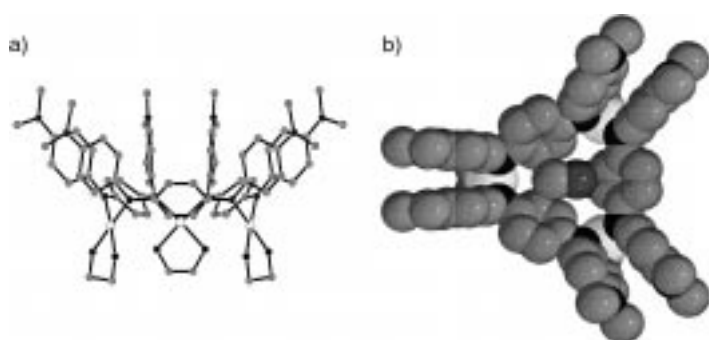


Figure 4. a) Molecular structure of **3** (H atoms, NO_3^- ions, and MeOH molecules omitted for clarity). b) Space-filling representation of the molecular structure of **3** (H atoms and NO_3^- ions omitted for clarity). Two MeOH molecules are located at the top and bottom of the cavity.

the distance between C_a and C_a' and between C_b and C_b' , are 3.66 and 6.72 Å, respectively (Figure 5). Due to π conjugation of **L**, the orientation of each phenylene ring should be nearly parallel to the quinonediimine plane. The steric interaction between the hydrogen atoms H_b and H_c , however, requires rotation of the phenylene ring of **L** away from this orientation, and results in dihedral angles of 43.2, 45.4, and 53.3° between the phenylene and quinonediimine planes (Figure 5). Another remarkable feature of the structure is the orientation of the phenylene rings of **L** in a face-to-face arrangement at a

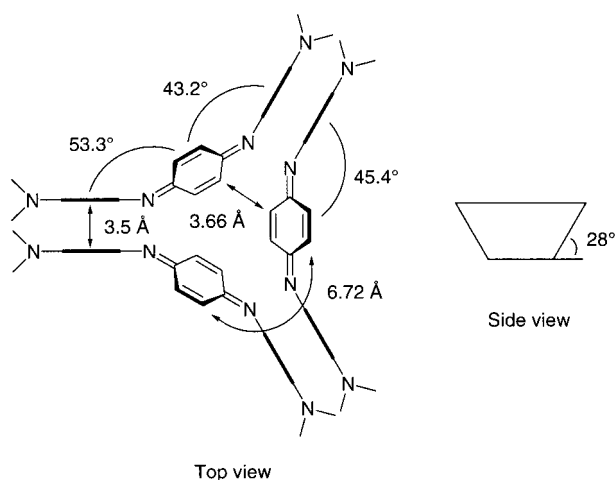


Figure 5. Schematic representation of **3**.

distance of about 3.5 Å at each corner of the triangle, which suggests a π – π stacking interaction. In addition to *cis* binding to $[\text{Pd}(\text{en})]$ units this π – π stacking interaction may partly contribute to the preferential formation of the trimetallic macrocycle over polymeric complexes. Architectural control of well-arranged metallomacrocycles based on transition metal directed assembly is a current research area in metal-directed chemistry.^[4, 8, 9] Considerable attention has been focused on the construction of a variety of metallosquares,^[4, 8] while metallotriangles have remained relatively unexplored.^[4c–e, 8h,m,o, 9] The angular strain at the metal center and the ligand flexibility resulted in the selective formation of the metallotriangle **3**.

Macrocycle **3** accommodates two MeOH molecules at the top and bottom of the cavity. This indicates the capability to encapsulate guest molecules. A preliminary experiment on guest binding in D_2O showed that the association constant for 1,2-dimethoxybenzene, calculated from ^1H NMR spectroscopic data, was $4.0 \times 10^3 \text{ M}^{-1}$.

In conclusion, an alternative coordination array of the redox-active π -conjugated bridging quinonediimine spacer **L** and a palladium unit led to the formation of the conjugated polymeric complex **2** or the conjugated trimetallic macrocycle **3**. In the latter case, the complexation is architecturally controlled by *cis* binding to $[\text{Pd}(\text{en})]$ fragments to give a trimetallic macrocycle with a cone conformation. A π – π stacking interaction between the phenylene rings of **L** may stabilize the triangular complex. An open cavity appears to offer a binding site for guest molecules. The present conjugated complexes are regarded as potential redox-active systems, which might be applied as catalysts and electronic materials. Further investigation, including redox and recognition properties of the trimetallic macrocycle **3**, is now in progress.

Experimental Section

3: A mixture of **L** (69.0 mg, 0.20 mmol) and $[\text{Pd}(\text{NO}_3)_2(\text{en})]$ (58.1 mg, 0.20 mmol) was stirred in acetonitrile (20 mL) under argon at room temperature for 2 h. After evaporation of the solvent, compound **3** was isolated in 92% yield by recrystallization from methanol. ^1H NMR (400 MHz, D_2O): δ = 9.17 (s, 6H), 6.91 (d, J = 8.7 Hz, 12H), 6.58 (s, 6H), 6.37 (d, J = 8.7 Hz, 12H), 3.01–2.95 (m, 6H), 2.94–2.88 (m, 6H), 2.90 (s, 36H); ^{13}C NMR (100 MHz, D_2O): δ = 158.1, 151.7, 136.9, 134.5, 129.2, 127.1, 112.6, 47.0, 39.7; ESI-MS: see Figure 2.

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- [6] The distinct signal broadening observed by NMR spectroscopy during the titration of L with [PdCl₂(MeCN)₂] indicated the formation of a conjugated polymeric complex. We have also demonstrated that the controlled complexation of the emeraldine base of poly(*o*-toluidine) with Pd^{II} compounds in an organic solvent affords cross-linked conjugated polymeric complexes.^[2b]
- [7] Crystal data for **3**: C₇₂H₉₆N₂₄O₁₈Pd₃·2CH₃OH, *M*_r = 1968.98, orthorhombic, space group *Pnma* (no. 62), *a* = 27.6315(9), *b* = 22.1045(7), *c* = 17.7133(6) Å, *V* = 10818.9(6) Å³, *Z* = 4, *T* = 23.0 °C, *ρ*_{calcd} = 1.209 g cm⁻³, *μ*(MoK_α) = 5.59 cm⁻¹, MoK_α radiation (*λ* = 0.71069 Å), *R* = 0.090, *R*_w = 0.253. 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-160516. 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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Directing Topoisomerase I Mediated DNA Cleavage to Specific Sites by Camptothecin Tethered to Minor- and Major-Groove Ligands**

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Hairpin polyamides containing pyrrole- and imidazole-carboxamide units represent convenient tools to target specific sequences in DNA.^[1] These compounds form dimeric motifs that fit snugly into the minor groove of B-DNA and recognize defined sequences through the formation of an array of hydrogen bonds and van der Waals contacts. They provide a paradigm for the design of small molecules that regulate genes.^[2]

The sequence-recognition properties of hairpin polyamides can be exploited for directing DNA-cleaving and -alkylating agents to particular sequences.^[3–7] By analogy, we reasoned that they could also be used to direct the action of DNA-cleaving proteins such as topoisomerases. These ubiquitous

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enzymes regulate DNA topology in cells and therefore are essential for many vital processes, such as replication, transcription, and chromosome segregation.^[8] Topoisomerases I and II both represent privileged targets for a number of cytotoxic anticancer drugs.^[9] In particular, the camptothecins (CPT), which include the clinically available derivatives topotecan and irinotecan, are the most potent inhibitors capable of stimulating DNA single strand cleavage by topoisomerase I at a variety of sites, essentially at T–G sites.^[10, 11]

DNA cleavage by topoisomerase I can be targeted to specific sequences by the linkage of topoisomerase inhibitors to sequence-recognition elements, such as oligonucleotides that form triple helices^[12–14] or small molecules related to the antibiotics netropsin and distamycin.^[3, 15, 16] The limited sequence-recognition properties of these antibiotics and analogues that form 1:1 drug:DNA complexes restrict their use as DNA-cleaving delivery systems. No sequence specificity was reported for the cleavage by the conjugates with the camptothecin derivatives.^[15] Here we present an alternative approach to direct camptothecin to specific sites in DNA and to introduce DNA breaks at a precise location by making use of the recognition of the target sequence by hairpin polyamide ligands that bind to the minor groove of DNA in a sequence-specific manner.^[1] We have compared these conjugates to camptothecin that is covalently linked to triplex-forming oligonucleotides that bind in the major groove at oligopyrimidine · oligopurine sequences.

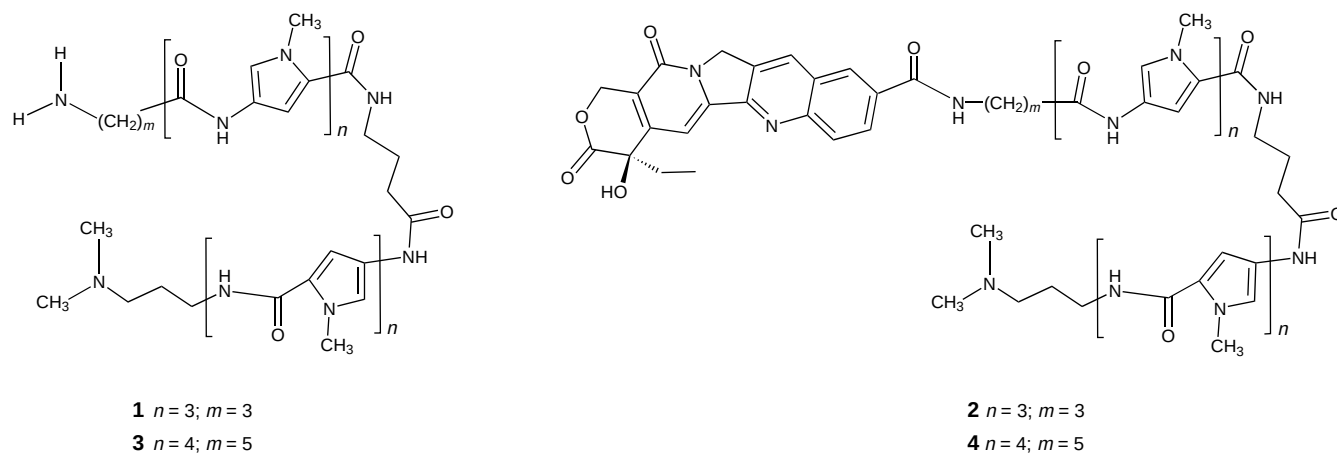
Two hairpin polyamides, each containing two series of three or four *N*-methylpyrrolicarboxamide units that form antiparallel dimers, were synthesized as previously described.^[17] Compounds **1** and **3** were linked to 10-carboxycamptothecin through aminoalkyl side chains of different lengths. The two minor-groove binder/camptothecin (MGB–CPT) conjugates **2** and **4** contain the same dimethylaminopropylamino cationic side chain on the C-terminal pyrrole heterocycle and an identical γ -aminobutyrate residue between the two oligopyrrole branches, but they differ in the number of *N*-methylated pyrrole units (2×3 or 2×4 pyrrole rings) and the nature of the alkyl connector (propyl in **2** or pentyl in **4**) between the MGB moiety and the topoisomerase I inhibitor. The coupling procedure recently described for the synthesis of oligonucleotide-aminoalkyl-CPT conjugates was employed.^[12, 13] Briefly,

the 10-carboxyl group of the camptothecin derivative was first treated with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to provide the corresponding active ester, which was then treated with the minor-groove binder **1** or **3**. The resulting MGB–CPT hybrid molecules **2** and **4** were separated from the remaining camptothecin molecules by successive precipitations from a mixture of DMSO and acetonitrile (1:4). The final compounds were characterized by UV spectroscopy and mass spectrometry.

The MGB–CPT conjugates were incubated with a 324 base pair (bp) ³²P-labeled DNA fragment prior to the addition of topoisomerase I to initiate the DNA cleavage reactions. Control experiments were performed in parallel with camptothecin alone and with the MGBs **1** and **3**, as well as with the noncovalent association of camptothecin plus each of the two MGBs (CPT + **1** and CPT + **3**). In all cases the DNA samples were treated with sodium dodecylsulfate (SDS) and proteinase K to remove any covalently bound protein and then resolved on an 8% polyacrylamide sequencing gel under denaturing conditions so as to identify the sequence of the drug-induced topoisomerase I cleavage sites. A typical example of a gel is shown in Figure 1a and a summary map showing the position of the main cleavage sites induced by CPT (asterisks) is presented in Figure 2. The positions of the AT-rich MGB-binding sites (boxed sequences in Figure 2) were inferred from DNase I footprinting experiments performed with the same *Eco*RI-*Pvu*II 324-bp restriction fragment (³²P-labeled at the 3'-end of the *Eco*RI site) incubated with the untethered polyamides **1** and **3** at 3 μ M (data not shown). This particular DNA fragment contains a 24 nucleotide long oligopyrimidine · oligopurine tract used previously for triple helix formation.^[12, 13] It was selected because it contained several CPT-sensitive sites, some of which are close to short AT tracts, thus providing exquisite binding sites for oligopyrrole polyamides such as compounds **1** and **3**. In addition it allowed us to compare the cleavage efficacy and selectivity of minor-groove and major-groove ligands conjugated to CPT.

With camptothecin alone, the position of seven cleavage sites (a–g) were determined with nucleotide resolution by various electrophoresis experiments (Figure 2).

As shown in Figure 1, the covalent linkage of a MGB to the CPT chromophore exerts a drastic effect on the sequence



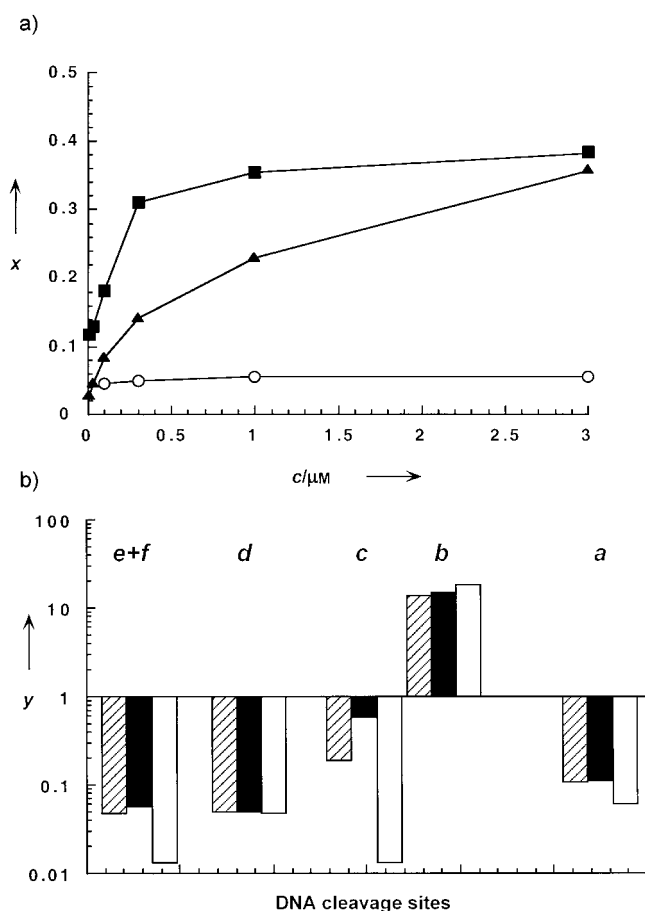


Figure 3. a) Quantification of the intensity of topoisomerase I mediated cleavage (x) at site b is reported as a function of the concentration (μM) for free camptothecin ($\circ$), hybrid **2** ($\blacktriangle$), and **4** ($\blacksquare$). x = fraction of cleaved DNA. b) Intensities of the cleavage observed in the presence of conjugates MGB-CPT **2**, **4**, and TFO-CPT ($3 \mu\text{M}$ each) are indicated by hatched, black, and white bars, respectively. The extent of the topoisomerase I mediated cleavage refers to the ratio between the intensity of the cleavage in the presence of the conjugate and that in the presence of free CPT (y) and is reported on a logarithmic scale. The position of the cleavage sites is indicated. y = ratio of cleavage intensity for conjugated drug versus free drug.

single site in the DNA fragment; cleavage at all the other sites disappeared upon conjugation of the CPT to the DNA ligand. Some cleavage at site c was still observed, since we used conjugate **4** for the comparison, which is in contrast to the conjugate TFO-CPT. It should be noted that the linkers were different for the two conjugates (Figure 1b). However, even though the CPT moiety is brought from the major-groove side of DNA by the TFO and from the minor-groove side by the MGB, both approaches provide a suitable route to guide a cytotoxic agent to a selected sequence in DNA (Figure 3b). The main advantage of MGB-CPT over TFO-CPT conjugates is that the binding of MGBs is not restricted to oligopyrimidine·oligopurine DNA sequences as observed with TFOs. In addition, MGBs are expected to be more cell permeable than TFOs and, therefore, might be more suitable for cell studies.

In summary, prominent cutting at site b attests that the covalent linkage of a hairpin polyamide to camptothecin provides an efficient system to direct DNA cleavage mediated

by topoisomerase I to specific sites. Hairpin polyamide/camptothecin conjugates represent a new category of single-strand-breaking artificial nucleases that could provide a basis for potential therapeutic applications.

Experimental Section

Synthesis of the CPT-MGB conjugates: 10-Carboxycamptothecin (0.005 mmol) was activated with a small excess of *N*-hydroxysuccinimide (0.007 mmol) in the presence of dicyclohexylcarbodiimide (0.007 mmol) for 8 h at room temperature in dry dimethylformamide (DMF). The camptothecin ester was then precipitated by diethyl ether, dissolved in DMF, and added to a solution of **1** or **3** (at 0.001 mmol) and triethylamine (4 μL) in dimethyl sulfoxide (DMSO; 50 μL) and left to stand at room temperature in the dark for 8 h. The product was isolated by precipitation with deionized water (1.5 mL). The pellet was dried, dissolved again in DMSO, and precipitated twice with 80% acetonitrile, then dried, and then dissolved in DMSO for subsequent biochemical experiments.

The resulting conjugate **2** or **4** was characterized by UV spectroscopy and by mass spectrometry. The $A_{379}:A_{310}$ ratio demonstrated a 1:1 stoichiometry of camptothecin to the minor groove binder in the conjugate. MS (matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)); found: 1381.7 (calcd: 1382.6) for **2**.

DNase I footprinting experiments: The protocol previously described was used.^[18] A 324-bp 3'-end radiolabeled DNA fragment was prepared as described in ref. [13]. The DNA was incubated with the conjugate at room temperature for 6 h.

Topoisomerase I cleavage assays were performed using the calf thymus enzyme at room temperature as described in refs. [13, 14].

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Caged Thiophosphotyrosine Peptides**

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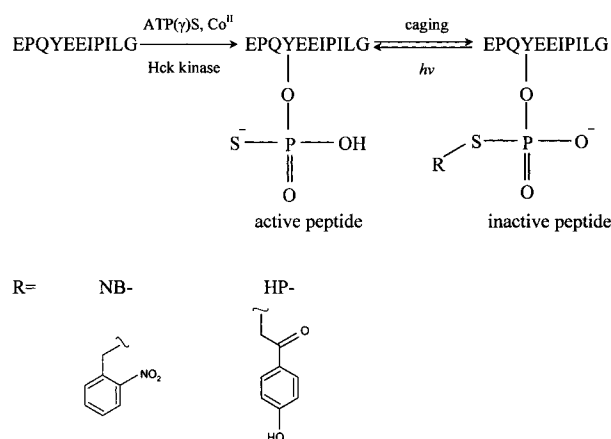
Caged reagents are molecules from which biological effectors are released by photolysis.^[1, 2] In recent studies, caged peptides and proteins have received attention because of their potential importance in investigations of processes such as cell signaling.^[3, 4] Although methods suited to specific situations have been explored,^[5–10] we have focused our attention on general procedures to cage unprotected peptides and proteins in aqueous solutions.

Because of the relative ease of nucleophilic substitution on cysteine, this amino acid has been introduced into peptides by chemical synthesis and into proteins by mutagenesis as a site for modification by caging reagents.^[3, 11–13] We recognized that most proteins involved in cell signaling are regulated by phosphorylation. Indeed, up to 30 % of proteins in mammalian cells are phosphorylated and well over one percent of mammalian proteins are protein kinases.^[14–16] Therefore, we reasoned that thiophosphoryl groups in peptides and proteins might also be utilized as sites for caging. This idea was readily demonstrated with thiophosphorylserine residues in peptides, by reaction with 2-nitrobenzyl bromide (NBB) to generate caged peptides that could be converted back into the thiophosphoryl peptides by near-UV light.^[17] Unfortunately, we encountered difficulties with thiophosphoryltyrosine residues, which was especially troublesome because of the pivotal role of tyrosine phosphorylation in cell signaling.^[18–23]

The initial difficulty centered around the enzymatic thiophosphorylation of model peptides. Herein, we show that tyrosine-containing peptides can be thiophosphorylated with cognate kinases by using divalent transition metal ions instead of Mg^{II} in the reaction buffer. The thiophosphorylated peptides can then be caged by reaction with electrophilic reagents such as NBB or *p*-hydroxyphenacyl bromide (HPB). The *p*-hydroxyphenacyl group has been used previously to cage molecules, including amino acids and oligopeptides, on carboxylate groups^[24–27] and, recently, a protein phosphatase on a cysteine residue.^[28] The mechanism of photorelease of substrates from *p*-hydroxyphenacyl carboxylate and phos-

phate esters has recently been examined.^[27, 29, 30] In the present context, HPB is shown to be superior to the prevailing 2-nitrobenzyl reagents.

In attempting to prepare thiophosphotyrosine peptides, we were, in most cases, unable to detect high extents of thiophosphorylation with ATP(γ)S by using tyrosine kinases under conditions that work well when ATP itself is used.^[31–33] For example, Abl, Src, and EGF and insulin receptor kinases^[23] failed to thiophosphorylate peptide substrates. To explore this problem more thoroughly, we focused on EPQYEEIPILG. A related peptide, EPQYEEIPIYL, binds to Src homology (SH2) domains when phosphorylated and phosphorylated EPQYEEIPIA acts as an activator of Src kinase.^[34] Divalent metal ions other than Mg^{II} have proved to be useful for promoting thiophosphorylation in other circumstances. For example, Cole and colleagues showed that k_{cat} for the phosphorylation of poly(Glu, Tyr) by C-terminal Src kinase (Csk) is hardly reduced for ATP(γ)S versus ATP in the presence of Ni^{II} and Co^{II} , while ATP(γ)S is far less effective in the presence of Mg^{II} and Mn^{II} .^[33] Following this lead, we found that EPQYEEIPILG could be thiophosphorylated with Hck kinase in the presence of Co^{II} (Scheme 1).^[35]



Scheme 1. Caging and uncaging of thiophosphotyrosine peptides. The caging reagents are 2-nitrobenzyl bromide ($R = NB$) and *p*-hydroxyphenacyl bromide ($R = HP$).

Thiophosphorylated EPQYEEIPILG was treated with a five-fold molar excess of HPB or NBB in the dark at room temperature in various aqueous buffers.^[36] The reaction products were separated by high-pressure liquid chromatography (HPLC) and identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). By using ^{35}S -labeled EPQY_{ps}EEIPILG, the yields of the two caged products could be determined by HPLC and scintillation counting: *p*-hydroxyphenacyl derivative EPQY_{ps}(HP)EEIPILG, 90 %; 2-nitrobenzyl derivative EPQY_{ps}(NB)EEIPILG, 75 %. In both cases, the final yield was little affected by the pH of the reaction buffer in the range pH 5.8 to 8.0. The stability of EPQY_{ps}(HP)EEIPILG and EPQY_{ps}(NB)EEIPILG was tested, after purification by HPLC. Both caged peptides were stable in aqueous solution at 37 °C for at least 24 h at pH values from 5.8 to 8.2.^[37]

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EPQY_p(HP)EEIPILG and EPQY_p(NB)EEIPILG were uncaged by photolysis at 312 nm.^[38] EPQY_pEEIPILG was released from EPQY_p(HP)EEIPILG in a yield of 50 to 70% at both pH 5.8 and pH 7.3. The $t_{1/2}$ values were 0.55 min at pH 5.8 and 0.52 min at pH 7.3, which correspond to product quantum yield (ϕ_p) values of 0.65 and 0.56 at the respective pH values. At pH 8.2, the yield was greatly reduced and several by-products were formed that could not be identified by MALDI-MS. Under the same conditions, EPQY_pEEIPILG was released from EPQY_p(NB)EEIPILG in 50 to 60% yield. The $t_{1/2}$ values were 5.5 min at pH 5.8 and 8.0 min at pH 7.3, which correspond to ϕ_p values of 0.37 and 0.25.

Finally, the caged peptides and uncaged products were tested in a biochemical assay, which measured the ability of the peptides to bind to an immobilized SH2 domain^[39] (Figure 1). When ³⁵S-EPQY_pEEIPILG was incubated with excess Src SH2-GST attached to agarose,^[40, 41] 27–31% of the radiolabel bound. When the caged ³⁵S-labeled peptides EPQY_p(HP)EEIPILG and EPQY_p(NB)EEIPILG were tested only 1–5% of the radioactivity was bound, which largely represents nonspecific binding, as determined with competing unlabeled peptide or by using an irrelevant peptide. After photolysis of EPQY_p(HP)EEIPILG and EPQY_p(NB)EEIPILG at 312 nm, in both cases, 25–31% of the radioactivity in the uncaged product, repurified by HPLC, was bound to the immobilized SH2 domains. When the uncaged product was not subjected to repurification, the extent of release as judged by Src SH2-agarose binding (corrected for binding efficiency) was consistent with the yield of 50–70% obtained by HPLC.

Here, we have shown that an SH2-binding peptide can be enzymatically thiophosphorylated on tyrosine and then caged with either the NB or HP protecting group. The caged peptides are no longer able to bind to an SH2 domain, but this activity is restored upon photolysis. The NB and HP groups serve their purpose equally well when caging efficiency, the stability of the caged peptide, and the yield of functional peptide after uncaging are considered. However, the HP-protected peptide is about 10 times more sensitive to near-UV radiation, which can be attributed largely to higher ϵ values in the wavelength range used here and to a lesser extent to higher ϕ_p values. While the

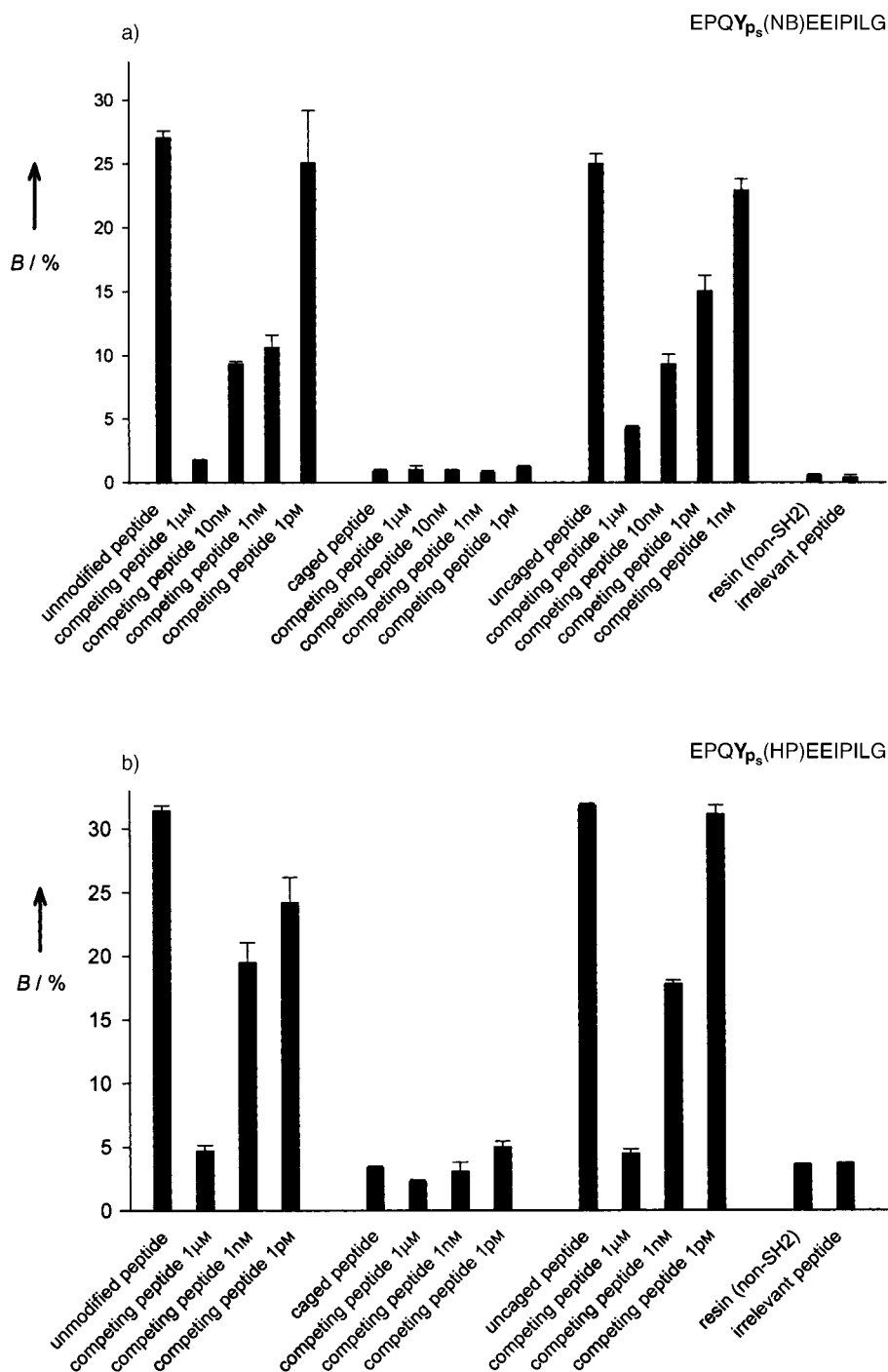


Figure 1. Binding of caged and uncaged peptides to a recombinant SH2 domain. a) EPQY_pEEIPILG caged with NBB; b) EPQY_pEEIPILG caged with HPB. ³⁵S-labeled caged or uncaged peptides were incubated with a Src SH2-SH3-GST fusion protein immobilized on glutathione-agarose beads. *B* is the percentage of the radioactivity retained by the beads. The bound peptides were eluted from the washed beads and assayed by scintillation counting. Competing peptide: EPQY_pEEIPILG; irrelevant peptide: ³⁵S-labeled thiophosphoryl kemptide (LRRASLG).

photosensitivity of a caged reagent ($\phi_{p\epsilon}$) is most important for experiments in cell biology, where photochemical damage to living cells and tissues must be avoided, ϕ_p itself can be of primary importance in certain biophysical experiments, where processes must be triggered with a single flash of light. The values of ϕ_p determined here were in the same range as those measured for *p*-hydroxyphenacyl carboxylate and phosphate

esters under various conditions in the neutral pH range (all $\phi_p > 0.2$).^[27, 29, 30] The HP group generally has the additional advantage of much faster departure after absorption of a photon^[27] compared with the NB group^[42] and we expect that this finding will apply to thiophosphate esters caged on sulfur. At pH values above 8.0, the photolysis of **EPQY_p(HP)EEI-PILG** produced by-products as well as the uncaged peptide. The pK_a of the *p*-hydroxyphenacyl group in water is ~ 8 . Zhang et al. found that the ϕ_p of *p*-hydroxyphenyl-2-oxoethyl acetate dropped to zero when the phenolic group was deprotonated in the ground state.^[29] It might be possible to adjust the pK_a of hydroxyphenacyl reagents, and thereby ϕ_p , by judicious substitution of the aromatic ring. That work is in progress. In any event, with ϕ_{pE} values of ~ 5000 , *p*-hydroxyphenacyl reagents are of indisputable utility and we are now extending our studies to signaling proteins thiophosphorylated on serine and tyrosine and to applications in cells.

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- [35] The **EPQYEEIPIILG** peptide (2 mm final) was thiophosphorylated with Hck kinase in buffer (100 μ L) containing 50 mM Tris·HCl, pH 7.3, 0.015% Brij 35, 50 μ M *N,N'*-(1,2-ethanediyl)bis[(*N*-carboxymethyl)glycine] (EDTA), 10 mM CoCl₂, ATP(γ S) (Sigma, in 100 μ L of water) was added in five-fold molar excess with respect to the peptide. Hck kinase (0.25–0.5 μ g in 1–2 μ L of storage buffer) was added and the mixture was incubated at 30 °C for 30–60 min. The thiophosphorylated peptide was purified by reverse-phase HPLC with a Vydac C18 column (linear gradient of 10–40% CH₃CN; buffer A: 0.1% CF₃COOH in water; buffer B: 0.075% CF₃COOH in CH₃CN) and identified by MALDI-MS. To prepare ³⁵S-labeled peptide, 100 μ Ci ³⁵S-ATP(γ S) (Amersham) was used, diluted to a specific activity of 177 Ci mmol⁻¹ with ATP(γ S).
- [36] The thiophosphorylated peptide **EPQY_pEEIPIILG** was chemically modified with HPB or NBB in 100 mM sodium acetate buffer (pH 5.8), or 100 mM Tris·HCl (pH 7.3 or 8.0), at 25 °C for 30 min in the dark. Both reagents were prepared as 50 mM stock solutions in 95% EtOH. The final concentration of the reagents was 1 mM, a five-fold molar excess with respect to the peptide. The caged products were purified by HPLC by using the reverse-phase column. The products were identified by MALDI-MS. **EPQY_pEEIPIILG** could not be modified cleanly with 2-bromo-2-(2-nitrophenyl)acetic acid (BNPA) or 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB) (data not given).
- [37] The radiolabeled caged peptides were stored at pH 5.8 (100 mM sodium acetate), pH 7.3 (100 mM Tris·HCl), and pH 8.2 (100 mM Tris·HCl) at both 4 °C and 37 °C for 24 h. No decomposition was detected by HPLC.
- [38] Photolysis of HPLC-purified ³⁵S-labeled caged peptides at 4 nm concentration was carried out in volumes of 200 μ L at pH 5.8 (100 mM sodium acetate), pH 7.3 (100 mM Tris·HCl), and pH 8.2 (100 mM Tris·HCl) in the wells of a 96-well plate (well size of 360 μ L: 0.36 cm² $\times$ 1 cm) by irradiation with a 30 W UV lamp (2200 μ W cm⁻², peak emission 312 nm, Cole-Parmer E-09815-22). The lamp was set 1.5 cm above the sample, which was shielded by a glass band-pass filter to provide a window at 280–370 nm (Oriel 59154). The light intensity was measured by the irradiation of 6 mM K₃Fe(C₂O₄)₃ as described previously.^[43] The photolysis products were removed at various time points during irradiation and subjected to HPLC analysis. The peptides were identified by MALDI-MS and quantified by scintillation counting. The half-time of photolysis ($t_{1/2}$) and product quantum yields (ϕ_p) were estimated by using $t_{1/2} = 0.3/\phi_p I_0$.^[3] Corrections were made for the spectral distribution of the lamp and absorption by the filter.
- [39] A Src SH2-SH3-GST fusion protein was expressed in *E. coli* and purified.^[44] The protein was immobilized on glutathione-Sepharose 4B beads (Pharmacia, No. 17–0756–01) as described previously.^[40] ³⁵S-labeled peptides (10 μ L, 50 nM), photolyzed where indicated, were incubated with SH2-SH3-GST agarose (20 μ L slurry, 1.6 μ M bound protein) in a total volume of 250 μ L containing 50 mM Tris·HCl, pH 7.3, 200 mM NaCl, 10 mM 1,4-dithio-threitol (DTT), 0.05% (w/v) bovine serum albumin for 5 h at room temperature and cooled to 4 °C. After collecting the flow-through, the beads were washed twice, within 1 min, at 4 °C with the incubation buffer (400 μ L). Bound peptide was then eluted with CF₃COOH (0.1%, 100 μ L) and assayed by scintillation counting.
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Adhesion Forces between Lewis^X Determinant Antigens as Measured by Atomic Force Microscopy**

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A carbohydrate coat named glycocalix covers the outer membrane of most types of cells. These carbohydrates at the cell surface are responsible for communication with other cells and with the extracellular matrix through interaction with proteins.^[1] Recently, carbohydrate to carbohydrate interactions have been proposed as the initial step of cell adhesion.^[2, 3] Embryogenesis, metastasis, and other proliferation processes are, according to Hakomori,^[2] mediated by carbohydrate–carbohydrate interactions. Some of the structures involved in this novel mechanism are the trisaccharide Lewis^X determinant (Gal β 1 $\rightarrow$ 4[Fuca1 $\rightarrow$ 3]GlcNAc β 1) and the glycosphingolipids GM3 (NeuAc α 2 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4Glc β 1Cer) and Gg3 (GalNAc β 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4Glc β 1Cer). The Lewis^X antigen (Le^X)—previously defined as the stage-specific embryonic antigen (SSEA-1)—is found in a wide variety of systems^[4] such as human cancers, pre-implantation mouse embryos, embryonic carcinoma cells, and human erythrocytes. This antigen mediates, by a homotypic interaction, morula compaction and teratocarcinoma metastasis in mouse cells.^[2, 5] A heterotypic interaction between GM3 and Gg3 also seems to be involved in metastasis of melanoma cells in mice.^[6, 7] Characteristic features of these interactions are a strong dependency on divalent cations (Ca²⁺), a high specificity and a low affinity. Nature solves these problems by a multivalent presentation of the carbohydrate antigen at the cell surface. These characteristics make it difficult to study and to quantify this process.^[8, 9] Nevertheless, in previous studies using synthetic receptors we have shown that the interaction between lipophilic patches of simple carbohydrate units in water contributes to the free energy of binding with a stabilization of up to 7.1 kJ mol⁻¹.^[10, 11]

Recently, atomic force microscopy (AFM)^[12] has been successfully applied to measure adhesion forces between complementary DNA strands,^[13] avidin–biotin derivatives,^[14–16] antigen–antibody systems,^[17] adhesion proteoglycans of marine sponges,^[18] and cadherins^[19] under physiological conditions. The study on the adhesion proteoglycans is, to

our knowledge, the only example in which a carbohydrate–carbohydrate interaction has been determined by AFM.^[18]

Herein, we present a quantitative AFM study of the specific interaction forces between individual Le^X-antigen molecules in aqueous solution using chemically well defined, two dimensional (2D), self-assembled monolayer (SAM) surfaces. This study demonstrates the self-recognition ability and selectivity of the Le^X antigen. To measure these adhesion forces, flat gold surfaces were functionalized with the Le^X determinant containing neoglycoconjugate **2** to form self-assembled monolayers. For comparison purposes a parallel study was carried out by similarly functionalizing gold surfaces with lactose (Gal β 1-4Glc) containing neoglycoconjugate **1** (Figure 1). Neoglycoconjugates **1** and **2** were

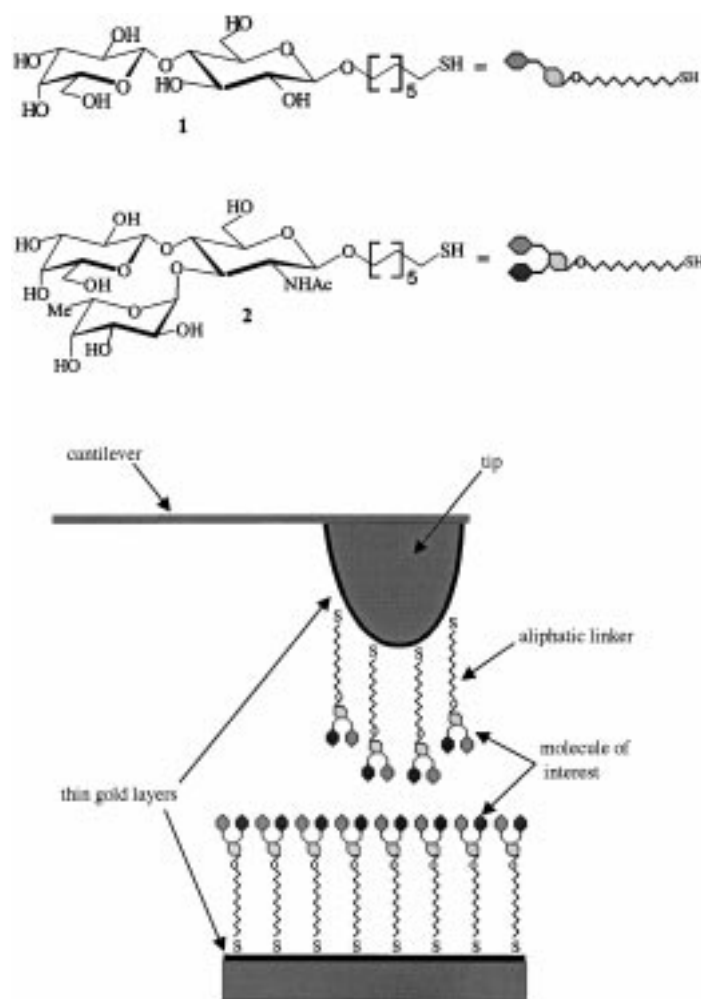


Figure 1. Neoglycoconjugates **1** and **2**, and schematic representation of the sample and the AFM tip which is functionalized with self-assembled monolayers of the neoglycoconjugates.

synthesized (manuscript in preparation) by glycosidation of conveniently protected lactose and Le^X derivatives, respectively, with 11-mercapto undecanol using the trichloroacetimidate method.^[20] The thiol group was incorporated to attach these molecules to the gold surfaces. A micro-fabricated AFM cantilever^[21] and a gold-surface sample were functionalized with neoglycoconjugates **1** and **2** by immersion for at least 2 h,

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in 1 mM solutions of **1** and **2** in methanol and water, respectively, then rinsed several times with the same solvent and dried under a stream of nitrogen.

The neoglycoconjugates organize themselves as monolayers both on the tip and on the sample.^[22] Although this model may be considered faraway from the natural glycosphingolipids presentation, it provides a polyvalent functionalized surface with chemically well-defined structure to evaluate carbohydrate–carbohydrate interactions. In a typical procedure, a series of approach and retract cycles with the Le^X functionalized tip and sample were carried out in water and in 10 mM aqueous calcium solution in a liquid cell. Unloading rates of about 100 nm s^{−1} were used. For each force scan, the functionalized tip was maintained in contact with the molecular surface at a constant force (150 pN) for two seconds before unloading to ensure intermolecular contact and chemical equilibrium. The unloading curve, representing the adhesion force as a function of the distance, shows a steplike behavior (Figure 2). Each step corresponds to an out of equilibrium cantilever movement because of the unbinding events among the molecules of the tip and the molecules of the sample. Measurement of the step height leads to the determination of the intermolecular unbinding force. For each sample, more than 300 curves over 15 different areas of the sample surface were recorded.

Typical force–distance curves obtained with a tip and a sample functionalized with Le^X determinant are presented in Figure 2A and 2D. The stepwise profile of the curves is characteristic of molecular interaction, and was observed in water as well as in calcium solution (Figure 2A and 2D, respectively). A statistical analysis of the step height^[14] reveals that the measured unbinding forces are entire multiples of 20 ± 4 pN (Figure 2B and 2E). The strong periodicity of the

auto-correlation function (Figure 2C and 2F) is an important evidence that the interaction observed in these experiments results exclusively from specific adhesion forces. The 20 ± 4 pN force has been assigned to the binding force between two individual Le^X-determinant molecules. The value of the force was independent of the presence of calcium ions in the solution. This result was not expected based on the described characteristics of carbohydrate–carbohydrate interactions. However, in experiments using many different tips and different samples the same 20 ± 4 pN interaction force was obtained.

Carbohydrate to carbohydrate interaction is based mainly on van der Waals contacts between the corresponding polyamphiphilic surfaces involved in the process.^[2, 23] The complementary nature of these surfaces makes the interaction highly specific and calcium ions seem to be crucial for this process to occur in biological and other model systems.^[2] The role of calcium in carbohydrate–carbohydrate interactions is not well understood. Calcium ions could be responsible for the approach and organization of the sugar moieties which provide the adequate surfaces for interaction, or may enhance the adhesion force between complementary surfaces, acting as a bridge between specific hydroxy groups.

In the AFM experiments presented here, the formation of a well-packed self-assembled monolayer provides the organization of carbohydrates on the surfaces. Furthermore, interaction between the molecules is established by applying an initial contact force in the cantilever which makes the Le^X-functionalized surfaces approach. Therefore, if the role of calcium ions is to favor the approach and organization of the molecules at the surface, it could not be detected in the AFM experiment. However, if the role of calcium is to reinforce the contact between the interacting molecules, an increase in the

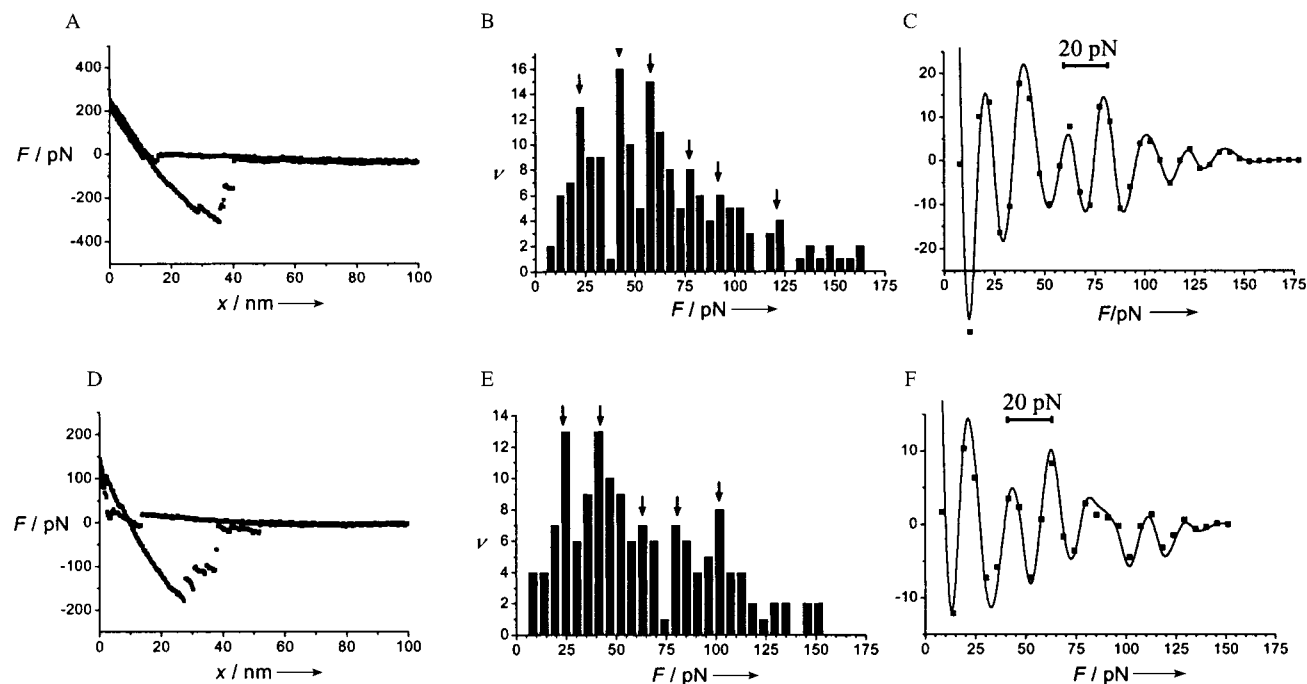


Figure 2. A) and D) Typical AFM force–distance curves obtained between an Le^X-functionalized tip and a SAM of Le^X molecules in water and in 10 mM calcium solution, respectively, B) and E) histogram of the unbinding force measured through 300 force–distance curves of Le^X molecules in water and in 10 mM calcium solution, respectively, and C) and F) corresponding auto-correlation function.

adhesion-force values in calcium solution should be observed.^[18] In these experiments, the presence of calcium ions does not contribute significantly to the adhesion force. This result may indicate that calcium is only responsible for the approach and organization of the carbohydrates in the cell membrane. This proposal should be considered as a model that can be refuted with new experimental evidence. It is interesting to note that in experiments with a three dimensional (3D) polyvalent Le^x model (glyconanoparticles) in aqueous solution, where the molecules move freely, we have observed that self-aggregation of the Le^x molecules only occurs in the presence of calcium cations.^[24]

Control experiments confirmed that the adhesion force of 20 pN corresponds to the Le^x–Le^x interaction. Force distance curves were measured between an Le^x-functionalized tip and a 11-mercapto undecanol functionalized sample. 500 force scans were performed over 12 different areas of the sample surface; in none of them was interaction observed among functionalized surfaces, thus indicating that the observed adhesion force is really because of the Le^x–Le^x specific interactions and not because of unspecific ones in which the linker could be involved. On the other hand, in control experiments, no interaction was observed between the lactose-functionalized tip and the sample, either in water or in aqueous calcium solution (Figure 3 A), showing the adhesive properties of the Le^x determinant in comparison with the lactose derivative. Additionally, AFM crossed experiments between lactose-functionalized tips and Le^x-functionalized samples or vice-versa gave the same force–distance curves as in the lactose–lactose experiments (Figure 3 B). No interactions were observed either in water or in aqueous calcium solution. In other words, the interaction between the Le^x determinant molecules appears to be highly selective, supporting the proposal that carbohydrate–carbohydrate inter-

actions could be an additional mechanism in specific recognition processes in nature.

The existence of an interaction force of 20 ± 4 pN between individual molecules of Le^x determinant has been clearly demonstrated in water, as well as in 10 mM aqueous calcium solution. This value is the smallest adhesion force reported to date. However, it is in the same order of magnitude as the forces of a single binding event measured in the proteoglycan of marine sponges^[18] and other systems such as cadherine–cadherine,^[19] adenine–thymine,^[25] glycosphingolipid GM1–cholera toxin B (CTB),^[26] and the ferritin–antiferritin antigen–antibody system.^[17] A further step to obtaining thermodynamic information would be to correlate the measured forces with the energy of binding. Moy et al.,^[15] and Chilkoti et al.^[27] found a linear correlation between experimental rupture forces and enthalpy in different avidin and biotin derivatives. Preliminary studies based on weak affinity chromatography have allowed us to measure a Le^x–Le^x binding constant around 10 M^{-1} at room temperature (unpublished results). This value corresponds to an energy of 5.7 kJ mol^{-1} and according to the equation $E = Fd$, the interaction distance could be determined taking into account that the maximum force (F) found by AFM is 20 pN.^[28] The distance (d) obtained, using this approach,^[28] is 0.5 nm which approximately corresponds to the size of a pyranose ring of the Le^x trisaccharide.

In summary, we have been able to quantify the adhesion forces between polyvalent surfaces of Le^x trisaccharide by using atomic force microscopy. The adhesion force measured between two Le^x molecules (20 ± 4 pN) represents a new limit in the sensitivity of force microscopy. The results obtained establish the capacity and selectivity of Le^x antigen for self-recognition supporting the concept of carbohydrate–carbohydrate interactions as a mechanism for cell adhesion and recognition. Furthermore, these results confirm AFM as a powerful tool to measure weak adhesion forces. The measurements of adhesion forces using other carbohydrate antigens are in progress.

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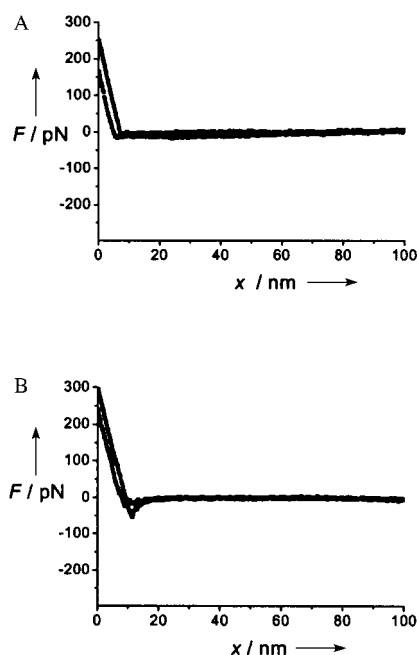


Figure 3. Typical AFM force–distance curve obtained with a lactose-functionalized tip and a SAM of A) lactose molecules. B) Le^x molecules or vice-versa.

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The CH_3N Diradical: Experimental and Theoretical Determinations of the Ionization Energies**

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Methylnitrene, the CH_3N diradical, is one of the most interesting of all organic free radicals, and it has drawn a lot of attention in both experiments^[1–5] and theory.^[6–11] As the simplest alkyl nitrene, the general class of molecules with the form RN , CH_3N is a dielectronic radical similar to carbene

CH_2 ; and is thought to be an important intermediate in many organic and inorganic reactions.^[12–14] Berry suggests^[1] that the alkyl nitrene should have electron states similar to NH which has been well characterized by spectroscopists. However, much less is known about alkyl nitrenes such as CH_3N , $\text{CH}_3\text{CH}_2\text{N}$, $(\text{CH}_3)_2\text{CHN}$, or $(\text{CH}_3)_3\text{CN}$. There are no reports on the ionization energy of the nitrenes until now, but these ionization energies, especially the first ionization energy, are important physical and chemical data.

When combined with ab initio molecular orbital calculations, HeI photoelectron spectroscopy (PES) gives a lot of information on the electronic and vibrational structures, the configuration, the bonding characteristics, as well as the relative photoionization cross sections of different molecular orbitals. Of course, the most important information obtained from the PES study is the ionization energy of the different molecular orbitals. Pople^[15] and Ng^[16] and their co-workers pointed out that the ab initio Gaussian2 (G2) calculation can correctly predict the first ionization energy.

We have shown how to generate a continuous flowing beam of short-lived species by the microwave discharge or pyrolysis of the parent species, allowing us to perform PES studies on reactive open-shell species such as NO_3 ,^[17] $(\text{CH}_3)_2\text{N}$,^[18] CH_3O ,^[19] CH_3S ,^[19] CH_3SS ,^[20] $\text{CH}_3\text{CH}_2\text{S}$,^[21] $(\text{CH}_3)_3\text{CO}$,^[22] and further demonstrated that the G2 calculation can also predict the ionization energies of different ionic states. Herein, we present the experimental and theoretical determinations of the ionization energies of different ionic states for the CH_3N diradical; this is also the first report on ionization energy for the nitrenes.

Calculations on the CH_3N_3 molecule indicate that the bond between the CH_3N and N_2 units is the weakest present and therefore pyrolysis of the CH_3N_3 offers a simple route to the CH_3N diradical through loss of the N_2 unit.^[23–26] However, in the pyrolysis study of CH_3N_3 carried out by Bock and co-workers,^[24–26] no PES signals of the CH_3N diradical could be detected and PES signals attributed to CH_2NH appeared when CH_3N_3 was heated to 770 K.

Through careful analysis of the experimental process used by Bock et al.^[24–26] it is clear that: 1) the distance from the point of pyrolyzation of the parent compound to the photo-ionized point for the new species generated is too far (maybe more than half meter); 2) the pyrolysis temperature (770 K) for maintaining the new species is also too high. Obviously, it is difficult to record the PES signals of transient species which have a short life-time, such as the CH_3N diradical. In fact, mixed PE spectra showing CH_3N_3 and CH_2NH (these spectra are the same as Figure 1 reported by Bock and Ralph in ref. [26]) were always obtained from the pyrolysis of the CH_3N_3 at $650(\pm 0.5)^\circ\text{C}$ with the heater installed outside our PE spectrometer.^[27] When the PES of the species generated by pyrolysis of CH_3N_3 are recorded in situ (see Experimental Section) a lot of the short-lived active species is detected at a lower temperature in our PE spectrometer.

Figure 1 gives the PE spectrum of the product obtained from the pyrolysis of CH_3N_3 at $145(\pm 0.5)^\circ\text{C}$, the spectrum is recorded in situ under protection of the NO species. Noteworthy is that the mixed PE spectra showing both CH_3N_3 and CH_2NH are also obtained at $215(\pm 0.5)^\circ\text{C}$ if NO is not

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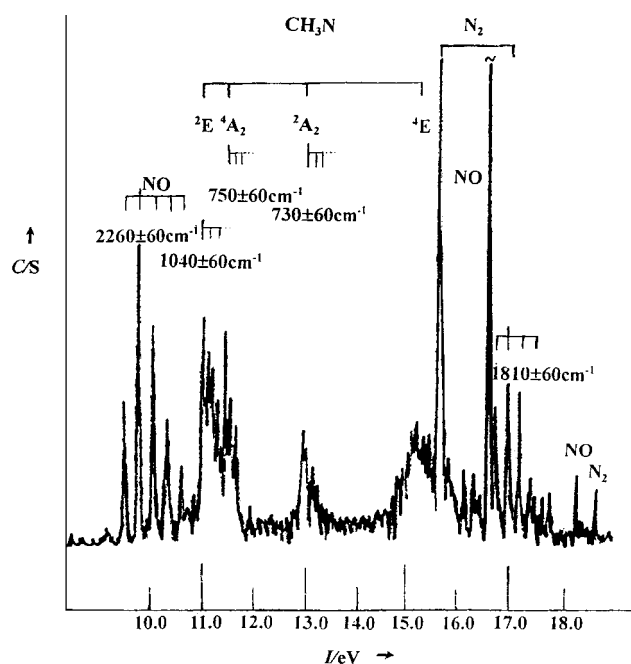


Figure 1. The PE spectrum (PES) of the CH_3N diradical obtained by pyrolysis of the CH_3N_3 at $145 (\pm 0.5)^\circ\text{C}$.

present.^[27] An expanded PE spectrum in which the fine vibrational structures could be clearly seen in the low ionization energy region (< 13.50 eV) is given in Figure 2.

For calibration purposes it is worth noting that the PES bands of the NO molecule (bands at 9.54 eV, with fine vibrational structure ($2260 \pm 60 \text{ cm}^{-1}$), at 16.56 eV (most intense band in Figure 1), and at 18.32 eV^[28]), are clearly displayed in the PE spectra (Figures 1 and 2). The PES peaks at 15.60 eV, 16.98 eV with vibrational structure ($1810 \pm 60 \text{ cm}^{-1}$), and 18.78 eV are characteristic of the N_2 molecule.^[28] Thus, the pyrolysis of the CH_3N_3 generates N_2 .

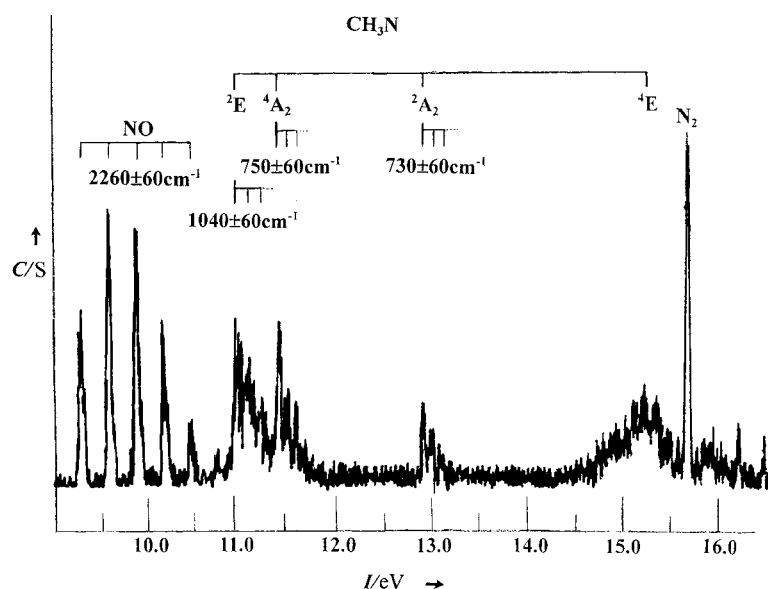


Figure 2. The expanded PE spectrum, in the lower ionization region (< 16.00 eV), of the CH_3N diradical obtained by pyrolysis of the CH_3N_3 at $145 (\pm 0.5)^\circ\text{C}$.

The PES bands at 11.04, 11.40, 12.85, and 15.53 eV are assigned to the CH_3N diradical. The peaks at 11.04, 11.40, and 12.85 eV are sharp with some structure to the higher ionization-energy side. This peak form is characteristic of the ionization of slightly bonding or slightly antibonding orbitals, i.e. the “vertical” ionization energy is equivalent to the adiabatic ionization energy of the PES band.^[28] The peak at 11.04 eV is assigned to the lowest energy PES peak of the CH_3N radical cation, because the vibrational spacing ($1040 \pm 60 \text{ cm}^{-1}$) is in excellent agreement with the reported vibrational frequency (1040 cm^{-1}) of the neutral CH_3N diradical,^[3, 11] and the vibrational spacing of the PES band resulting from electronic ionization of a nonbonding orbital should be close to the vibrational frequency of the neutral species. The position of the first CH_3N PES peak matches well with the results of both G2 and DFT calculations, and is assigned as the ^2E state of the CH_3N radical cation (see Table 1). According to the Frank–Condon principle, the

Table 1. The experimental vertical ionization energies (I_v), calculated ionization energies (E_v) according to C_{3v} symmetry from the G2 and DFT methods, and relative intensities of the PES signals observed from different ionic states of the CH_3N diradical.

PES experimental I_v [eV] ^[a]	G2 energies E_v [eV]	DFT energies E_v [eV]	Cationic states	Relative intensity this work ^[b]	statistical ratio
11.04	11.063	11.006	$^2\text{E}^{[c]}$		
11.40	11.301	11.492	$^4\text{A}_2^{[d]}$	1.95	2
12.85	12.880	13.263	$^2\text{A}_2^{[d]}$	1.00	1
15.53	15.495	15.361	$^4\text{E}^{[e]}$		

[a] ± 0.02 eV. [b] Corrected for analyzer sensitivity ± 0.02 . [c] Ionic states come from electron ionization of the HOMO(2e) for CH_3N radical. [d] Ionic states come from electron ionization of the SHOMO($5a_1$) for the CH_3N diradical. [e] Ionic states come from electron ionization of the 1e orbital of the CH_3N diradical.

energies of the ionic states are given using the vertical ionization energy (I_v), chosen as the maximum of the PES bands, and the computed ionization energy (E_v) should be obtained from the difference of the total energies of the resulting cation/neutral radical in C_{3v} symmetry.

The assignment of the PES bands for a new species can be supported by theoretical analysis. According to C_{3v} symmetry and a $^3\text{A}_2$ ground state for the CH_3N diradical the molecular orbitals would be, in the order of increasing energy:

$$(1a_1)^2(2a_1)^2(3a_1)^2(4a_1)^2(1e)^4(5a_1)^2(2e)^2$$

The removal of an electron from the HOMO will leave the ion in the singlet state ^2E , whereas removal of an electron from each of the other valence shell orbitals in turn can leave both quartet and doublet states of the cation.

The HOMO(2e) of the CH_3N diradical has a dominant contribution from the lone-pair electrons of the nitrogen atom with a small degree of the C–N antibonding character. This means that removal of

an electron from the HOMO(2e) of CH₃N, leading to the cation in the singlet state ²E, should give a sharp PES peak with fine structure of 1040 ± 60 cm⁻¹ which is in excellent agreement with the reported vibrational frequency 1040 cm⁻¹ of the C–N stretch mode of the neutral CH₃N diradical.^[3, 11]

The orbital character of the 5a₁ of the CH₃N diradical is essentially that of a lone-pair orbital of the nitrogen atom. This means that the removal of the electrons of the 5a₁ orbital should lead to two sharp peaks corresponding to ⁴A₂ and ²A₂ ionic states of the CH₃N radical cation, and the intensity of the PES band for the ⁴A₂ ionic state should be two times that of the ²A₂ ionic state, because the intensity of the PES band represents the relative ionic statistical weights.

A careful analysis of the intensities of the two peaks at 11.40 and 12.85 eV for the PE spectrum of the pyrolyzed CH₃N₃ shows that the intensity of the peak at 11.40 eV is about 1.95 times that of the peak at 12.85 eV. That is to say, the intensity ratio of the two peaks is in good agreement with the ratio of relative ionic statistical weight led by electron ionization of the 5a₁ orbital for the CH₃N diradical, that is, they correspond to the ⁴A₂ and ²A₂ ionic states of the CH₃N radical cation. The vibrational spacing (750 ± 60 cm⁻¹) of the peak at 11.40 eV is very close to the vibrational spacing (730 ± 60 cm⁻¹) of the peak at 12.85 eV. This fact also shows that the two PES peaks should arise from the result of electron ionization of the same orbital. The PES band centered near 15.53 eV could be attributed to removal of the electrons from the 1e orbital or maybe also includes the contribution of more orbitals in the deep valence shell of CH₃N, because the band is very broad. As indicated in Table 1, PES experimental and both G2 and DFT theoretical results agree reasonably well.

Experimental Section

Methyl azide CH₃N₃ was prepared according to the methods reported in refs. [26, 29]. The purity of CH₃N₃ was checked by mass spectrometry and NMR spectroscopy. The PE spectrum of the CH₃N₃ obtained is the same as that reported^[26] and did not vary from day to day before each experiment.

The PES experiment was performed in a double-chamber UPS Machine-II which was built specially, as described elsewhere,^[17] to detect transient species. A continuous flowing CH₃N diradical beam is produced in situ by pyrolysis of CH₃N₃ at 145(±0.5) °C in a quartz tube by using a double heater inlet system, the heater was capable of producing 1200 °C. Thus, the new species was produced within 1–2 cm of the photoionization region of the spectrometer, it then passed through the photoionization point and was quickly pumped out. The double heaters were installed inside of the sample chamber. The PE spectrum of the CH₃N diradical was measured at a resolution of about 30 meV as indicated by the Ar⁺(³P_{3/2}) photoelectron band. Experimental vertical ionization energies (*E_v* in eV) were calibrated by simultaneous addition of a small amount of NO to the sample, the first PES band with NO fine vibrational structure can be used for calibration the of the fine vibrational structure of the new species, NO can also stabilize the new species.^[22]

Gaussian 2 and improved DFT calculations: to assign the PES bands of the CH₃N diradical, both G2 and DFT calculations were performed on the ³A₂ ground state of the CH₃N diradical in the C_{3v} symmetry and the ground and several ionic states of the CH₃N radical cation. The G2 theoretical procedure has been described in detail by Curtiss et al.^[15] The improved density functional theory (DFT) calculations are described in refs. [17–22]. The computed ionization energies (*E_v* in eV) were obtained from the difference between the total energy of the resulting radical cation ³A₂ ground state in the C_{3v} symmetry to the total energy of the CH₃N diradical.

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A Hydrogen-Bonded [(Mercuracarborand–Water)₂–Benzene] π -Sandwich Complex**

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Benzene–water, π -hydrogen bond complexes have been extensively studied by a variety of theoretical and experimental methods.^[1–5] The structural basis of such complexes is a π -hydrogen bond involving hydrogen atom(s) of water and the π -electron cloud associated with the benzene ring. These benzene–water complexes serve as prototypes for the generic O–H $\cdots\pi$ interaction.^[3–6] The number of hydrogen atoms of water directed toward the benzene π -electron cloud is yet to be clearly determined.^[5–7] Most of these experiments involved gas-phase results and the main structural information was obtained by comparison with data from ab initio calculations. The structure derived from these gas-phase studies is a benzene/(water)₂ complex with dimeric H₂O bound to only one side of the aromatic ring.

Mercuracarborands, cyclic multidentate Lewis acids composed of alternating units of carborane cages and mercury atoms (Figure 1), readily form complexes with electron-donor

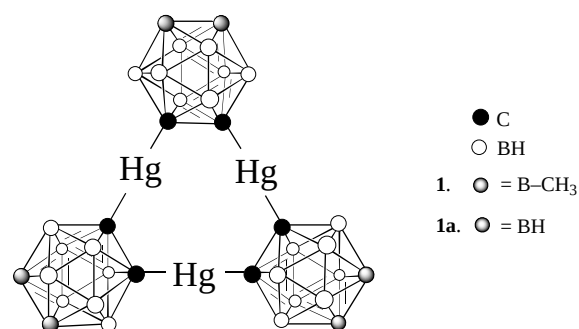


Figure 1. Representation of *B*-hexamethyl-[9]mercuracarborand-3 (**1**) and [9]mercuracarborand-3 (**1a**).

molecules such as tetrahydrofuran,^[8] acetonitrile,^[9] nitrate ion,^[10] halide anions (Cl[−], Br[−], I[−]),^[11, 12] and the polyhedral *closo*-B₁₀H₁₀^{2−} ion among others.^[12, 13] Also, mercuracarborands have been demonstrated to complex and activate an uncharged nucleophilic species for catalysis.^[14]

Here we report the structural characterization of trimeric 9-mercuracarborand-3, (C₂B₁₀H₁₀Hg)₃, (**1a**) in which the mercuracarborand is η^3 -coordinated by the oxygen atom of a water molecule, and the first solid-state structure of a benzene–water complex, [(**1**·water)₂·benzene]. The latter is composed of two electroneutral trimeric *B*-hexamethyl-9-mercuracarborand-3, (9,12-(CH₃)₂-C₂B₁₀H₈Hg)₃, (**1**) recep-

tors, each of which hosts a single water molecule. The benzene molecule is situated between two such water molecules in a sandwich fashion.

A single crystal of the simple benzene-free complex, [**1a**·(OH₂)·((CH₃)₂CO)₃], (**2**) grown from acetone/water (50:1) was selected for an X-ray diffraction study.^[15, 19b] Compound **2** is an air- and moisture-stable solid which crystallized in the rhombohedral space group $R\bar{3}$. The cyclic tridentate host **1a** is coordinated to water and to three acetone molecules (Figure 2).

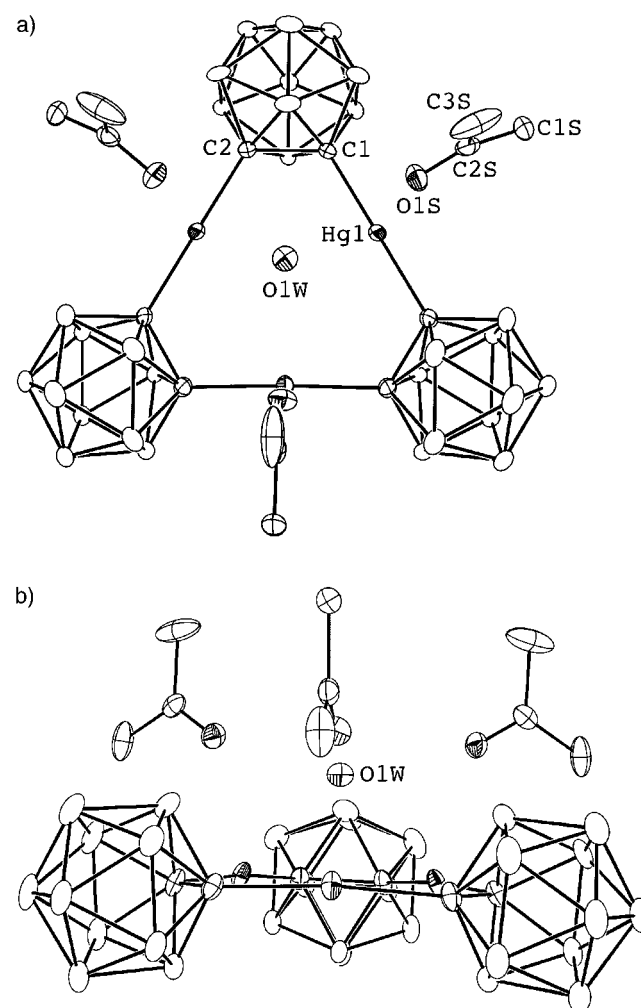


Figure 2. Structure of **2** (ORTEF plot; hydrogen atoms omitted for clarity). a) Top view. b) Side view. Selected distances [Å]: Hg1–O1W 2.915(5), Hg1–O1S 2.762(4), Hg $\cdots$ Hg 3.7329(6), O1W $\cdots$ O1S 3.014(4), Hg₃(centroid) $\cdots$ O1W 1.963(7).

The three mercury atoms present in complex **2** are arranged in an equilateral triangle (Hg $\cdots$ Hg 3.7329(6) Å; Hg–Hg–Hg 60°); the Hg₃C₆ ring is nearly planar and the Hg–C–C–Hg torsion angle is 1.0(6)°. The water molecule lies on the threefold axis and is located 1.824 Å above the Hg₃ plane. The oxygen atom of water is η^3 -coordinated to the three mercury atoms (Hg1 $\cdots$ O1W 2.915(5) Å). This distance is within the sum of the van der Waals separation of 3.13 Å (Hg = 1.73^[16] and O = 1.40^[17] Å). The hydrogen atoms of water were

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crystallographically disordered and not located. The carbonyl oxygen atom of each acetone molecule is coordinated to its corresponding mercury center ($\text{Hg1} \cdots \text{O1S}$ 2.762(4) Å) forming a 1:1 acetone to mercury center complex. The observed $\text{Hg} \cdots \text{O}=\text{C}$ distance is consistent with previously reported $\text{Hg} \cdots \text{O}=\text{C}$ intermolecular distances.^[18] Interestingly, water and acetone molecules are located on the same cavity face of **1a**. A short $\text{O1W} \cdots \text{O1S}$ distance of 3.014(4) Å may indicate the presence of a hydrogen bond between the water and acetone molecules. The oxygen atom of water is η^3 -coordinated to the mercury centers and is in close proximity to the carbonyl oxygen atom of acetone ligands. The water molecule may serve as a template for the acetone molecules.

Compound **1** dissolves in a hot dichloromethane/benzene/water (20:10:1) solution. A single crystal of $[(\mathbf{1} \cdot \text{OH}_2)_2 \cdot \text{C}_6\text{H}_6]$ (**3**) was grown by slow evaporation of solvent and was selected for an X-ray diffraction study.^[19] Species **3** is an air- and moisture-stable crystalline solid which crystallized in the rhombohedral space group $R\bar{3}$. Each water molecule is η^3 -coordinated at the center of a host, **1**, forming a $[\mathbf{1} \cdot \text{OH}_2]$ complex, similar to the $[\mathbf{1a} \cdot \text{OH}_2]$ component of complex **2**. The benzene ring of **3** is sandwiched between two $[\mathbf{1} \cdot \text{OH}_2]$ complexes (Figure 3) with the acidic water protons of the complexed water molecules forming hydrogen bonds with available π electrons of the benzene ring.

The three mercury atoms present in each of the two hosts in complex **3** are arranged in an equilateral triangle ($\text{Hg} \cdots \text{Hg}$ 3.7346(7) Å; Hg-Hg-Hg 60°) and the Hg-C-C-Hg torsion angle (2.8(8)°) shows these four atoms are not coplanar. Each water molecule lies on a threefold axis and is located 2.08(1) Å from its respective Hg_3 plane in **3**. The oxygen atom of water is equidistant to three mercury atoms ($\text{Hg} \cdots \text{O}$ 2.994(8) Å), which is less than the sum of the van der Waals radii (3.13 Å). The hydrogen atoms of water are crystallographically disordered and not located. However, in the solid state, both hydrogen atoms of water must be oriented toward and bonding with the π system of benzene. The benzene centroid $\cdots$ oxygen distance is 2.99(1) Å, which is closer than the reported spectroscopic (3.32–3.35 Å)^[2] and theoretically calculated distances (3.20–3.32 Å)^[2, 5] of a benzene–water complex (single $\text{O-H} \cdots \pi$ interaction). Thus, host **1** increases the acidity of the hydrogen atoms of η^3 -coordinated water in the $[(\mathbf{1} \cdot \text{OH}_2)_2 \cdot \text{C}_6\text{H}_6]$ complex which, in turn, increases the $\text{O-H} \cdots \pi$ hydrogen bond affinity. This increased bond affinity may explain the apparent ability of benzene to simultaneously coordinate four hydrogen atoms.

The two trimeric hosts of **3** are inverted with respect to each other and separated by 10.126(3) Å. For comparison, the two trimeric hosts of the $[\mathbf{1}_2 \cdot \text{I}]^-$ sandwich complex are separated by 4.902 Å.^[11] The distance between the C–C centroid of benzene and the mercury centers (5.19 Å) is much longer than those (3.36 Å) reported by Tsunoda and Gabbai^[20] and presumably, the mercury centers of **3** are not directly coordinated to the benzene ring.

The correct formulation of the hydrogen bonding present in **3** is not possible without knowledge of the disposition of the water protons. However, in the solid state, we assume both hydrogen atoms of water must be oriented toward and bonding with the π system of benzene.

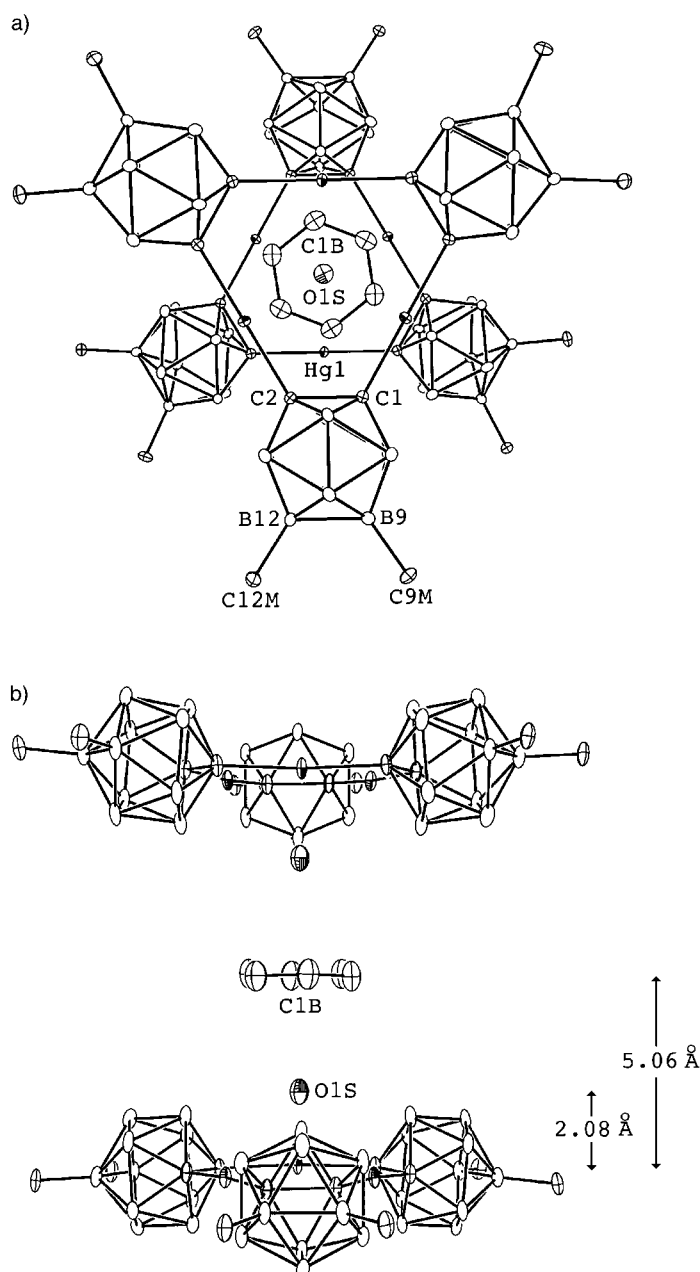


Figure 3. Structure of **3** (ORTEP plot; hydrogen atoms omitted for clarity). a) Top view. b) Side view. Selected distances [Å]: Hg1-O1S 2.994(8), $\text{Hg}_3(\text{centroid}) \cdots \text{O1S}$ 2.08(1), $\text{Hg} \cdots \text{Hg}$ 3.7346(7), $\text{O1S} \cdots \text{C1B}$ 3.28(2), $\text{O1S} \cdots \text{C1B}'$ 3.32(2), $\text{O1S} \cdots \text{benzene centroid}(\pi)$ 2.99(1).

Experimental Section

$[\mathbf{1a} \cdot (\text{OH}_2) \cdot ((\text{CH}_3)_2\text{O})_3]$ (**2**): Compound **1a**^[9] (20 mg, 19 μmol) was dissolved in an acetone/water (1 mL/0.02 mL) mixture. Slow evaporation of the solvent yielded clear crystals.

$[(\mathbf{1} \cdot \text{OH}_2)_2 \cdot \text{C}_6\text{H}_6]$ (**3**): Compound **1**^[21] (10 mg, 9 μmol) was dissolved in a hot methylene chloride/benzene/water (1 mL/0.5 mL/0.02 mL) mixture. Slow evaporation of the solvent yielded clear crystals.

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- [15] Crystallographic data collection for $[1\mathbf{a} \cdot (\text{H}_2\text{O}) \cdot ((\text{CH}_3)_2\text{O})_3]$ (**2**): $\text{C}_{15}\text{H}_{48}\text{B}_{30}\text{Hg}_3\text{O}_4$, $M_r = 1218.6$, crystal dimensions = $0.2 \times 0.25 \times 0.5 \text{ mm}^3$, rhombohedral, space group $R\bar{3}$, $a = 21.565(3)$, $c = 15.455(3) \text{ \AA}$, $V = 6225(2) \text{ \AA}^3$, $Z = 6$, $\rho_{\text{calc}} = 1.951 \text{ mg cm}^{-3}$, $T = 100(2) \text{ K}$, absorption coefficient $\mu = 11.098 \text{ mm}^{-1}$. Data were collected on a Bruker SMART CCD diffractometer, using $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). Unit cell parameters were determined from a least-squares fit of 976 accurately centered reflections ($6.345^\circ < 2\theta < 55.785^\circ$). A total of 3361 unique reflections were measured, of which 2990 reflections were considered observed with $I > 2\sigma(I)$. All reflections were used for structure analysis. The intensity data were corrected for Lorentz and polarization effects, absorption, and secondary extinction. Atoms were located by use of statistical methods. All non-hydrogen atoms were refined with anisotropic parameters. With the exception of the hydrogen atoms of water (not located), all hydrogen atoms were included in structure factor calculations but parameters were not refined. The hydrogen atoms were assigned isotropic displacement values based approximately on the value for the attached atom. The final discrepancy index was $R = 0.032$, $R_w = 0.074$ ($w = 1/\sigma^2(|F_o|)$) for 2990 independent reflections with $I > 2\sigma(I)$. The largest peak maximum and minimum on a final difference electron density map were 2.19 and $-3.43 \text{ e \AA}^{-3}$, both near Hg. Data were processed by using programs supplied with the Bruker SMART CCD diffractometer.^[19b]
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- [19] a) Crystallographic data collection for $[(1 \cdot \text{H}_2\text{O})_2 \cdot \text{C}_6\text{H}_6]$ (**3**): $\text{C}_{15}\text{H}_{45}\text{B}_{30}\text{Hg}_3\text{O}$, $M_r = 1167.58$, crystal dimensions = $0.15 \times 0.15 \times 0.05 \text{ mm}^3$, rhombohedral (obverse), space group $R\bar{3}$, $a = 20.763(3)$, $c = 15.944(4) \text{ \AA}$, $V = 5953(2) \text{ \AA}^3$, $Z = 6$, $\rho_{\text{calc}} = 1.954 \text{ mg cm}^{-3}$, $T = 100(2) \text{ K}$, absorption coefficient $\mu = 11.594 \text{ mm}^{-1}$. Data were collected on a Bruker SMART CCD diffractometer, using $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). Unit cell parameters were determined from a least-squares fit of 959 accurately centered reflections ($6.794^\circ < 2\theta < 56.532^\circ$). A total of 3202 unique reflections were measured, of which 2505 reflections were considered observed with $I > 2\sigma(I)$. All reflections were used for structure analysis. The intensity data were corrected for Lorentz and polarization effects, absorption, and secondary extinction. Atoms were located by use of statistical methods. All non-hydrogen atoms were refined with anisotropic parameters. With the exception of the hydrogen atoms of water (not located), all hydrogen atoms were included in structure factor calculations but parameters were not refined. The hydrogen atoms

were assigned isotropic displacement values based approximately on the value for the attached atom. The final discrepancy index was $R = 0.041$, $R_w = 0.105$ ($w = 1/\sigma^2(|F_o|)$) for 2505 independent reflections with $I > 2\sigma(I)$. The largest peak maximum and minimum on a final difference electron density map were 3.37 and $-3.41 \text{ e \AA}^{-3}$, both near Hg. Data were processed using programs supplied with the Bruker SMART ccd diffractometer. 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-160758 (**2**) and CCDC-160759 (**3**). 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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An Efficient Synthesis of (±)-Narwedine and (±)-Galanthamine by an Improved Phenolic Oxidative Coupling

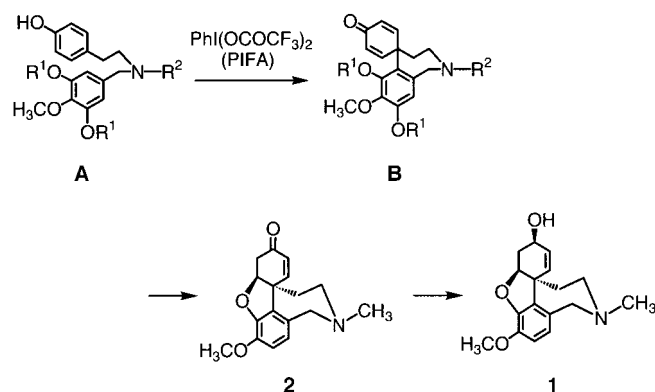
Manabu Node,* Sumiaki Kodama, Yoshio Hamashima, Takahiro Baba, Norimitsu Hamamichi, and Kiyoharu Nishide

(–)-Galanthamine (**1**; Scheme 1),^[1] an alkaloid isolated from the *Amaryllidaceae* family, has been approved in Austria and the United Kingdom for the treatment of Alzheimer's disease as a selective acetylcholinesterase inhibitor.^[2, 3] Because of the limited supplies and the high cost of its isolation from natural sources^[4], several syntheses of galanthamine have been reported which use a phenolic oxidative coupling.^[5] Recent syntheses make use of an asymmetric Heck reaction.^[6a] (–)-Galanthamine (**1**) is considered to be synthesized biologically by the phenolic oxidative coupling^[7] of norbelladine derived from L-tyrosine. Since the first synthesis of galanthamine by Barton and Kirby^[8a] in 1960 by the biomimetic phenolic oxidative coupling of the biogenetic precursor norbelladine by using potassium ferricyanide, several methods have been designed to improve the low yield of this coupling reaction: for example, blocking one of the *ortho* positions on the norbelladine derivative with a bromo^[5b,e,g,i,j, 8b] or a trimethylsilyl^[5h] group, which could be easily be substituted by a hydrogen atom, changing the oxidant,^[5c,d,h] and using symmetrical substrates.^[5c,d] Despite these efforts, the yield of the pivotal step in these syntheses still remains only moderate (~50%). Therefore, an efficient synthesis of **1** is highly desirable and useful.

Since (–)-galanthamine (**1**) has been efficiently obtained from (±)-narwedine (**2**)^[8] by crystallization-induced chiral

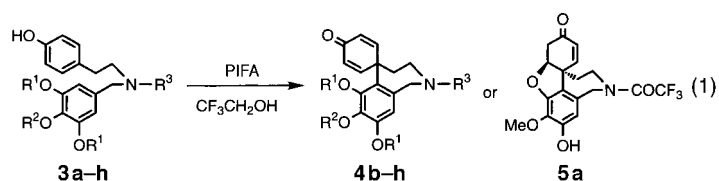
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conversion,^[4, 5i,j] it would seem that an efficient synthesis of **1** should involve an improvement in the phenolic oxidative coupling of the norbelladine derivative **A** to form **2**. We describe herein an efficient formal synthesis of **1** by using an improved phenolic oxidative coupling reaction that uses **A** as the symmetrical substrate^[5c,d] and phenyliodine(III) bis(trifluoroacetate) (PIFA)^[5h] as the oxidant to give spirodienone **B** (Scheme 1).



Scheme 1. Synthetic strategy for (–)-galanthamine (**1**).

For the substrate in the coupling reaction, we chose the norbelladine-type derivatives **3a–h**,^[9] which contain a pyrogallol moiety [Eq. (1)]. The oxidizing agent, PIFA (1.1 equivalents), is degraded to iodobenzene and trifluoroacetic



acid. These byproducts can be removed from the product mixture under reduced pressure and thus an extraction procedure is not required. Results of the oxidative coupling reaction of **3a–h** with PIFA are summarized in Table 1. The reaction of the ideal substrate **3a** with a 2-methoxyresorcinol moiety which can give the narwedine-type product **5a** directly, was carried out in trifluoroacetic acid at -20°C ; however, the yield of **5a** was low (Table 1, entry 1). To increase the

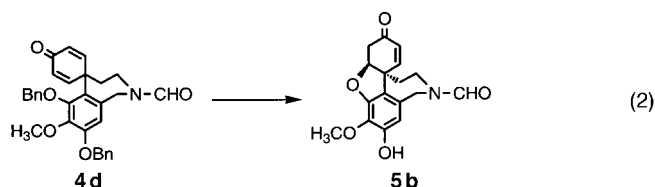
Table 1. Phenolic oxidative coupling of **3** with PIFA.

Entry	Substrate				Product			
	R ¹	R ²	R ³	Temp. [°C]	Time [min]	Yield [%] ^[a]		
1 ^[b]	3a	H	Me	COCF ₃	−20	20	5a	12
2	3b	Bn	Bn	CHO	−40	120	4b	90
3	3c	Me	Me	CHO	−40	15	4c	95
4	3d	Bn	Me	CHO	−40	60	4d	82
5	3d	Bn	Me	CHO	RT	15	4d	85
6	3e	Allyl	Me	CHO	−40	30	4e	48
7	3f	MOM	Me	CHO	−40	10	4f	43
8	3g	Me	Me	COCF ₃	−40	120	4g	75
9	3h	Bn	Me	COCF ₃	−40	60	4h	53

[a] Yield of isolated product. [b] The reaction was carried out in CF₃CO₂H.

solubility of the substrates in trifluoroethanol, we protected the two phenolic hydroxy moieties as the Me, Bn (benzyl), allyl, and MOM (methoxymethyl) ethers **3b–h** (Table 1, entries 2–9). Substrates **3b–d** afforded the desired spirodienones **4b–d** in excellent yields (Table 1, entries 2–4). The formyl group was a better protecting group than the trifluoroacetyl group for the secondary amine (Table 1, entries 3, 8 and 4, 9). The allyl ether (**3e**) and the MOM ether (**3f**) gave the corresponding spirodienones **4** in moderate yields (Table 1, entries 6, 7). Product **4d** was especially easy to crystallize, and was separated mainly by crystallization from the reaction mixture.^[10] Even at room temperature, the coupling reaction of **3d** proceeded successfully and showed a shorter reaction time than those carried out at a lower temperature (Table 1, entries 4 and 5). The yield of the phenolic oxidative coupling reaction of **3c** (95%) is the highest recorded to date among such reactions.

Because selective demethylation of the spirodienone **4c** was difficult, compound **4d** was consequently chosen as the suitable substrate for conversion into the narwedine-type compound **5b** [Eq. (2)]. The use of relatively weak acids was



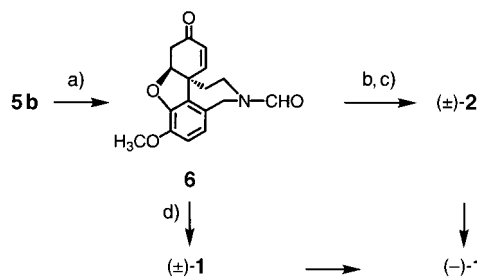
considered to avoid a dienone–phenol rearrangement^[11] and to allow the two benzyl ethers to be dealkylated in the presence of the methyl ether. Trifluoroacetic acid in dimethyl sulfide^[12] required a long reaction time, but the yield was satisfactory (Table 2, entry 1). Methanesulfonic acid^[13] gave the desired product **5b**; however, the yield was only moderate (Table 2, entry 2). The reaction with boron trichloride^[14] at low temperature afforded the desired product **5b** in excellent yield (Table 2, entry 3).

Table 2. Debenzylation of **4d** to form narwedine-type compound **5b**.

Entry	Reagent	Temp.	Time	Yield [%] ^[a] of 5b
1	CF ₃ CO ₂ H in Me ₂ S (2:1)	RT	4 d	81
2	CH ₃ SO ₃ H (10 equiv) in Me ₂ S	RT	2 h	62
3	BCl ₃ (3 equiv) in CH ₂ Cl ₂	-78°C	20 h	95

[a] Yield of isolated product.

The extra hydroxy group on **5b** was reduced quantitatively by its conversion into a triflate group, followed by the palladium(0)-catalyzed reduction with formic acid^[15] (Scheme 2). Although the above synthetic route to **6** from **3d** requires four steps, the overall yield (77%) represents a significant improvement over the reported yield.^[5] The narwedine-type compound **6** was converted efficiently into (±)-narwedine (**2**) by protection of the ketone group, followed by reduction of the *N*-formyl group to an *N*-methyl group. Furthermore, compound **6** was transformed into (±)-galanthamine (**1**) by the diastereoselective reduction of the



Scheme 2. a) 1) TiF_2O , pyridine, 99%; 2) $\text{Pd}(\text{OAc})_2$, PPh_3 , Et_3N , HCO_2H , 97%; b) ethylene glycol, PPTS, 92%; c) 1) LiAlH_4 , 2) HCl , 83% (2 steps); d) 1) L-Selectride, 2) LiAlH_4 , 61% (2 steps). PPTS = pyridinium *p*-toluenesulfonate.

carbonyl group, as previously reported.^[5g] (±)-Galanthamine has been optically resolved previously,^[5g] and (±)-narwedine (**2**) has been converted into (–)-galanthamine (**1**).^[5i, 8c] Therefore, the described efficient route to (±)-narwedine (**2**) and (±)-galanthamine implies a formal total synthesis of (–)-galanthamine (**1**).

In conclusion, we were able to improve the pivotal phenolic oxidative coupling reaction of norbelladine-type derivatives, which had been limited to moderate yields over 40 years, and have provided an efficient synthetic route for the industrial production of (–)-galanthamine (**1**).

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- [9] The norbelladine-type derivatives **3a–h** were easily prepared from methyl gallate and tyramine in high yields, according to ref. [5d] and [5e].

- [10] Typical procedure for the improved phenolic oxidative coupling: To a solution of **3d** (2.0 g, 4.0 mmol) in 2,2,2-trifluoroethanol (100 mL) was added a solution of PIFA (1.9 g, 4.4 mmol) in 2,2,2-trifluoroethanol (10 mL) at -40°C under nitrogen, and the resulting mixture was stirred for 1 h. After the evaporation of the solvent under reduced pressure, the product **4d** (1.29 g, 65%) was isolated by crystallization of the residue from ethyl acetate. The additional crop of **4d** (341.5 mg, 17%) was obtained by column chromatography (chloroform/methanol 20:1) of the condensed mother liquor, followed by crystallization from ethyl acetate. **4d**: colorless needles; m.p. $187\text{--}189^\circ\text{C}$ (dec.) (ethyl acetate); ^1H NMR spectra of a mixture of two conformational isomers were measured. The signals for the minor isomer are shown in parentheses. ^1H NMR (400 MHz, CDCl_3 , 25°C , TMS): δ = (8.21) 8.19 (s, 1H; CHO), 7.47–7.22 (m, 10H), (7.05) 6.98 (d, J = 10.2 Hz, 2H), (6.76) 6.57 (s, 1H; Ar-H), 6.14 (d, J = 10.2 Hz, 2H), 5.15 (5.14) (s, 2H), 4.88 (4.84) (s, 2H), (4.69) 4.60 (s, 2H), 3.75 (3.74) (s, 3H), 3.71 (3.65) (t, J = 6.2 Hz, 2H), (2.29) 2.26 (t, J = 6.2 Hz, 2H); IR (CHCl_3): $\tilde{\nu}$ = 1663 ($\text{C}=\text{O}$, dienone), 1620 and 1593 ($\text{C}=\text{O}$, formyl), 1123 cm^{-1} ($\text{C}-\text{O}$); EI-MS (70 eV): m/z (%): 465 (7) [M^+], 404 (6), 91 (100), 65 (7); HRMS calcd for $\text{C}_{31}\text{H}_{29}\text{NO}_5$ [M^+]: 495.2045, found: 495.2052; elemental analysis: calcd (%) for $\text{C}_{31}\text{H}_{29}\text{NO}_5$: C 75.13, H 5.90, N 2.83; found: C 75.26, H 5.98, N 2.92.
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Aqueous One-Pot Synthesis of Derivatized Cyclopentadienyl–Tricarbonyl Complexes of $^{99\text{m}}\text{Tc}$ with an In Situ CO Source: Application to a Serotonergic Receptor Ligand**

Joachim Wald, Roger Alberto,* Kirstin Ortner, and Lukas Candreia

The radionuclide $^{99\text{m}}\text{Tc}$ is among the most widely used isotopes in diagnostic nuclear medicine.^[1] It is cheap, exhibits good decay characteristics, and typically burdens the patient with a low radiation dose. Whereas in the past $^{99\text{m}}\text{Tc}$ -complexes were preferentially applied as perfusion agents,^[2] a challenge now lies in combining a $^{99\text{m}}\text{Tc}$ complex with a targeting molecule such as a tumor-specific peptide or a small

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CNS receptor-binding molecule, as recently highlighted.^[3] The numerous technetium or rhenium complexes that have been investigated in either case are essentially of the Werner type, comprising tetradentate N,S-ligands or polyamino polycarboxylates.^[4] One such complex, which has become the most important myocardial imaging agent, is an organometallic compound.^[5] Since any clinically useful diagnostic agent ultimately has to be prepared in water, the difficulty in using sensitive organometallic ligands such as cyclopentadienyl (Cp) is evident.

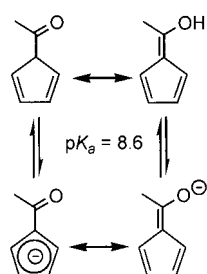
Apart from these feasibility considerations, the Cp ligand offers very attractive properties as a ligand for radiopharmaceutical purposes. Its inherent advantages are the small size, the low molecular weight, and the stability of half-sandwich complexes, [CpM(CO)₃] (**1**; Me = Re and Tc). These highly crucial prerequisites minimize steric interference with the receptor binding part of a labeled biomolecule. Complexes **1** are highly lipophilic, which makes them particularly interesting as labeled, blood–brain barrier-crossing molecules.

The fact that **1** (M = Re) can be coupled to biomolecules by classical organometallic methods without affecting the bioactivity has been demonstrated by several groups.^[6] The physiological stability of the complexes [(R-Cp)Re(CO)₃] (**2**; R = steroid hormone,^[7] antibody,^[8] etc.) initiated investigations into the preparation of [(R-Cp)^{99m}Tc(CO)₃] (**2a**). Wenzel reported a double ligand-transfer reaction, which ultimately led to the formation of **2a** starting from [^{99m}TcO₄][−].^[9] Based on this approach, further improvements towards a general synthesis of **2** and **2a** have recently been published^[10] and octreotide was labeled in a five-step procedure.^[11] Both approaches still suffer from unacceptable reaction conditions for routine use.

We recently reported the synthesis of *fac*-[^{99m}Tc(OH)₂(CO)₃]⁺ (**3a**) directly from [^{99m}TcO₄][−] in water.^[12] Precursor **3a** is a very versatile source of the “*fac*-[^{99m}Tc(CO)₃]⁺” moiety which accepts different chelators for the remaining three positions. Attempts to coordinate cyclopentadienyl or fulvene derivatives to **3a** failed or resulted in very low yields. This lack of reactivity is in agreement with the low tendency of neutral alkenes to compete with water in [M(OH)₂(CO)₃]⁺ (M = ^{99m}Tc, Re). Alkyl η^1 coordination of deprotonated cyclopentadiene as the synthetic key step,^[13] preceding ring slippage to η^5 -coordination, is unlikely owing to its prohibitively high pK_a of about 14.

The key step in the preparation of **2a** in water lies in the ability of a Cp-derivative to be deprotonated to a reasonable extent at physiological pH. This can be achieved by introducing electron-withdrawing substituents. The requirements of delocalizing the resulting negative charge over the Cp system prompted us to investigate the well-known acetyl cyclopentadiene (**4**; ACp),^[14] in which the keto–enol tautomerism stabilizes the deprotonated form (Scheme 1).

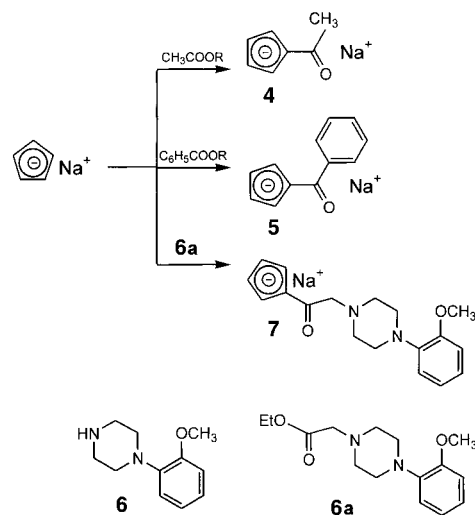
The pK_a value of formyl cyclopentadiene is about 8.^[15] More accurately, we



Scheme 1. Acidification of acetyl cyclopentadiene **4** by keto–enol tautomerism. Several isomers are possible.

found that the pK_a value of **4** is 8.62(1) in 0.1M NaCl (potentiometric titration), which means an increase in acidity by about 5 orders of magnitude relative to cyclopentadiene. Thus, about 5 % of **4** is dissociated at physiological pH. The enolate form of **4** should be able to coordinate efficiently to **3a** or its Re analogue, either through the alkoxide group, with subsequent intramolecular rearrangement, or directly to the negatively charged cyclopentadienyl ring, to afford the desired half-sandwich complex as the thermodynamic product.

The preparation of differently substituted carbonyl cyclopentadienyl compounds (Na[R-Cp]) by the reaction of NaCp with an ester functionality is straight forward and gives typically 50–60 % yields. As a model for steroids, we introduced a phenyl group instead of the methyl group to get benzoyl-Cp (BCp) **5**. To assess the possibility of attaching Cp to more complicated (bio)molecules, we have chosen a lead structure of the class of aryl piperazines, 1-(2-methoxyphenyl)piperazine **6**, which are among the most thoroughly studied ligands for the 5-HT_{1A} subclass of serotonergic receptors (Scheme 2).^[16] Its Cp derivative **7** was prepared in

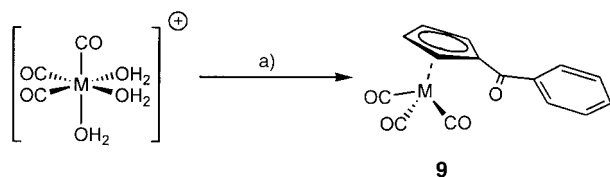


Scheme 2. Derivatization of the cyclopentadienyl group with different organic (bio)molecules.

the same way, after alkylation of the piperazine ring in **6** with ethyl bromoacetate to give **6a**. The pK_a values for **7** were found to be >12.5, 8.71(1), and 6.37(1). The dissociation constants pK_{a1} and pK_{a3} were assigned to the tertiary amine groups, and pK_{a2} represents the dissociation constant of cyclopentadiene. In contrast to NaCp, **4**, **5**, and **7** are reasonably soluble in water and are stable for hours, even when exposed to air.

To demonstrate the possibility of preparing complexes with the general composition **2** (M = Tc or Re) directly in water, **3** was treated in phosphate buffer at pH 7.4 with **4**. Within 2 h at 85 °C, [(ACp)M(CO)₃] (**8**) formed in about 15–25 % yield for Re, and 40 % for Tc. This is one of the first examples in which a Cp derivative reacts in water with an organometallic precursor to yield the corresponding half-sandwich complex. We anticipate that this convenient strategy can be extended to the preparation of other half-sandwich complexes, probably

containing water ligands. As depicted in Scheme 3, the preparation of **2** directly in water is not restricted to **4**; compound **5** and the receptor binding molecule **7** also reacted comparably in water with **3** to yield the corresponding complexes $[(\text{Cp}')\text{M}(\text{CO})_3]$ (**9**, $\text{Cp}' = \mathbf{5}$; **10**, $\text{Cp}' = \mathbf{7}$).



Scheme 3. Synthesis of cyclopentadienyl complexes $[(\text{R-Cp})\text{M}(\text{CO})_3]$ ($\text{M} = \text{Re}$) in water. Reagents and conditions: a) **5**, phosphate buffer, pH 7.4, 2 h, 85 °C.

The observed dimerization of the ligands at elevated temperatures and higher concentrations can be compensated by a slight ligand excess. The limitation of the yield essentially arose from the competing hydrolytic reaction of **3** with the very stable cluster compound $[\text{M}(\mu_3\text{-OH})(\text{CO})_3]_4$, which represents the only rhenium-containing by-product.^[17] The rhenium compounds of **9** and **10** were crystallized, and their structures were elucidated by X-ray studies. A SCHAKAL representation of **10** is given in Figure 1.^[18, 19]

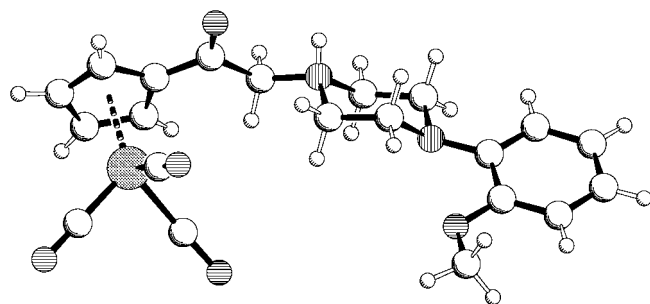


Figure 1. SCHAKAL representation of complex **10** (Re) with protonated piperazine shown without the counterion Cl^- .^[19]

Complex **9** ($\text{M} = \text{Re}$) has been prepared by Friedel–Crafts acylation of **1** by Fischer et al.^[20] The crystal structures of **9** and **10** exhibit the typical piano–stool arrangements. The average bond lengths of $\text{Re–C}(\eta^5)$ are 2.31 Å in both cases.

The preparation of $[(\text{R-Cp})\text{Re}(\text{CO})_3]$ directly in water offers a convenient alternative to the classical approach, which starts from $[\text{ReBr}(\text{CO})_3]$ in benzene.^[21] This is particularly important for the syntheses of half-sandwich complexes with a pendant biomolecule, since the latter are often insoluble in common organic solvents. The availability of a “cold” compound is essential for radiopharmaceutical application of the labeled “hot” biomolecules, as their characterization is only possible by chromatographic comparison.

To examine the potential of this novel approach for routine preparations of Cp-derivatized biomolecules such as **7** labeled with $^{99\text{m}}\text{Tc}$, we transferred the reaction conditions to the no-carrier-added (n.c.a.) level. For kinetic reasons, cluster formation as the major competing reaction is not expected,

since the concentration of $^{99\text{m}}\text{Tc}$ is very low (nM–μM range). The precursor **3a** was prepared as previously reported,^[12] buffered (phosphate buffer, pH 7.4, 1–2 cm³), and then mixed with an aqueous solution containing, for example, 0.1–1 μmol of **4**. The reaction was carried out for 15 min at 90 °C to afford the corresponding complex **8** ($^{99\text{m}}\text{Tc}$) in quantitative yield. Its identity was confirmed by coinjecting **8**(Re) and **8**($^{99\text{m}}\text{Tc}$) into the HPLC (high-pressure liquid chromatograph) (Figure 2), thus allowing the simultaneous detection by UV absorption and radiodetection (retention times 21.5 min for the Re-species and 21.9 min for the $^{99\text{m}}\text{Tc}$ -species; the delay is a result of the separation of the two detectors).

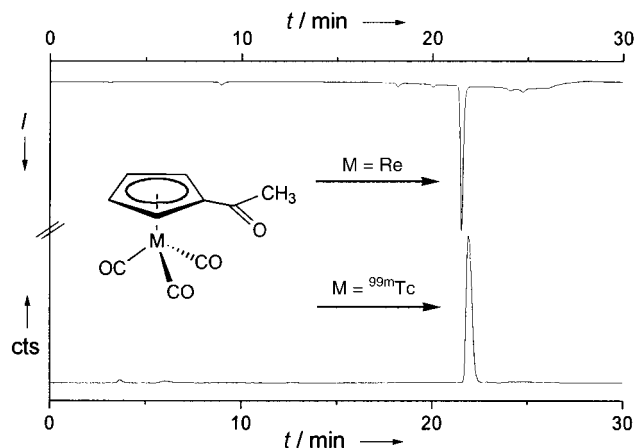
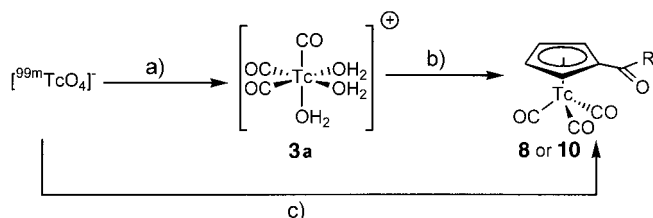


Figure 2. The upper trace shows the UV absorption at 250 nm for **8** (Re); the lower trace shows the radioactive trace for the corresponding $^{99\text{m}}\text{Tc}$ complex, proving their identity (delay due to detector separation).

This synthetic pathway was also applied to the Cp-derivatized serotonergic receptor ligand **7** with identical results. Excess **7** dimerizes during the reaction and the precipitate can be removed by filtration, thus increasing the specific activity efficiently, which may be of importance for preventing a saturation of in vivo receptors with a “cold” substrate. This unique reaction clearly reveals the high potential behind the chosen approach, which allows the preparation and use of half-sandwich $^{99\text{m}}\text{Tc}$ -complexes in physiological solutions for the first time, in quantitative yield and convenient for routine use.

At this stage, the reaction consisted of two steps, the initial preparation of **3a** and subsequent reaction with the respective Cp-derivatized (bio)molecule. To achieve a true one-pot synthesis for $[(\text{R-Cp})^{99\text{m}}\text{Tc}(\text{CO})_3]$, we introduced potassium boranocarbonate (**11**, $\text{K}_2\text{H}_3\text{BCO}_2$), the preparation and properties of which were recently reported by us.^[22] Boranocarbonate **11** is an air-stable reducing agent, and acts as an in situ CO source in water at elevated temperatures. The simple addition of $[\text{Cp}^{99\text{m}}\text{TcO}_4]^-$ in a saline solution to a vial that contained a small amount of **11**, is sufficient to give **3a** in quantitative yield. If, in addition to **11**, a small amount of the Cp-derivative **4** or **7** was present, 40 min at 90 °C was sufficiently long to produce the corresponding half-sandwich complexes **8** or **10** ($^{99\text{m}}\text{Tc}$) in a single step in >95% yield (Scheme 4).



Scheme 4. One- and two-step synthesis of complexes **8** and **10** (^{99m}Tc) directly in water and $[^{99m}\text{TcO}_4]^-$. Reagents and conditions: a) CO (1 atm), NaBH_4 , pH 12 or $\text{K}_2[\text{H}_3\text{BCO}_2]$ only, >98%. b) **4** or **7** (0.1–1 μmol), 15 min 90 °C, >98%. c) 0.1–1 μmol **4** or **7**, $\text{K}_2[\text{H}_3\text{BCO}_2]$ (**11**; 4 mg), $\text{Na}_2\text{B}_4\text{O}_7$, pH 10, 40 min 90 °C, >95%.

Besides the major product, a second complex was formed in minor amounts, with a similar retention time. It is likely that **11** reduced the carbonyl group in **8** to a hydroxy group. We assume that the one-pot reaction is consecutive $[^{99m}\text{TcO}_4]^- \rightarrow \mathbf{3a} \rightarrow \mathbf{8}$ or **10**. All the compounds labeled with ^{99m}Tc were stable in phosphate buffer for at least 24 h at 37 °C without any significant decomposition, thus confirming the highly robust nature of $[(\text{R-Cp})\text{M}(\text{CO})_3]$.

We demonstrated herein that half-sandwich complexes $[(\text{R-Cp})\text{M}(\text{CO})_3]$ can easily be synthesized if the acid dissociation constant of the cyclopentadiene ring is increased. The reaction of acetyl cyclopentadiene and derivatives with $[^{99m}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ directly yielded the radiopharmaceutically relevant complexes $[(\text{R-Cp})^{99m}\text{Tc}(\text{CO})_3]$ in good yields. The major impact of this work emerges from the general possibility of introducing the very small and highly lipophilic $[\text{Cp}^{99m}\text{Tc}(\text{CO})_3]$ moiety in a wide variety of small receptor-binding biomolecules. Furthermore, the direct reaction of acidic and water-soluble cyclopentadiene compounds with aqua ions could lead to interesting and novel species in aqueous organometallic chemistry.

Experimental Section

7: Alkylation of 1-(2-methoxyphenyl)piperazine (Fluka) with ethyl bromoacetate for 24 h at room temperature in the presence of Hünig's base, and purification on silica gel (CH_2Cl_2 /ethylacetate 4:1 v/v) afforded **6a** in 65–70% yield. NaCp was freshly prepared in situ from Na (0.3 g, 12.7 mmol) and the corresponding amount of freshly distilled dicyclopentadiene. Compound **6a** in THF (1.8 g, 6.4 mmol) was added to the NaCp solution and heated at about 60 °C for 6 h to afford a precipitate, which was isolated by filtration under N_2 . The solid was washed several times with *n*-hexane to leave an almost colorless powder, which was recrystallized from hot THF to give analytically pure **7**. ^1H NMR (200 MHz, D_2O): δ = 7.0 (m, 4H), 6.60 (brs, 2H), 6.15 (brs, 1H), 6.06 (brs, 1H), 3.74 (s, 3H), 3.50 (s, 2H), 2.95 (brs, 4H), 2.70 (brs, 4H); ^{13}C NMR (300 MHz, D_2O): δ = 182.4, 150.4, 138.2, 122.5, 122.2, 119.1, 116.9, 116.5, 115.9, 114.2, 111.3, 109.7, 59.8, 53.0, 50.5, 48.1.

8: Compound **8** can be synthesized according to published routes, starting from $[\text{ReBr}(\text{CO})_5]$ or directly from an aqueous buffer solution. $(\text{NEt}_4)_2[\text{ReBr}_3(\text{CO})_3]$ (130 mg, 0.15 mmol) in phosphate buffer (15 mL) was added dropwise over a period of 2 h to compound **7** (70 mg, 0.18 mmol) dissolved in phosphate buffer (15 mL, pH 7.4). The mixture was heated for 3 h at 75 °C, causing a brownish precipitate containing the product, as shown by using HPLC. The precipitate was filtered and dissolved in CH_2Cl_2 . Separation of the product was performed by silica-gel column chromatography. Elution with CH_2Cl_2 gave nonpolar side products, whereas subsequent elution with CH_2Cl_2 /2-propanol (80:1 v/v) gave compound **8** (20 mg, 23%). The product was recrystallized from an acidic mixture of EtOH/water by slow evaporation of ethanol. ^1H NMR (200 MHz, CD_3CN): δ = 6.9 (m, 4H), 6.24 (t, 2H), 5.53 (t, 2H), 3.79 (s, 3H), 3.48 (s, 2H), 3.05 (t,

J = 4.8 Hz, 2H), 2.65 (t, J = 4.8 Hz, 2H); ^{13}C NMR (300 MHz, CD_3CN): δ = 193.7, 153.3, 142.4, 124.1, 121.9, 119.2, 112.0, 96.1, 90.2, 89.7, 87.4, 86.9, 65.6, 54.2, 50.9. FAB-MS (fast-atom bombardment mass spectrometry): $[M^+]$ 567.8 (567).

$[(\text{R-Cp})^{99m}\text{Tc}(\text{CO})_3]$: The starting material $[^{99m}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ (**3a**) was prepared as described elsewhere.^[12,22] An aqueous solution of **7**, for example, in phosphate buffer was added to **3a** (final concentration of **7** = 10^{-3} – 10^{-4} M). Subsequent heating at 95 °C for 15 min afforded $[(\text{R-Cp})^{99m}\text{Tc}(\text{CO})_3]$ in overall yields >95%. The HPLC retention times for **8** and **10** are 21.9 and 20.5 min, respectively.

For the one-pot synthesis of **8** or **10** ($\text{M} = ^{99m}\text{Tc}$), a vial was charged with **11** (4 mg, reducing agent and CO source) and $\text{Na}_2\text{B}_4\text{O}_7$ (5.5 mg), sealed and flushed with dry N_2 . Generator eluate (2 cm³) containing $[^{99m}\text{TcO}_4]^-$ was added, followed by an aqueous solution of **4**. Heating at 90 °C for about 40 min gave **8** or **10** in high yields ($\approx 80\%$ and $\approx 20\%$ reduced form). The yield and time course of the reaction were not influenced by the total amount of radioactivity (up to 20 GBq of ^{99m}Tc).

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- [18] Crystallographic analysis: **10** ($\text{C}_{21}\text{H}_{22}\text{ClN}_2\text{O}_5\text{Re}$), colorless column, $0.19 \times 0.08 \times 0.03$ mm³, monoclinic, space group $P2_1/c$, a = 22.8795(14), b = 14.0402(11), c = 7.1351(4) Å, β = 94.495(7)°, V = 2285.0(3) Å³, Z = 4, ρ_{calc} = 1.756 g cm⁻³, R_1 ($I \geq 2\sigma(I)$) = 0.0325, wR_2 (F^2) = 0.1090 for 22230 data (5468 independent, 1263 observed).

($I \geq 2\sigma(I)$), 275 parameters, 6 restraints, numerical absorption correction, $\mu = 5.468 \text{ mm}^{-1}$, $T_{\text{min}} = 0.5802$, $T_{\text{max}} = 0.8524$. Single crystals of **10** were obtained by dissolving the product in a slightly acidic EtOH/water mixture and slow evaporation of ethanol. Data were collected on a Stoe IPDS diffractometer using graphite-monochromated $\text{MoK}\alpha$ ($\lambda = 0.71073 \text{ \AA}$) radiation, and were corrected for Lorentz, polarization, and absorption effects. The structure was solved by direct methods with SHELXS97 and refined with SHELXL97 on F^2 using all data with all non-hydrogen atoms anisotropically defined.^[23] The hydrogen atoms were placed in calculated positions and isotropically refined with thermal parameters at $1.5 \times U(\text{eq})$ of the parent carbon atom, except for the piperazyl-N(H) which was found in the difference Fourier map and isotropically refined. 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-160745 (**9**) and CCDC-160746 (**10**). 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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A Chemosensing Ensemble for Selective Carbonate Detection in Water Based on Metal–Ligand Interactions**

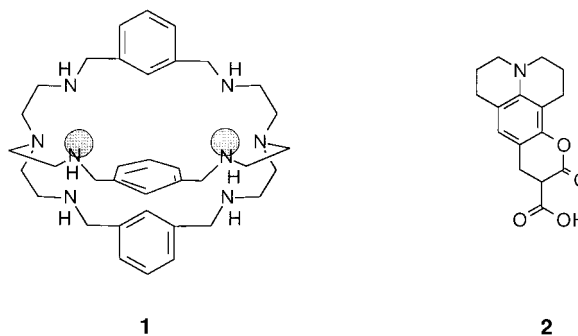
Luigi Fabbrizzi,* Antonella Leone, and Angelo Taglietti

The classical approach to the design of a fluorescent sensor involves the covalent linking of a fluorescent fragment (the “signaling unit”) to a receptor, which displays specific binding tendencies towards a given analyte. In order to communicate the occurrence of the recognition event to the outside world, the receptor/analyte interaction must affect, to some extent, the emission properties of the signaling unit—either quenching the fluorescence through a defined mechanism (usually energy or electron transfer) or enhancing it by suppressing a pre-existing quenching process.^[1]

Recently, Anslyn and co-workers^[2] have taken inspiration from antibody-based biosensors in immunoassays and developed an efficient “competition” approach to the design of chemosensors: according to the so-called “chemosensing ensemble” approach, the plain receptor interacts in solution

with a colorimetric or fluorescent indicator, namely, a highly colored or light-emitting substrate. Two main requirements have to be fulfilled: the receptor/indicator interaction must not be too strong and the indicator must show significantly different optical properties when bound to the receptor and when dispersed in solution. Thus, the indicator is displaced from the host cavity on titration with the desired analyte and released to the solution, where it displays drastically different optical features. Hence, the occurrence of the recognition event is communicated by either a substantial color change or a dramatic modification of the light emission. In their competition studies Anslyn and co-workers considered anion receptors capable of interacting with the analyte through formation of hydrogen bonds. Typical investigated analytes were citrate, tartrate, malate, and inositol triphosphate. The procedure of competitive spectrophotometry has been treated in detail by Connors.^[3] Ueno and co-workers later extended the approach by covalently linking the dye to the receptor.^[4]

We now extend the competition approach to the fluorescent sensing of anions by making use of a different type of receptor–analyte interaction: metal–ligand (coordinative) interactions. Coordinative interactions present some substantial advantages compared to hydrogen bonding and, in general, to electrostatic interactions.^[5] In fact, they can be highly energetic as a result of the strong ligand-field stabilization energy effects that may be induced by coordination. Moreover, transition metal ions present definite stereochemical preferences which can be addressed to impart selective binding tendencies towards anions of a given shape. In order to benefit from these features, metal–ligand interactions have been utilized in the design of anion receptors operating in highly polar media, including pure water. In this connection, we have shown that the dicopper(II) cage complex **1** is capable of including polyatomic anions



within its intermetallic cavity in an aqueous solution buffered at pH 8. The ion selectivity is determined by the bite length of the anion, that is, the distance between two consecutive donor atoms.^[6] In particular, receptor **1** showed a definite preference towards the triangular HCO_3^- ion^[7] and the two linear triatomic ions N_3^- and NCO^- , whose bite length range between 2.28 and 2.42 Å. The intrinsic limit of receptor **1** as a proper molecular sensor is that only small changes in the absorption spectra and in the color are observed following anion recognition. For example, in the case of HCO_3^- ions, the color turns from the pale blue of the void receptor to the pale

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green of the inclusion complex. The sole exception is represented by the inclusion of the N_3^- ion, which induces the development of a rather intense ligand-to-metal charge transfer band centered at 400 nm. Thus, we considered the opportunity to teach the "old" receptor **1** new ways to act as a sensor, in particular, as a fluorescent sensor, by taking advantage of the chemosensing ensemble approach.

As a fluorescent indicator we chose coumarine 343 (**2**), which possesses a carboxylate function capable of bridging the two Cu^{II} centers of receptor **1**. It is also strongly fluorescent in its anionic form, which is the dominating species in an aqueous solution adjusted to pH 7. Complete quenching of the coumarine emission was observed ($\lambda_{\text{exc}} = 424 \text{ nm}$, $\lambda_{\text{em}} = 487 \text{ nm}$) on titrating a degassed solution of **2** (10^{-7} M), buffered to pH 7 (HEPES; 0.05 M), with a standard solution of receptor **1**.

Curve fitting of the titration profile (fluorescent intensity versus concentration of **1**; Figure 1) by a nonlinear least-squares procedure was consistent with the formation of an

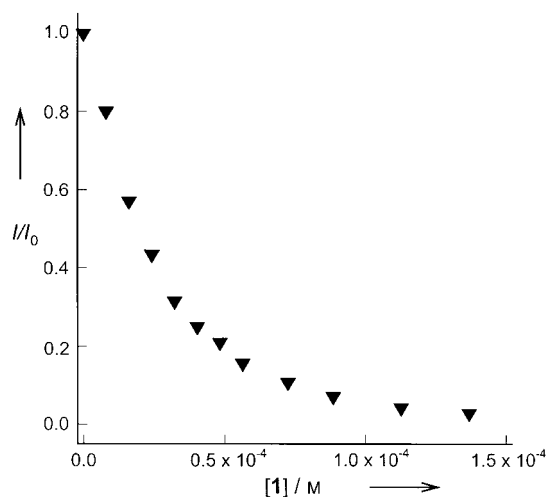


Figure 1. Titration of a solution of the fluorescent indicator coumarine 343 (**2**) with the dimetallic receptor **1** in an aqueous solution buffered to pH 7. A 1:1 adduct forms, whose association constant is $4.8 \pm 0.1 \text{ log units}$. Quenching is ascribed to the occurrence of an intramolecular electronic energy transfer which involves the photoexcited coumarine subunit and the Cu^{II} center(s) within the 1:2 adduct.

adduct of 1:1 stoichiometry, whose association equilibrium constant K_{ass} was $4.8 \pm 0.1 \text{ log units}$.^[8] Fluorescence quenching is ascribed to an intracomplex electronic energy transfer process involving the photoexcited coumarine fragment and the Cu^{II} center(s). The occurrence of a transfer of energy rather than a transfer of electrons was demonstrated by the fact that quenching persisted even when an ethanolic solution of the 1:2 adduct was vitrified at 77 K .^[9]

A chemosensing ensemble was then generated by making a degassed aqueous solution containing $2 \times 10^{-4} \text{ M} **1** and $10^{-7} \text{ M} **2** buffered to pH 7 with HEPES (0.05 M). Titration of the resulting chemosensing ensemble solution with a standard solution of carbonate (Figure 2, $\blacktriangle$) resulted in an almost complete recovery of the coumarine emission. This result indicates there is successful competitive binding of the HCO_3^- ions and displacement of the indicator from the host cavity.$$

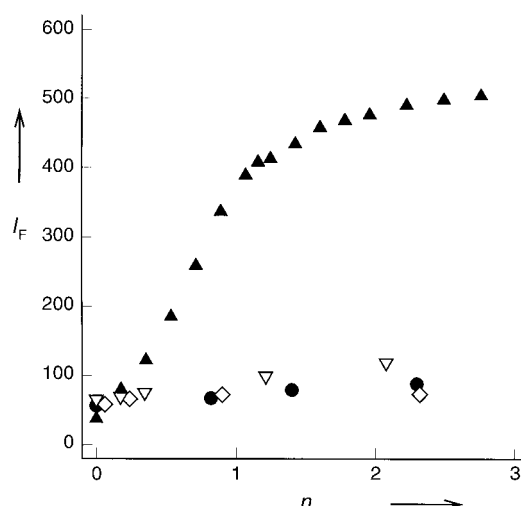


Figure 2. Competitive titration of an aqueous solution of $2 \times 10^{-4} \text{ M} **1** and $10^{-7} \text{ M} **2**, buffered to pH 7, with standard solutions of selected anions. HCO_3^- ($\blacktriangle$) is able to displace the indicator **1** from receptor **2**. The indicator is released into the solution and displays its full emission. Other anions, for example, phosphate (∇), acetate ($\bullet$), and sulfate ($\diamond$) do not compete successfully with **2** for **1** and induce only a slight fluorescence enhancement. n = anion equivalents.$$

Full regeneration of the coumarine emission was observed also when the solution of the chemosensing ensemble was titrated with N_3^- and NCO^- ions. However, titration with a variety of ambidentate anions (NCS^- , NO_3^- , SO_4^{2-} , HPO_4^{2-} , HCOO^- , CH_3COO^-) induced only a slight enhancement of the fluorescence (Figure 2).

The selectivity pattern described above could be tentatively explained by assuming that HCO_3^- , N_3^- , and NCO^- ions exhibit association constants with **1** that are distinctly larger than that of the coumarine indicator (4.8 log units), whereas all the other investigated anions must show substantially lower $\text{log } K_{\text{ass}}$ values. This hypothesis was confirmed through direct spectrophotometric titration experiments, that is, by adding a standard solution of the anion to a solution of **1** buffered at pH 7 and looking at the development/modification of the pertinent charge-transfer absorption bands. Association constants for the formation of 1:1 receptor:anion adducts were determined through a least-squares procedure,^[8] and their values plotted versus the anion bite length (Figure 3). A selectivity pattern was observed, which is similar to that previously found in somewhat different conditions (aqueous solution buffered to pH 8 with 0.1 M lutidine).^[6] Figure 3 shows that the HCO_3^- , N_3^- , and NCO^- ions exhibit association constants with **1** higher than that of coumarine 343 (whose value is indicated as a dashed horizontal line), and are therefore able to displace the fluorescent indicator from the receptor cavity. The remaining anions, however, whose association constants are well below that of coumarine 343, cannot compete successfully with the indicator and cannot, therefore, be detected spectrofluorimetrically through the chemosensing ensemble approach.

We validated the approach by determining the $\text{log } K_{\text{ass}}$ value for the association equilibrium of HCO_3^- ions with **1** from the combination of the spectrofluorimetric titration data (direct titration of Figure 1 and competitive titration of Figure 2), by

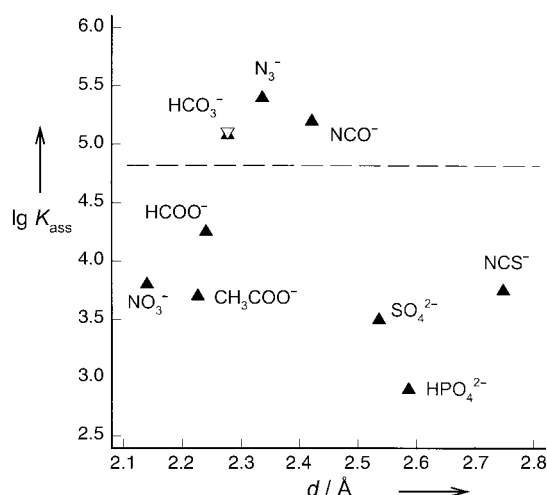


Figure 3. Selectivity pattern for the inclusion of polyatomic anions into the dimetallic receptor **1**: $\blacktriangle$ indicates the $\lg K_{\text{ass}}$ value for the association equilibrium at pH 7 determined through spectrophotometric titrations. The dashed horizontal line corresponds to the $\lg K_{\text{ass}}$ value for the association of **1** with the fluorescent indicator **2**. Only the anions whose $\lg K_{\text{ass}}$ value is above the horizontal line are able to displace **2** from **1** and can be detected spectrofluorimetrically through the “chemosensing ensemble” method. d = bite length.

following a published calculation procedure.^[3] The $\lg K_{\text{ass}}$ value obtained from a competition spectrofluorimetric titration (gray triangle in Figure 3) is coincident with that obtained by direct spectrophotometric titration.

Of the anions that could be detected spectrofluorimetrically by the “chemosensing ensemble” method, carbonate is surely the most relevant from the point of view of practical applications. Thus, a calibration curve was obtained over the range 2×10^{-5} – 2×10^{-4} M (Figure 4) and subsequently used for the quantification of carbonate in samples of commercial mineral water. In a typical measurement, a small amount of the sample (10–500 μL) was added to the chemosensing ensemble solution (10 mL, 2×10^{-4} M **1** and 10^{-7} M **2**). The fluorescence intensity was measured and the amount of

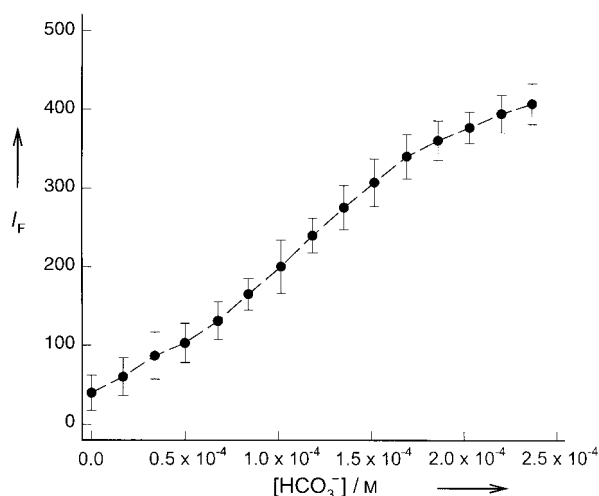


Figure 4. Calibration curve for the determination of the concentration of HCO_3^- ions by measurement of the fluorescent intensity of a solution containing the “chemosensing ensemble” **1:2**.

carbonate was then calculated from the calibration curve. A substantial range of carbonate concentration can be explored by varying the volume of the sample used. For example, by taking a sample of 10–500 μL , it is possible to span a 4×10^{-4} – 2×10^{-1} M range of HCO_3^- ions (corresponding to 24–1222 mg L^{-1} of hydrogencarbonate), which covers all the commercially available mineral waters. The present procedure seems easier and more selective than that currently used to determine the concentration of HCO_3^- ions in water which is based on volumetric titration of the alkalinity with standard hydrochloric or sulfuric acid.^[10] In fact, the latter procedure does not distinguish HCO_3^- ions from other bases present in solution.

The marriage of a dimetallic cage and the chemosensing ensemble approach seems very promising for the fluorimetric determination of anions. The utilization of a smaller cavity cage may, for example, allow the fluorimetric determination of monoatomic anions such as halides.

Experimental Section

Complex **1** was prepared as described elsewhere.^[6,7] Compound **2** and other chemicals were purchased from Aldrich–Fluka and used without any further purification.

UV/Vis determinations of association constants between anions and **1** were performed in water buffered at pH 7 with 2-[4-hydroxyethyl]-1-piperazinyl]ethanesulfonic acid (HEPES; 0.05 M). Degassed standard solutions of anions were added to a 5×10^{-4} M degassed solution of **1**. Data fitting was performed on absorbance data at the appropriate wavelength.

The determination of the association constants between **1** and **2** was performed in degassed water solution buffered at pH 7 with HEPES (0.05 M) and containing 10^{-7} M **2**. Aliquots of a fresh and degassed standard solution of **1** were added, and the emission spectra of **2** were recorded ($\lambda_{\text{exc}} = 424$ nm, $\lambda_{\text{em}} = 487$ nm). The chemosensing ensemble solutions for competition assays were prepared by adding **1** to a degassed solution of **2** in water buffered at pH 7 with HEPES (0.05 M), to avoid any carbonate contamination. The chemosensing ensemble solution (**1**: 2×10^{-4} M, **2**: 10^{-7} M) was titrated, under a nitrogen atmosphere, with standard solutions of anions. Points on the calibration curve for carbonate detection were obtained from four competition assays and the mean value calculated for every concentration used. Error bars were calculated as the double of the standard deviations of the values.

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Combinatorial Templates Generated by Dip-Pen Nanolithography for the Formation of Two-Dimensional Particle Arrays**

Linette M. Demers and Chad A. Mirkin*

A general method for organizing micro- and nanoparticles on a substrate could facilitate the formation and study of photonic band-gap arrays for analysis of the relationship between pattern structure and catalytic activity and enable formation of arrays of single protein molecules for proteomics research. While several methods have been reported for assembling collections of particles onto patterned surfaces,^[1, 2] a major challenge lies in the selective immobilization of single particles into predetermined positions with respect to adjacent particles.

A strategy for chemically and physically immobilizing a wide variety of particle types and sizes with a high degree of control over particle placement calls for a soft lithographic technique capable of high-resolution patterning, as well as one with the ability to form patterns of one or more molecules with precise alignment. Dip-pen nanolithography (DPN) has emerged as one such tool.^[3] DPN is a scanning probe nanopatterning technique in which an atomic-force microscopy (AFM) tip is used to deliver molecules to a surface through a water meniscus, which naturally forms in the ambient atmosphere. Significantly, DPN also can be used to generate many customized templates formed from the same or different chemical inks which can be screened under identical conditions for a particular application. Herein, we report an example of this new combinatorial approach, and focus on the problem of particle assembly in the context of colloidal crystallization.

Recently, conventional sedimentation methods for preparing colloidal crystals consisting of close-packed layers of polymer or inorganic particles have been combined with polymer templates, fabricated by electron-beam lithography, to form high-quality single-component structures.^[1] However, sedimentation or solvent evaporation routes do not offer the element of chemical control over particle placement.^[4] Herein, we describe a DPN-based strategy for generating charged chemical templates to study the assembly of single particles into two-dimensional square lattices.

Our general method (Figure 1) is to form a pattern on gold composed of an array of dots of a "molecular ink" which will attract and bind a specific type of particle. For these studies we

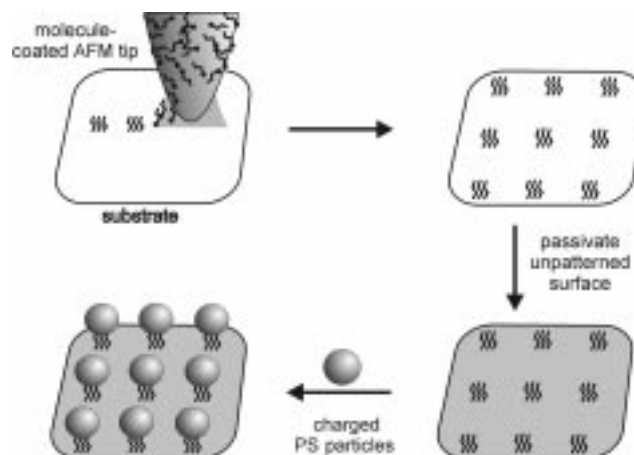


Figure 1. A schematic representation of the DPN-based particle organization strategy.

used 16-thiohexadecanoic acid (16-mercaptohexadecanoic acid, MHA) to make templates, and positively-charged protonated amine- or amidine-modified polystyrene (PS) spheres as particle building blocks. The unpatterned regions of the gold were passivated by immersing the substrate in a 1 mM ethanolic solution of another alkanethiol, such as 18-octadecanethiol (ODT), or cystamine for 9 min. Minimal, if any, exchange takes place between the MHA molecules and the ODT or cystamine in solution during this treatment, as evidenced by lateral force microscopy of the substrate before and after treatment with ODT (no change). Finally, particle assembly was accomplished by placing a 20- μ L droplet of dispersed particles (10% wt/vol in deionized water) onto the horizontal substrate in a humidity chamber (100% relative humidity). Gentle rinsing with deionized water completes the process. In this particular study, it is important to note that the carboxylic acid groups in the MHA patterns are deprotonated, thus providing an electrostatic driving force for particle assembly.^[5]

In a typical experiment involving 0.93 μ m diameter particles, multiple templates are monitored simultaneously for particle assembly by optical microscopy. In these experiments, the diameter of the template dot is varied to search for optimal conditions for particle-template recognition (Figure 2). After 1 h of particle assembly, the substrates are rinsed with deionized water, dried under ambient laboratory conditions, and then imaged by optical microscopy (Figure 3).

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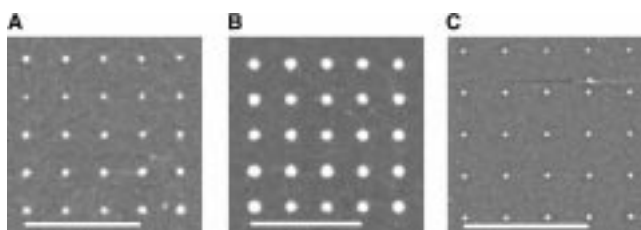


Figure 2. A–C) Patterns generated on gold thin films by DPN and imaged by lateral-force microscopy (MHA light areas, ODT dark areas). MHA dots (diameters: A: 540, B: 750, and C: 240 nm, center-to-center distance 2 μm) deposited by holding the AFM tip at a series of x,y coordinates (5, 10, and 15 s for A–C, respectively). Scale bars represent 6 μm .

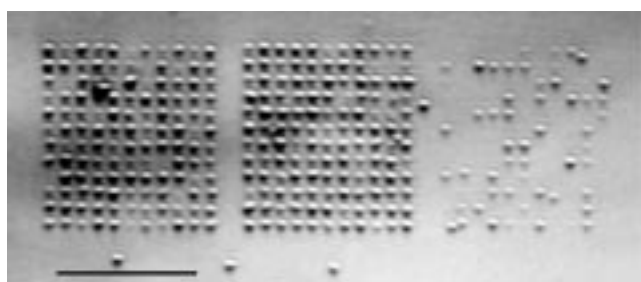


Figure 3. Optical micrograph of particle arrays in a combinatorial experiment on a MHA-patterned substrate (as shown in Figure 2). Scale bar represents 20 μm .

The combinatorial experiment reveals that the optimum size of the template pad with which to immobilize a single particle of this type in high registry with the pattern is approximately 500–750 nm. It is important to note that drying the substrate tends to displace the particles from their preferred positions on the template, an effect that has been noted by others with larger scale experiments.^[6] Indeed, evidence for better, in fact near-perfect, particle organization (using 700-nm template dots) is obtained by in situ imaging of the surface after the template has been treated with 1- μm amine-modified particles for 1 hour (Figure 4).

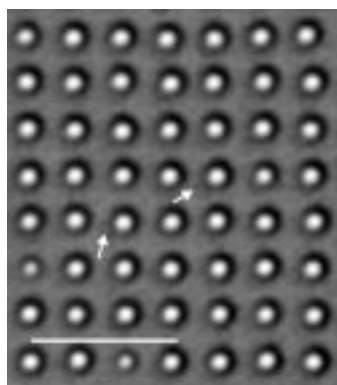


Figure 4. In situ optical micrograph of 1.0 μm diameter amine-modified polystyrene particles organized into a square array with a lattice constant of 2 μm . The dark fuzzy dots are particles in solution that have not reacted with the template (white arrows). Scale bar represents 6 μm .

Spatial organization of single particles on the micron length-scale has been achieved by physical means, for example, by using optical tweezers,^[7] or by sedimentation

onto electron-beam lithographically patterned polymer films.^[1] However, the DPN-based method described here offers an advantage over previous methods because it provides flexibility of length scale and pattern type as well as a means to achieve more robust particle array structures. For example, we have used DPN to construct chemical templates which can be utilized to prepare square arrays of 190 nm diameter amidine-modified polystyrene particles. Screening of the dried particle arrays by noncontact AFM or scanning electron microscopy (SEM) imaging after 3 hours of particle drying reveals that 300-nm template dots of MHA, spaced 570 nm apart, with a surrounding repulsive monolayer of cystamine are suitable for immobilizing single particles at each site in the array (Figure 5 A). However, MHA dots of 700 nm diameter and 850-nm spacing result in immobilization of multiple particles at some sites (Figure 5 B).

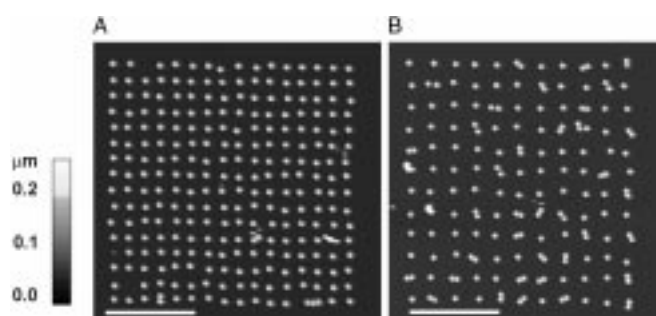


Figure 5. Two regions of a gold substrate with 190 nm diameter amidine-modified polystyrene particles selectively organized on MHA regions of the patterned surface, imaged by intermittent-contact AFM. Particle arrays formed on: A) 300 nm diameter MHA dots and B) 700 nm diameter dots. Also, note that the AFM tip in some case drags the particles from their preferred locations. Scale bars represent 3 μm (A) and 4 μm (B), respectively.

Similar particle assembly experiments conducted at $\text{pH} < 5$ or > 9 result in random, nonselective particle adsorption, presumably as a result of protonation of the surface acid groups, or deprotonation of the amine or amidine groups on the particles. These experiments suggest that the particle assembly process is induced by electrostatic interactions between charged particles and patterned regions of the substrate.

We have demonstrated that DPN can be used as a tool for generating combinatorial chemical templates with which to position single particles in two-dimensional arrays. It is important to note that the DPN approach will allow one to systematically vary the chemical composition of the array, spot size, spot shape, and interfeature distances. The specific example of charged alkanethiols and latex particles described here will provide a general approach for creating two-dimensional templates for positioning subsequent particle layers in predefined crystalline structures that may be composed of single or multiple particle sizes and compositions. In a more general sense, the combinatorial DPN method will allow researchers to efficiently form patterned substrates with which to study particle–particle and particle–substrate interactions of dielectric spheres which comprise

certain photonic band-gap materials, metal or semiconductor particles with potential catalytic or electronic properties, or even living biological cells and macromolecules.

Experimental Section

Gold-coated substrates (60 nm Au, 10 nm Ti on Si) were prepared according to previously reported procedures.^[8] For in situ imaging experiments requiring transparent substrates, coverslip glass (Corning No. 1 thickness, VWR, Chicago, IL) was cleaned with Ar/O₂ plasma for 1 min, then coated with 2 nm of Ti and 15 nm of Au.

All DPN patterning experiments and lateral force imaging experiments were carried out under ambient laboratory conditions (30 % relative humidity, 23 °C) and as previously reported.^[8]

Optical microscopy was performed using the optics on a Park Scientific CP AFM (Thermomicroscopes, Sunnyvale, CA) or, for in situ imaging, an inverted optical microscope (Axiovert 100A, Carl Zeiss, Jena, Germany) operated in differential interference contrast mode. Images were captured with a Penguin 600 CL digital camera (Pixera, Los Gatos, CA). Intermit-tant-contact imaging of particles was performed with a Thermomicroscopes M5 AFM using silicon ultra-levers (Thermomicroscopes, spring constant = 3.2 N m⁻¹).

Suspensions of charged polystyrene latex particles in water were purchased from either Bangs Laboratories (0.93 µm, Fishers IN, USA) or IDC Latex (1.0 µm and 190 nm, Portland OR, USA). Particles were rinsed free of surfactant by centrifugation and redispersion (twice) in distilled deionized water (18.1 MΩ) purified with a Barnstead (Dubuque, IA) NANOpure water system.

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Orthogonal Assembly of Nanoparticle Building Blocks on Dip-Pen Nanolithographically Generated Templates of DNA**

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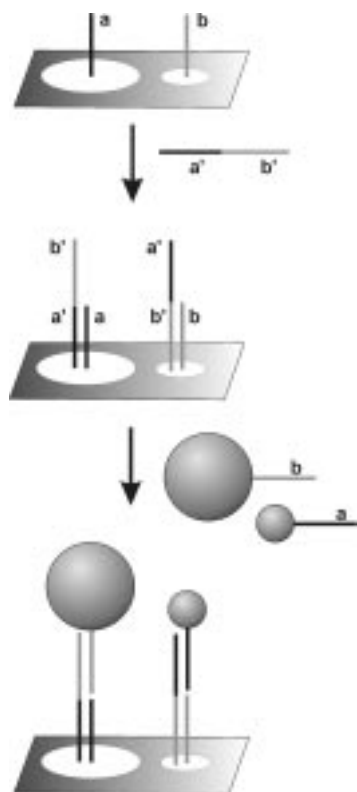
The development of methods for organizing nanostructures into functional materials with addressable nanoscopic components represents a significant challenge in nanoscience. A variety of methods have been employed to control the assembly of nanoparticles into ordered two- and three-dimensional (2D and 3D) architectures in solution and on surfaces. These involve three general approaches: 1) the use of organic linker molecules and covalent bonding to generate meso- and macroscopic architectures with control over particle placement within an assembled network of particles,^[1] 2) the use of external physical forces (e.g. Langmuir–Blodgett techniques, electric fields) and weak interactions to form ordered 2D particle arrays,^[1, 2] or 3) the use of biological molecules and their molecular-recognition properties to guide the assembly of polymeric-network structures either on a surface or in solution.^[1, 3] However, at present there are no efficient methods for chemically directing the assembly of *multicomponent* nanostructures on surfaces with precise control over the placement of the nanoscale building blocks. An intriguing possibility for the biomolecule-based approach to particle assembly would be to learn how to pattern biological molecules on surfaces with nanoscale resolution, one could literally chemically program or encode such surfaces with information based upon the biorecognition elements used in the patterning process. For example, in the case of a synthetic sequence of DNA that is 20 bases long, there are 4²⁰ possible recognition elements that could be used for guiding the assembly of nanoscale building blocks functionalized with the appropriate complementary sequences. As the length of the sequence increases, the number of recognition elements increases dramatically providing an almost limitless number of interaction pairs that can be designed to guide a given nanostructure-assembly process. In contrast, if ordinary organic molecules were used in either surface-modification chemistry or covalent organic methods for directing such processes, there are a limited and small number of interaction pairs that could be designed and employed. Indeed, Wrighton and co-workers, in studying the assembly of redox-active molecules on micron-scale electrode surfaces made of In₂O₃–SnO₂ (ITO) and Au, respectively, showed that it is difficult to design even two interaction pairs by using coordination chemistry to guide such processes in a *perfectly orthogonal manner*.^[4] Herein, we show how Dip-Pen

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Nanolithography (DPN) can be used to create nanostructures of DNA on a surface, which can be used subsequently to guide the assembly of discrete nanoparticle building blocks with complementary DNA in an orthogonal manner. Significantly, this strategy could lead to a new and general way for preparing multicomponent nanostructures for a wide-range of applications ranging from biological diagnostics to nanoelectronics to the preparation of colloidal crystals for use in catalysis and photonics.^[5]

In a typical experiment, a gold surface is patterned with 1,16-mercaptohexadecanoic acid (MHA) by DPN (contact force: 0.5 nN, contact time: 1 s per 100 nm dot radius, $r \sim t^{1/2}$, and 55 % relative humidity).^[6] The area surrounding the MHA pattern is modified with 1-octadecanethiol (ODT) by immersing the substrate in a 1 mM ethanolic solution of ODT for 5 min. This allows one to passivate the bare gold with minimal ODT exchange with the MHA patterns (not detectable by lateral force microscopy (LFM)). Alkylamine-modified DNA ("a" in Scheme 1) is then coupled to the MHA



Scheme 1. A schematic representation of orthogonal assembly with DNA nanostructures and nanoparticles functionalized with complementary DNA. From the 5' end, sequences are **a**: TCTCAACTCGTAA₁₀, **b**: A₁₀CGCATTCAGGAT, **a'**: TACGAGTTGAGAATCCTGAATGCG; white ellipsoid = MHA pattern.

pattern by the formation of an amide bond. This linking is accomplished by immersing a patterned substrate in an aqueous buffer solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC; 10 mg mL⁻¹ in 0.1 M morpholine/ethanesulfonic acid (MES), pH 4.5) for 15 min to activate the surface carboxyl moieties, followed by rinsing with 0.1 M sodium borate/boric acid buffer, pH 9.5. A 10 μ L

droplet of the alkylamine DNA (25 μ M) in borate buffer is placed on the patterned substrate for 12 h. The substrate is rinsed with a phosphate buffered saline (PBS; 0.3 M NaCl, 0.01 M phosphate, pH 7), and then deionized water, dried in air and imaged by atomic force microscopy (AFM; Figure 1 A).

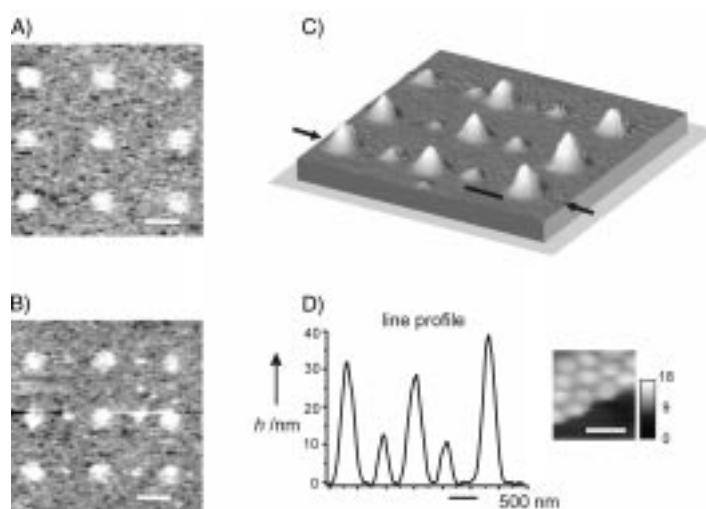


Figure 1. AFM images of patterned gold substrates. A) Topographical image (contact mode) of the substrate after DNA has been coupled to the DPN-generated MHA pattern. B) Topographical image of the two-component DNA pattern. C) Noncontact AFM topography image of particles after the orthogonal assembly process. Scale bars in A, B, and C are 1 μ m. D) Line scan of the first row of particles in the image in Figure 1C. The inset shows a high resolution, tapping-mode image of one of the nanostructures comprising 13 nm particles (scale bar is 30 nm).

A second MHA pattern, offset from the first DNA pattern and with smaller features (200 nm), is generated by DPN. Significantly, in a DPN experiment MHA will replace the areas modified with ODT in this manner under normal writing conditions (0.5 nN, 55 % humidity, 23 °C), as evidenced by LFM. This allows a second nanopattern of MHA to be placed in near-perfect registration with the first pattern without chemically modifying the first pattern.^[6b] A second alkylamine oligonucleotide ("b" in Scheme 1) can be immobilized on the second pattern of MHA by using the procedure described above. This creates two nanopatterns comprising two distinct oligonucleotide sequences that can guide the assembly of randomly dispersed nanoparticles, which are each functionalized with different oligonucleotides, Figure 1 B and Scheme 1.

To demonstrate the concept of orthogonal assembly of nanostructures by using DPN-generated oligonucleotide templates, a droplet of an oligonucleotide linker strand (0.3 μ M in PBS buffer) was placed on top of the patterned substrate for 2.5 h. This linker strand was designed to bind in an orthogonal manner to the two different oligonucleotide patterns (3'–5' and 5'–3', respectively from the surface) generating floppy ends that are complementary to the predesigned oligonucleotide-modified particles (Scheme 1). The substrates are rinsed with PBS buffer prior to particle-assembly experiments. Then, a droplet of solution containing DNA-derivatized 13 and 30 nm particles (particle concentrations $\sim$ 1 nM) was placed on the substrate for 4 h then rinsed

with PBS buffer and 0.3 M aqueous ammonium acetate, pH 7. The latter, which is a volatile salt, is used to prepare the substrates for imaging by reducing imaging complications that result from salt crystallization on the substrate. AFM images of the resulting structures show that the 13 and 30 nm particles are only immobilized on the complementary oligonucleotide patterns (Figure 1 C). Note the line scan shows the expected change in height as the tip traverses the nanoparticle-modified areas (Figure 1 D). Significantly, under the conditions used, there is no evidence of cross-reactivity between the nanoparticles and the DNA nanopatterns (see inset, Figure 1, no 30 nm particles are evident.) Indeed, when the patterning is performed in the reverse order, that is, the DNA patterns that bind the 13 nm particles are formed first, followed by patterns that bind the 30 nm particles, AFM images after particle assembly show no evidence of the larger particles bound to the first DNA pattern. In addition, control experiments without the linker show no particle assembly takes place, and that with the linker, each particle can be introduced separately to analogous substrates to generate one component nanostructures (either 13 or 30 nm particles) on the complementary regions of the substrate (Figure 2 A and B).

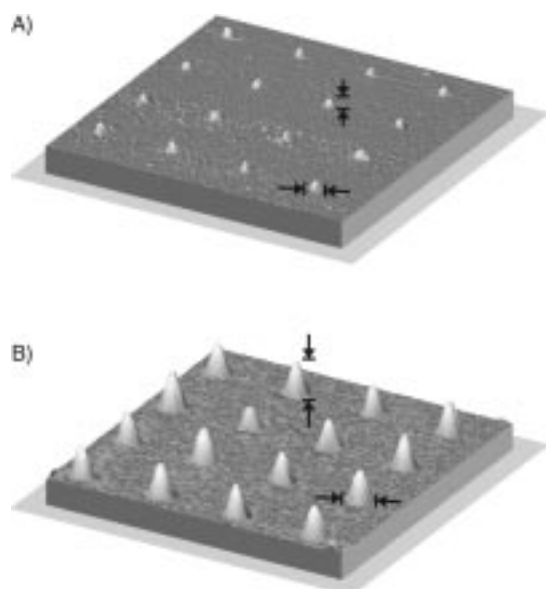


Figure 2. Noncontact AFM topography images of A) 13 nm and B) 30 nm diameter gold particles hybridized to complementary DNA patterns in the presence of linker DNA. Horizontal arrows indicate widths of 200 and 400 nm, and vertical arrows indicate heights of approximately 13 and 31 nm, for A and B, respectively.

Noteworthy is that the choice of conditions and modification procedures used in these experiments are important for reducing nonspecific particle adsorption. Indeed, although this process is clearly dominated by the specific interactions between the DNA patterns and complementary nanoparticles, it is not perfect. High resolution AFM images show that a few stray particles nonspecifically bind to the areas surrounding the oligonucleotide patterns. Indeed, in any sort of

experiment geared towards controlling nanostructure placement on a surface, non-specific binding of the nanoscale building blocks to the patterned surface will dictate the quality of the structures that can be generated. This is why it is essential to use direct-write methods such as DPN to immobilize the strong-binding agents on the substrate to be patterned. Indirect methods that rely on resists or exchange chemistry will ultimately lead to surface contamination and could increase particle nonspecific binding. This work points toward the need to develop ways of using oligonucleotides as inks that are directly patterned by the DPN experiment. Efforts in this direction are underway.^[5]

Experimental Section

Gold coated substrates were prepared according to reported procedures.^[7] All DNA was synthesized and purified by literature methods.^[8] ODT, MHA, and EDAC were purchased from Aldrich (Milwaukee, WI). MES was purchased from Sigma (St. Louis, MO). Gold nanoparticles were purchased from Ted Pella (Redding, CA; 30 nm diameter) or prepared by reduction of HAuCl₄ as previously described (13 nm diameter).^[9] Nanoparticles were modified with thiol-functionalized DNA as previously described.^[8]

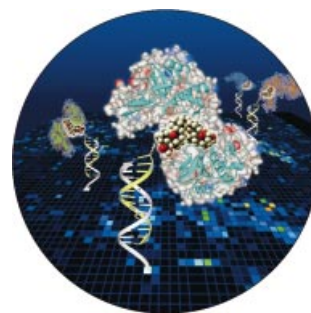
Noncontact and lateral-force imaging experiments were performed with a Park Scientific Instruments CP (Thermomicroscopes, Sunnyvale, CA) with silicon ultralevers (Thermomicroscopes, spring constant = 3.2 N m⁻¹) or silicon nitride microlevers (Thermomicroscopes, spring constant = 0.3 N m⁻¹). Tapping-mode images of nanoparticles were obtained using a Digital Instruments (Santa Barbara, CA) Nanoscope IIIa with silicon cantilevers from Digital Instruments, spring constant = ~40 N m⁻¹. All AFM images were processed by applying first-order flattening and a low-pass filter when needed.

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COVER PICTURE

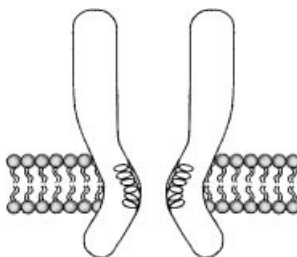
The cover picture shows a new method of arraying small molecules and its application in proteomic profiling. Each small molecule (CPK model) is tethered to a unique peptidonucleic acid (PNA) (yellow ribbon), which encodes its synthetic history. The library is screened against a sample of interest and then hybridized to an oligonucleotide microarray to determine the active compounds and/or the activity of the proteins. More details about this method are provided by Schultz and co-workers on page 3152 ff. Artwork by Nicolas Winssinger, a co-author.



REVIEWS

Key biological information is processed by ligand-gated ion channels (see schematic representation). These proteins comprise, among others, important neurotransmitter receptors of the nervous system. Recent crystal structures of channel domains and of related voltage-gated ion channels reveal their functional mechanisms.

Angew. Chem. **2001**, *113*, 3194–3211



F. Hucho,* C. Weise 3100–3116

Ligand-Gated Ion Channels

Keywords: ion channels •
neurotransmitters • proteins •
receptors • structure elucidation

Is rational design dead? The desire to create highly selective receptor molecules is being confronted with the prospect of multicomponent arrays of differential receptors (see schematic representation). The creation of a series of cross-reactive, nonspecific sensors is the basis behind this approach. The sensing capabilities arise from the study of the pattern created from an array of such receptors.



Specific



Differential

Angew. Chem. **2001**, *113*, 3212–3225

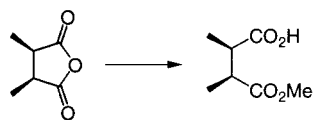
J. J. Lavigne, E. V. Anslyn* 3118–3130

Sensing A Paradigm Shift in the Field of Molecular Recognition: From Selective to Differential Receptors

Keywords: analytical methods • combinatorial chemistry • molecular recognition • receptors • sensors

HIGHLIGHTS

The single symmetry-breaking transformation of a relatively simple *meso* compound can provide highly expedient access to a wide variety of usefully functionalized chiral building blocks. Recent advances in asymmetric desymmetrization, through the development of tertiary amines as nonenzymatic catalysts for the ring opening of *meso* cyclic anhydrides with alcohols [for example, see Eq. (1)], are discussed here.



(1)

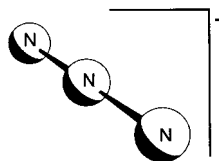
Angew. Chem. **2001**, *113*, 3227–3230

A. C. Spivey,* B. I. Andrews 3131–3134

Catalysis of the Asymmetric Desymmetrization of Cyclic Anhydrides by Nucleophilic Ring-Opening with Alcohols

Keywords: alkaloids • anhydrides • asymmetric catalysis • desymmetrization • ring opening

Azidometalates are not explosive if the solid salts contain sufficiently large cations. Structure determinations on single crystals of such compounds elucidate how the azide ions (see picture) act as covalently bound ligands and have several different bridging modes.



Angew. Chem. **2001**, *113*, 3231–3232

A. Kornath* 3135–3136

Homoleptic Azidometalates

Keywords: azides • azidometalates • nitrogen

VIPs

The following communications are “Very Important Papers” in the opinion of two referees. They will be published shortly (those marked with a diamond will be published in the next issue). Short summaries of these articles can be found on the *Angewandte Chemie* homepage at the address <http://www.angewandte.com>

Zero-Strain Intercalation Cathode for Rechargeable Li-Ion Cell

J. Cho, Y. J. Kim, T.-J. Kim, B. Park* ◆

High Turnover Numbers for Catalytic Selective Epoxidation of Alkenes with 1 atm Molecular Oxygen

Y. Nishiyama, Y. Nakagawa, N. Mizuno*

Experimental Detection of Tetraoxygen

F. Cacace,* G. de Petris, A. Troiani

[12.12]Paracyclophanedodecaynes $C_{36}H_8$ and $C_{36}Cl_8$: The Smallest Paracyclopynes and Their Transformation into the Carbon Cluster Ion C_{36}^-

Y. Tobe,* R. Furukawa, M. Sonoda, T. Wakabayashi

Fluorescence Quenching via Sequential Hydrogen, Electron, and Proton Transfer in the Proximity of a Conical Intersection

A. Sinicropi, R. Pogni*, R. Basosi, M. A. Robb, G. Gramlich, W. M. Nau,* M. Olivucci*

Solvent-induced self-assembly of oligomer-substituted shape-persistent macrocycles leads to the formation of cylindrical aggregates (see the schematic representation), which have been fully characterized in solution. These aggregates, bound by nonspecific interactions, can be described as supramolecular hollow polymer brushes.



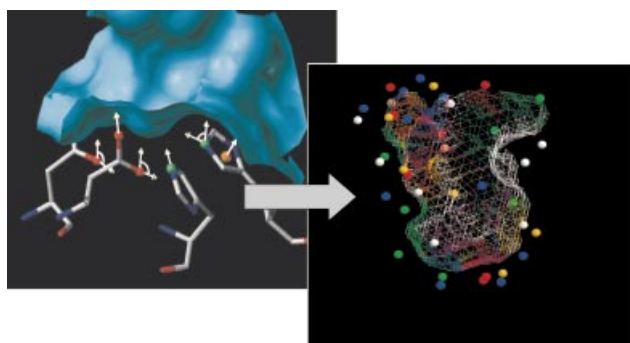
Angew. Chem. **2001**, *113*, 3234–3237

S. Rosselli, A.-D. Ramminger, T. Wagner,
B. Silier, S. Wiegand, W. Häussler,
G. Lieser, V. Scheumann,
S. Höger* 3138–3141

Coil–Ring–Coil Block Copolymers as
Building Blocks for Supramolecular
Hollow Cylindrical Brushes

Keywords: aggregation • liquid crystals •
macrocycles • solvent effects •
supramolecular chemistry

Protein function is almost invariably linked with the specific recognition of substrates or endogenous ligands in particular binding pockets; proteins of related function should, therefore, share comparable recognition pockets. On the basis of this idea a new computer method has been developed to detect functional relationships among proteins, independent of a particular sequence or fold homology, in which the functionality of the residues is translated into simple physicochemical descriptors (see picture). By this method novel ligands in drug design can be suggested.



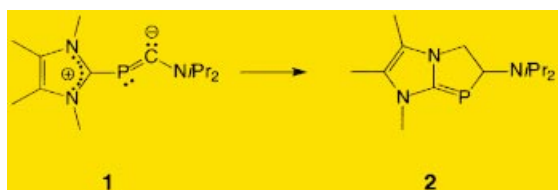
Angew. Chem. **2001**, *113*, 3237–3241

S. Schmitt, M. Hendlich,
G. Klebe* 3141–3144

From Structure to Function: A New
Approach to Detect Functional Similarity
among Proteins Independent from
Sequence and Fold Homology

Keywords: computer chemistry •
molecular recognition • protein–ligand
interactions • protein structures

 **Intramolecular C–H insertion** into the methyl group of the amino substituent of **1** is shown by density functional theory calculations to stabilize this intermediate in the formation of the novel 1:1 carbene–phosphaalkyne adduct **2**. Compound **2** is formed in near quantitative yield by reaction of 1,3,4,5-tetramethylimidazol-2-ylidene with $P\equiv CNiPr_2$.



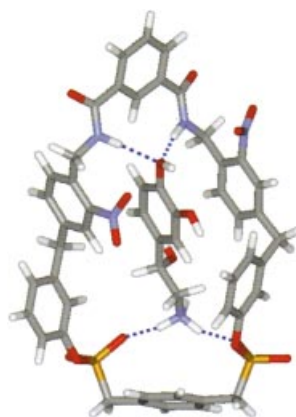
Angew. Chem. **2001**, *113*, 3241–3244

F. E. Hahn,* D. Le Van, M. C. Moyes,
T. von Fehren, R. Fröhlich,
E.-U. Würthwein 3144–3148

Unusual Formation of an
Azaphospholene from 1,3,4,5-
Tetramethylimidazol-2-ylidene and
Di(isopropyl)aminophosphaalkyne

Keywords: carbenes • density functional
calculations • insertion •
phosphaalkynes • phosphorus
heterocycles

A new rationally designed receptor molecule binds adrenaline derivatives in water. Its binding pattern (see picture) imitates the interplay of noncovalent interactions operating in the natural receptor. High shape selectivity is achieved for the slim dopamine skeleton, and leads to rejection of substrates with an α -substituent, such as amino acid derivatives.



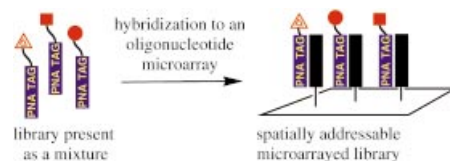
M. Herm, O. Molt,
T. Schrader* 3148–3151

Towards Synthetic Adrenaline
Receptors—Shape-Selective Adrenaline
Recognition in Water

Keywords: adrenaline • hormones •
molecular recognition • receptors •
supramolecular chemistry

Angew. Chem. **2001**, *113*, 3244–3248

Small molecules encoded with peptidonucleic acid (PNA) were used to probe protein function in a microarray format. The PNA tag served to encode the synthetic history of the small molecule and to positionally encode the identity of the small molecule by its location upon hybridization to an oligonucleotide microarray (see picture).



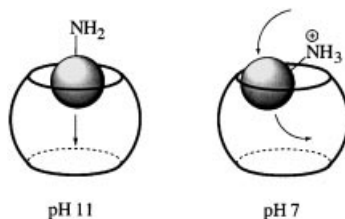
N. Winssinger, J. L. Harris, B. J. Backes,
P. G. Schultz* 3152–3155

From Split-Pool Libraries to Spatially
Addressable Microarrays and Its
Application to Functional Proteomic
Profiling

Keywords: combinatorial chemistry •
cysteine protease • functional genomics •
hydrolases • split-pool synthesis

Angew. Chem. **2001**, *113*, 3254–3258

20 times more rapid is the complexation of the organic ammonium ion receptor cucurbit[6]uril (CB6) with cyclohexylmethylamine than with the cyclohexylmethylammonium species (see scheme). Within the narrow pH region around the pK_a -value of the amine, the complexation kinetics are accelerated but the binding constant remains essentially unaffected. In this region, the ammonium complex is formed through binding of the amine form followed by fast protonation and not through direct complexation of the ammonium form. Molecular dynamics calculations suggest that the amine form undergoes a direct inclusion into CB6 while the ingress of the ammonium form is retarded by the formation of an association complex, which reacts through a different, higher lying transition state to the inclusion complex.



C. Marquez, W. M. Nau* 3155–3160

Two Mechanisms of Slow Host–Guest
Complexation between Cucurbit[6]uril
and Cyclohexylmethylamine:
pH-Responsive Supramolecular Kinetics

Keywords: cucurbituril • host–guest
systems • kinetics • protonation
equilibria • supramolecular chemistry

Angew. Chem. **2001**, *113*, 3248–3254

Doped with up to 70 different metal salts, three semiconductor oxides (TiO_2 , SnO_2 , and WO_3) formed the basis of three libraries of mixed oxides. From a high-throughput method, over 30 oxides were found to have activities similar to the well-studied Pt-doped TiO_2 towards the catalyzed decomposition of a model pollutant 4-chlorophenol in water on irradiation with visible light. Two of the new materials (Ir-doped WO_3 and Ce-doped SnO_2) have been reproduced by conventional synthesis, and their catalytic activity was confirmed.

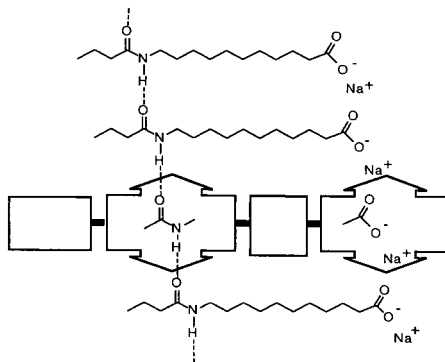
C. Lettmann, H. Hinrichs,
W. F. Maier* 3160–3164

Combinatorial Discovery of New
Photocatalysts for Water Purification
with Visible Light

Keywords: combinatorial chemistry •
photocatalysts • tin • titanium •
tungsten • water purification

Angew. Chem. **2001**, *113*, 3258–3262

N-Acyl amino acids $\text{RCONH}(\text{CH}_2)_n\text{COOR}^1$ represent a new family of organogelators that can be obtained in a combinatorial approach from libraries of activated acids and amino acids. The amounts required for gel formation (see picture) in solvents such as DMF are less than 1 mg mL^{-1} and the gels formed are very stable. $n = 5, 7, 10, 12$; R = aromatic or aliphatic group; $\text{R}^1 = \text{H}$ or alkali metal (Na).



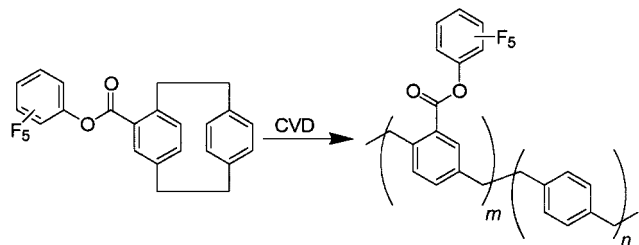
Angew. Chem. **2001**, *113*, 3266–3267

G. Mieden-Gundert, L. Klein, M. Fischer, F. Vögtle,* K. Heuzé, J.-L. Pozzo, M. Vallier, F. Fages* 3164–3166

Rational Design of Low Molecular Mass Organogelators: Toward a Library of Functional N-Acyl-1, ω -Amino Acid Derivatives

Keywords: amino acids • fatty acids • gels • self assembly • supramolecular chemistry

By using chemical vapor deposition (CVD) a range of substrates can be coated with a highly reactive polymer containing pentafluorophenol ester groups (see scheme). A biotin-modified pattern is generated on the substrate by microcontact printing; such a pattern interacts specifically with fluorescein-conjugated streptavidin and thereby the pattern becomes observable with fluorescence microscopy.



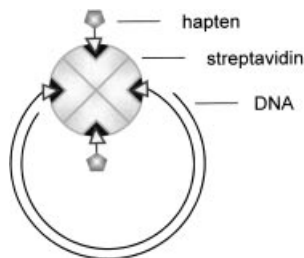
Angew. Chem. **2001**, *113*, 3273–3276

J. Lahann, I. S. Choi, J. Lee, K. F. Jensen, R. Langer* 3166–3169

A New Method toward Microengineered Surfaces Based on Reactive Coating

Keywords: CVD • cyclophanes • immobilization • microcontact printing • reactive coating

Analysis with nanorings: The endogeneous proteins of supramolecular DNA nanocircles, obtained in high yields from oligomeric precursors containing bisbiotinylated DNA and streptavidin, are conveniently functionalized with biotinylated hapten moieties (see schematic representation). These modular conjugates can be used as reagents in a novel competitive immunoassay for the high-sensitivity detection of low molecular weight analytes.



Angew. Chem. **2001**, *113*, 3262–3265

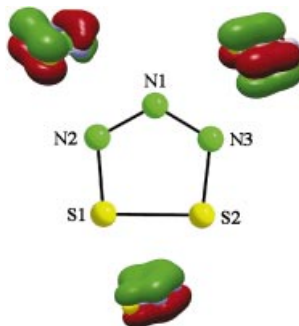
C. M. Niemeyer,* R. Wacker, M. Adler 3169–3172

Hapten-Functionalized DNA-Streptavidin Nanocircles as Supramolecular Reagents in a Competitive Immuno-PCR Assay

Keywords: analytical methods • DNA conjugates • nanostructures • streptavidin • supramolecular chemistry



Surprisingly stable is the first example of a binary six π electron aromatic SN^+ cation with an N_3 unit, S_2N_3^+ (see picture). It can be isolated on a macroscopic scale when a large counter anion is present. The structure determined by X-ray investigations is in good agreement with theoretical data. The unequivocal identification was supported by Raman and infrared studies. The structure and bonding are discussed on the basis of MO (molecular orbital) and AIM (atoms-in-molecules) analysis.



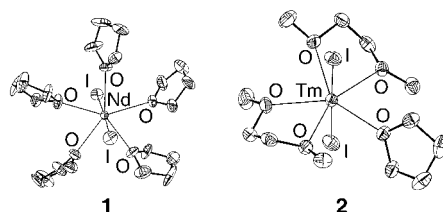
Angew. Chem. **2001**, *113*, 3270–3273

S. Herler, P. Mayer, H. Nöth, A. Schulz,* M. Suter, M. Vogt 3173–3175

S_2N_3^+ : An Aromatic SN^+ Cation with an N_3 Unit

Keywords: ab initio calculations • bond theory • cations • nitrogen • sulfur

Two new starting materials for a new chemistry of low-valent lanthanide compounds are the first crystallographically characterized molecular complexes of the rare Nd^{2+} ion, $[\text{NdI}_2(\text{thf})_5]$ (**1**), and $[\text{TmI}_2(\text{thf})(\text{dme})_2]$ (**2**; dme = 1,2-dimethoxyethane). These have a pentagonal-bipyramidal structure in which the two iodine atoms are in axial positions.



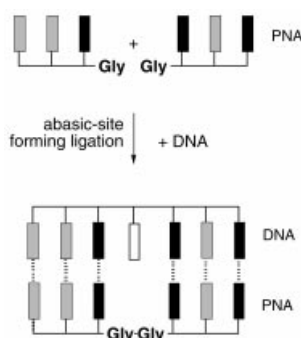
M. N. Bochkarev,* I. L. Fedushkin,
S. Dechert, A. A. Fagin,
H. Schumann* 3176–3178

$[\text{NdI}_2(\text{thf})_5]$, the First Crystallographically
Authenticated Neodymium(II) Complex

Keywords: lanthanides • neodymium •
structure elucidation • subvalent
compounds • thulium

Angew. Chem. **2001**, *113*, 3268–3270

An abasic-site-forming ligation reaction might allow multiplex analysis of single-base mutations to be performed in homogeneous solution. The ligation strategy capitalizes upon the use of the non-ionic DNA-analogue peptide nucleic acids (PNAs) and combines the highly sequence-selective base pairing of short-length PNA (see scheme) with its ease and accuracy of detection by MALDI-TOF mass spectrometry.



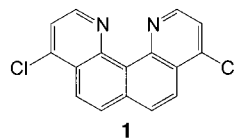
A. Mattes, O. Seitz* 3178–3181

Mass-Spectrometric Monitoring of a
PNA-Based Ligation Reaction for the
Multiplex Detection of DNA Single-
Nucleotide Polymorphisms

Keywords: DNA recognition • mass
spectrometry • multiplex detection •
nucleic acids • peptide nucleic acids

Angew. Chem. **2001**, *113*, 3277–3280

An extremely high thermal and chemical stability as well as a unique “out-of-plane” position of the metal atom is shown by the first stable transition metal complexes formed with the proton sponge **1** as the ligand. The properties of these complexes await wide application in catalysis and medicine.



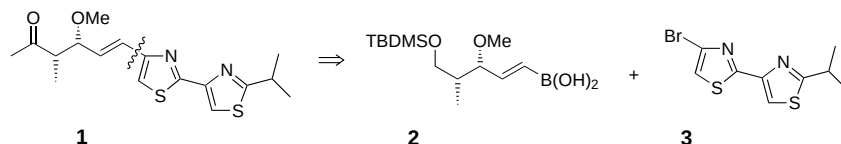
H.-U. Wüstefeld, W. C. Kaska,*
F. Schüth,* G. D. Stucky, X. Bu,
B. Krebs 3182–3184

Transition Metal Complexes with the
Proton Sponge 4,9-Dichloroquinoline:
Highly Twisted Aromatic Systems and an Extreme “Out-
of-Plane” Position of the Coordinated
Transition Metal Atom

Keywords: N ligands • platinum • proton
sponge • rhenium

Angew. Chem. **2001**, *113*, 3280–3282

No problems have been encountered while using bithiazoles in Pd-catalyzed cross-coupling reactions. It was thus possible to achieve the first synthesis of cystothiazole E (**1**) with a Suzuki coupling of the building blocks **2** and **3** as the pivotal C–C bond-formation step (94% yield, TBDMS = *t*BuMe₂Si). The bithiazole **3** could be prepared very conveniently from 2,4-dibromothiazole by regioselective cross-coupling reactions.



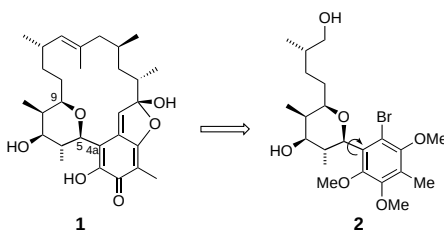
T. Bach,* S. Heuser 3184–3185

Regioselective Cross-Coupling Reactions
as an Entry into Biologically Relevant
Bithiazoles: First Total Synthesis of
Cystothiazole E

Keywords: aldol reaction • asymmetric
synthesis • cross-coupling • heterocycles •
total synthesis

Angew. Chem. **2001**, *113*, 3283–3284

The left-hand fragment **2** of the novel antibiotic kendomycin (**1**) has been synthesized by an aldol addition and a Michael-type 1,4-addition of a C5 alcohol with a C9–C11 enone. Compound **2** shows an interesting atropisomerism around the C4a–C5-sp²–sp³ bond. The atropisomers can be separated in pure forms by low-temperature high-pressure liquid chromatography.



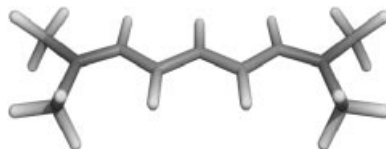
H. J. Martin, M. Drescher, H. Kählig, S. Schneider, J. Mulzer* 3186–3188

Synthesis of the C1–C13 Fragment of Kendomycin: Atropisomerism around a C–Aryl Glycosidic Bond

Keywords: aldol reaction • antibiotics • atropisomerism • glycosides • Michael addition

Angew. Chem. **2001**, *113*, 3287–3289

Sometimes the simplest systems need the most sophisticated treatment! Only with multiconfigurational SCF methodology including second-order correction (CASPT2) is it possible to quantitatively reproduce the position of the high-intensity methine band of streptocyanine dyes such as the one shown.



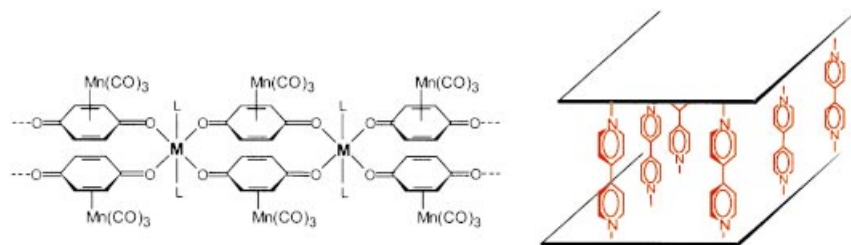
V. Buss,* M. Schreiber, M. P. Fülcher 3189–3190

Nonempirical Calculation of Polymethine Excited States

Keywords: ab initio calculations • chromophores • computer chemistry • cyanines • polymethines

Angew. Chem. **2001**, *113*, 3284–3286

Coordination of Mn(CO)₃⁺ to the π system in hydroquinone facilitates proton loss to afford benzoquinone complexes (see figure). Subsequent σ coordination of the benzoquinone oxygen atoms to added metal ions results in neutral one-, two-, or three-dimensional quinoid polymers. The geometrical requirements of the metal ion and the presence of added “spacer” ligands dictate the type of polymer formed.



M. Oh, G. B. Carpenter, D. A. Sweigart* 3191–3194

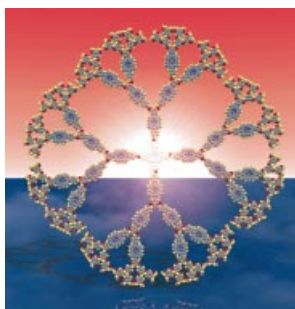
Metal-Mediated Self-Assembly of π-Bonded Benzoquinone Complexes into Polymers with Tunable Geometries

Keywords: host–guest systems • manganese • polymers • quinone complexes • self assembly

Angew. Chem. **2001**, *113*, 3291–3294



π-Electronic conjugation is not necessary to facilitate long-range energy migration and transfer: A rapid ($k_{\text{ENT}} = 1.04 \times 10^9 \text{ s}^{-1}$) and efficient ($\Phi_{\text{EXT}} = 71\%$) energy transfer from the 28 photoexcited zinc porphyrin units to the focal free-base porphyrin unit occurs in a large, star-shaped dendritic multiporphyrin array in which the chromophore units are linked through flexible ether groups (see picture).



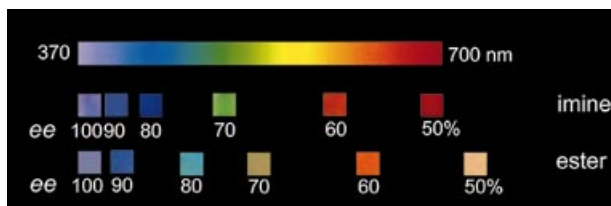
M.-S. Choi, T. Aida,* T. Yamazaki, I. Yamazaki 3194–3198

A Large Dendritic Multiporphyrin Array as a Mimic of the Bacterial Light-Harvesting Antenna Complex: Molecular Design of an Efficient Energy Funnel for Visible Photons

Keywords: dendrimers • energy transfer • fluorescence spectroscopy • photosynthesis • porphyrinoids

Angew. Chem. **2001**, *113*, 3294–3298

A simple color test for enantiomeric excess requires no chiral auxiliaries and only microgram quantities of chiral products. The method is based on a chirality-dependent color generation in doped LC films on formation of an imine or ester (see picture) from an amine and carbaldehyde or alcohol and acid chloride, respectively, and will enable the rapid screening of libraries of nonracemic compounds.



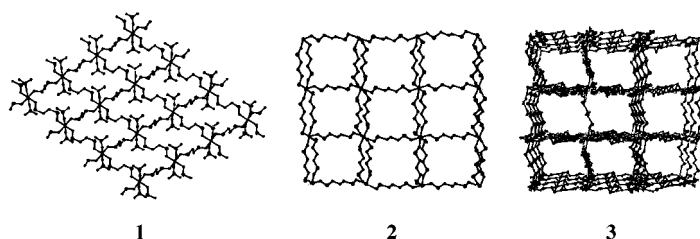
Angew. Chem. **2001**, *113*, 3298–3300

R. A. van Delden,
B. L. Feringa* 3198–3200

Color Indicators of Molecular Chirality
Based on Doped Liquid Crystals

Keywords: asymmetric catalysis •
chirality • combinatorial chemistry •
enantioselectivity • liquid crystals

Fillings and cavities: Three novel pseudo-octahedral metal–organic frameworks, **1–3**, consisting of macrometallacyclic noninterpenetrating *meso* networks and exhibiting weak antiferromagnetic interactions, have been constructed from Cu^{II} centers and structurally flexible *R,S*-bis(sulfinyl) ligands. Varying the chain length of ligands is found to control the cavity sizes of the networks.



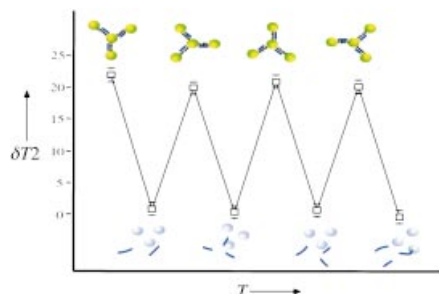
Angew. Chem. **2001**, *113*, 3301–3303

X.-H. Bu,* W. Chen, S.-L. Lu,
R.-H. Zhang, D.-Z. Liao, W.-M. Bu,
M. Shionoya, F. Brisse,
J. Ribas 3201–3203

Flexible *meso*-Bis(sulfinyl) Ligands as
Building Blocks for Copper(II)
Coordination Polymers: Cavity Control
by Varying the Chain Length of Ligands

Keywords: coordination polymers •
copper • crystal engineering • magnetic
properties • S ligands

Superparamagnetic nanoparticles conjugated to oligonucleotides exert sensitive and reversible effects on the spin–spin relaxation rates of adjacent water protons when the conjugates hybridize to target oligonucleotides (see temperature cycle in the picture). The fluid-phase magnetic sensors provide the basis for detecting specific nucleic acid sequences in turbid media and potentially in tissues in vivo.



Angew. Chem. **2001**, *113*, 3304–3306

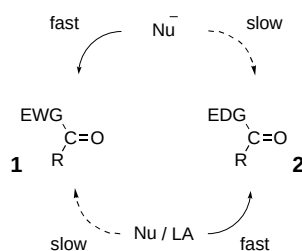
L. Josephson, J. M. Perez,
R. Weissleder* 3204–3206

Magnetic Nanosensors for the Detection
of Oligonucleotide Sequences

Keywords: DNA recognition • magnetic
properties • nanostructures •
oligonucleotides



A crucial role in the chemoselective synthesis of primary, secondary, and homoallylic alcohols and of Diels–Alder adducts is played by Lewis acids (LA) in the addition of nucleophiles (Nu) to carbonyl compounds (see scheme; EWG = electron-withdrawing group, EDG = electron-donating group).



Angew. Chem. **2001**, *113*, 3306–3308

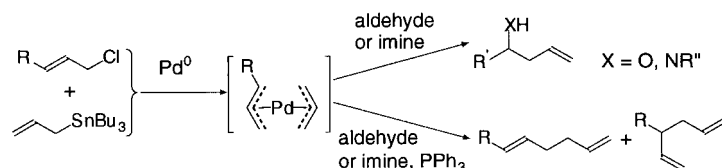
N. Asao, T. Asano,
Y. Yamamoto* 3206–3208

Do More Electrophilic Aldehydes/
Ketones Exhibit Higher Reactivity
toward Nucleophiles in the Presence of
Lewis Acids?

Keywords: chemoselectivity •
electrophiles • Lewis acids •
nucleophilic addition



The mere presence or absence of PPh_3 suffices to control the reactivity of bis(η^3 -allyl)palladium complexes. In the absence of PPh_3 they undergo chemoselective allylic addition to aldehydes or imines, even in the presence of allylic chlorides, whereas in the presence of PPh_3 the Stille coupling reaction takes place chemoselectively, even when aldehydes or imines are also present (see scheme).



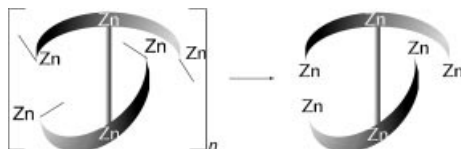
Angew. Chem. **2001**, *113*, 3308–3310

H. Nakamura, M. Bao,
Y. Yamamoto* 3208–3210

The Fate of Bis(η^3 -allyl)palladium Complexes in the Presence of Aldehydes (or Imines) and Allylic Chlorides: Stille Coupling versus Allylation of Aldehydes (or Imines)

Keywords: allylation • allyl ligands • C–C coupling • palladium • Stille coupling

The controlled cleavage of coordination polymers consisting of well-defined large units can be employed for the rational synthesis of high nuclearity clusters. This synthetic concept is demonstrated by the conversion (schematically shown) of a three-dimensional Zn^{II} coordination polymer containing repeating hexameric units with a “twin-anchor” arrangement of the metal ions into the corresponding hexanuclear cluster by reaction with a donor solvent.



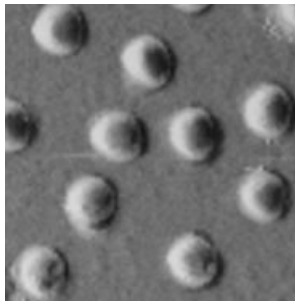
Angew. Chem. **2001**, *113*, 3311–3314

N. Lalioti, C. P. Raptopoulou, A. Terzis,
A. E. Aliev, I. P. Gerothanassis,*
E. Manessi-Zoupa,*
S. P. Perlepes* 3211–3214

High Nuclearity $\text{Zn}^{\text{II}}/\text{MeCO}_2^-/(\text{C}_5\text{NH}_4)_2\text{CO}_3^{2-}$ Clusters by “Depolymerization”: Conversion of a Three-Dimensional Coordination Polymer Containing Hexameric Units into Its Constituent Hexanuclear Complex

Keywords: cluster compounds • coordination polymers • N ligands • synthetic methods • zinc

A reactor for the synthesis of gold nanoparticles (see atomic force microscopy image) is one of the uses of a poly(styrene)-*block*-poly(2-vinylpyridine)-*block*-poly(ethylene oxide) triblock copolymer (PS-*b*-P2VP-*b*-PEO) which forms core-shell-corona micelles in water. Very low polydispersity spherical micelles are observed that consist of a PS core surrounded by a pH-sensitive P2VP shell and a corona of PEO chains end-capped by a hydroxyl group. The corona can act as a site for attaching responsive or sensing molecules.



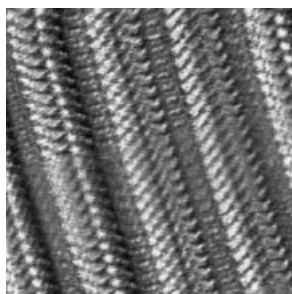
Angew. Chem. **2001**, *113*, 3314–3316

J.-F. Gohy, N. Willet, S. Varshney,
J.-X. Zhang, R. Jérôme* 3214–3216

Core–Shell–Corona Micelles with a Responsive Shell

Keywords: gold • micelles • nanostructures • polymers • self assembly

Enantiomorphous monolayers of enantiopure formamides form on a graphite surface, while the racemic compound does not undergo a spontaneous resolution under similar conditions. This difference in the two-dimensional behavior has been studied by scanning tunneling microscopy (see image of the racemate adsorbed on graphite). A study of the three-dimensional crystallization of the racemic formamide by X-ray crystallographic analysis also shows that it forms racemic tapes.



Angew. Chem. **2001**, *113*, 3317–3320

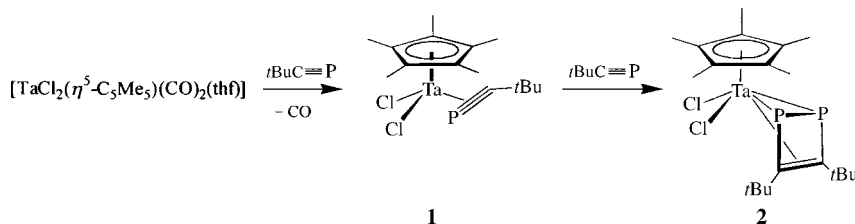
S. De Feyter, A. Gesquière, K. Wurst,
D. B. Amabilino, J. Veciana,*
F. C. De Schryver* 3217–3220

Homo- and Heterochiral Supramolecular Tapes from Achiral, Enantiopure, and Racemic Promesogenic Formamides: Expression of Molecular Chirality in Two and Three Dimensions

Keywords: chirality • scanning tunneling microscopy • self assembly • surface chemistry • X-ray diffraction



Only the 1,2-diphosphacyclobutadiene and none of its 1,3 isomer was obtained in the reaction of **1** with $t\text{BuC}\equiv\text{P}$ to give **2**. In complex **1**, which was prepared from $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)(\text{CO})_2(\text{thf})]$ and $t\text{BuC}\equiv\text{P}$, the phosphalkyne adopts an $\eta^2(4e)$ bonding mode according to NMR data, crystal structure analysis, and theoretical calculations.



Angew. Chem. **2001**, *113*, 3321–3324

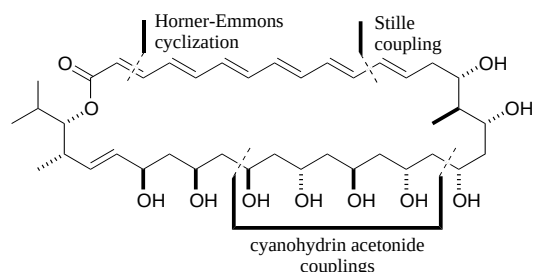
A. D. Burrows, A. Dransfeld, M. Green,*
J. C. Jeffery, C. Jones, J. M. Lynam,
M. T. Nguyen* 3221–3224

Mononuclear $\eta^2(4e)$ -Bonded
Phosphalkyne Complexes;
Selective Formation of a
1,2-Diphosphacyclobutadiene Tantalum
Complex

Keywords: density functional
calculations • phosphalkynes •
phosphorus heterocycles • tantalum



An oxo-hexaene macrolide antibiotic, dermostatin A (see picture) has been synthesized. Key features of the synthesis include the application of cyanohydrin acetonide couplings for the synthesis of the polyol portion, and the convergent introduction of the polyene segment by means of a Stille coupling.



Angew. Chem. **2001**, *113*, 3324–3327

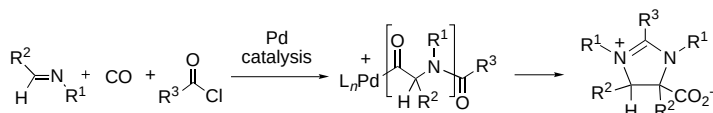
C. J. Sinz,
S. D. Rychnovsky* 3224–3227

Total Synthesis of Dermostatin A

Keywords: alkylation • asymmetric
synthesis • cross-coupling • polyenes •
total synthesis



One of the most facile routes to prepare carboxylate-substituted imidazolines is by the palladium-catalyzed coupling of an imine, carbon monoxide, and an acid chloride. In this reaction, a peptide unit is constructed and directly incorporated into the heterocyclic core (see scheme).



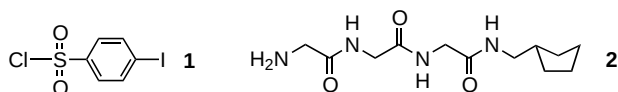
Angew. Chem. **2001**, *113*, 3328–3330

R. D. Dghaym, R. Dhawan,
B. A. Arndtsen* 3228–3230

The Use of Carbon Monoxide and Imines
as Peptide Derivative Synthons: A Facile
Palladium-Catalyzed Synthesis of
 α -Amino Acid Derived Imidazolines

Keywords: homogeneous catalysis •
multicomponent reactions • nitrogen
heterocycles • palladium • peptides

44 members are in the trisaccharide library synthesized by the method described here. Six differently protected glucoside building blocks were sulfonated with **1**, coupled to the solid support **2** (a crown compound) by Pd⁰-catalyzed carbonylative amidation, glycosylated twice, and cleaved from the sulfonate linker with four nucleophiles.



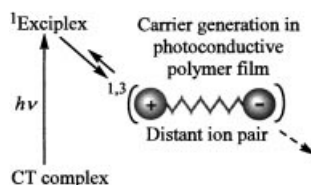
Angew. Chem. **2001**, *113*, 3330–3333

T. Takahashi,* H. Inoue, Y. Yamamura,
T. Doi 3230–3233

Synthesis of a Trisaccharide Library by
Using a Phenylsulfonate Traceless Linker
on Synphase Crowns

Keywords: carbohydrates •
combinatorial chemistry • solid-phase
synthesis • trisaccharides

Photoconduction in charge transfer materials apparently occurs through a distant geminate ion pair with an interionic separation of about 8 Å, as demonstrated by time-resolved EPR spectroscopy of a doped poly(*N*-vinylcarbazole) film (see scheme).



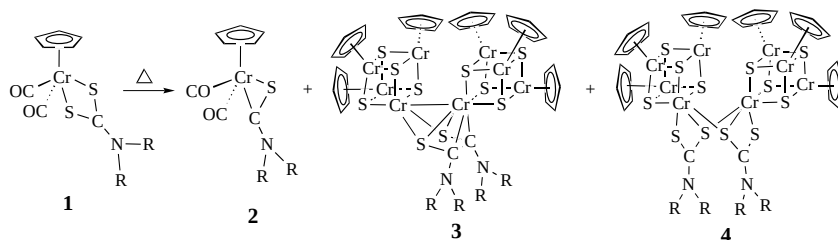
T. Ikoma,* M. Nakai, K. Akiyama,
S. Tero-Kubota,* T. Ishii 3234–3236

Direct Observation of a Distant Ion Pair
Generated in a Photoconductive Poly-
(*N*-vinylcarbazole) Film with Dopant

Keywords: electron transfer •
EPR spectroscopy • ion pairs •
photoconductive polymers • thin films

Angew. Chem. **2001**, *113*, 3334–3336

Thermolysis of the dithiocarbamate complex 1 led to the formation of the thioarbenoid complex **2** and two double cubanes: **3**, which has an intercubane Cr–Cr bond and a dithiooxamide ligand in the $\mu\text{-}\eta^2, \eta^4$ bonding mode, and **4**, which contains two dithiocarbamate ligands in the $\mu\text{-}\eta^1, \eta^2$ bonding mode.



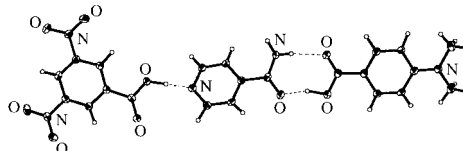
L. Y. Goh,* Z. Weng, W. K. Leong,*
P. H. Leung 3236–3239

C–S Bond Cleavage and C–C Coupling in
Cyclopentadienylchromium Complexes
To Give the First Dithiooxamide-Bridged
and Doubly Dithiocarbamate-Bridged
Double Cubanes: $[\text{Cp}_6\text{Cr}_8\text{S}_8\{(\text{C}(\text{S})\text{N}(\text{Et})_2)_2\}]$
and $[\text{Cp}_6\text{Cr}_8\text{S}_8(\text{S}_2\text{CNEt}_2)_2]$

Keywords: chromium • cluster
compounds • cubanes • S ligands

Angew. Chem. **2001**, *113*, 3336–3339

Getting the right balance between intermolecular interactions is crucial for the synthesis of supermolecules in a preconceived manner. The three-component supermolecule (see picture) in the ternary cocrystal of 3,5-dinitrobenzoic acid, isonicotinamide, and 4-(dimethylamino)benzoic acid (1:1:1) assembles through a “primary” (between the stronger acid and pyridine) and a “secondary” hydrogen-bonding interaction (between the weaker acid and amide).



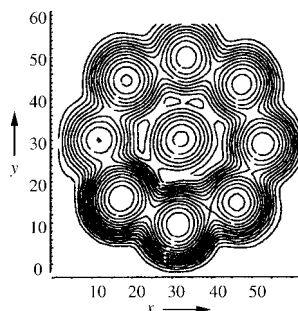
C. B. Aakeröy,* A. M. Beatty,
B. A. Helfrich 3240–3242

“Total Synthesis” Supramolecular Style:
Design and Hydrogen-Bond-Directed
Assembly of Ternary Supermolecules

Keywords: cocrystals • crystal
engineering • hydrogen bonds •
molecular recognition • supramolecular
chemistry

Angew. Chem. **2001**, *113*, 3340–3342

A reduction of a factor of 10 in the time needed to measure highly resolved NMR spectra of multiple samples can be achieved with any NMR spectrometer by using a combination of chemical shift imaging and a compartmented detection volume consisting of a bundle of capillaries. The picture shows the cross-sectional image of a bundle of nine 1-mm capillaries after Fourier transformation along the spatial dimensions only.



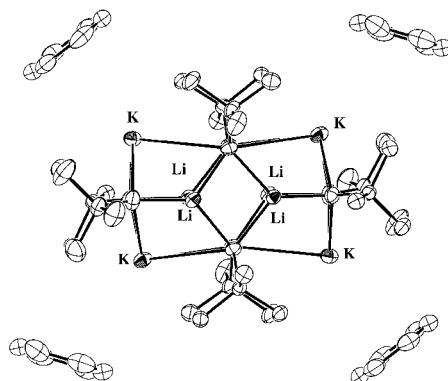
A. Ross,* G. Schlotterbeck, H. Senn,
M. von Kienlin 3243–3245

Application of Chemical Shift Imaging for
Simultaneous and Fast Acquisition of
NMR Spectra on Multiple Samples

Keywords: analytical methods •
chemical shift imaging • high-throughput
screening • NMR spectroscopy

Angew. Chem. **2001**, *113*, 3343–3345

At long last an experimentally utilized superbase has been crystallographically characterized. Isolated from a lithium amide–potassium alkoxide–arene mixture, this landmark compound adopts a novel sixteen-vertex “carousel” arrangement stabilized by two distinct types of benzene ligation (see structure).



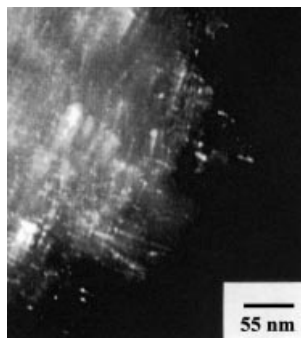
Angew. Chem. **2001**, *113*, 3345–3347

A. R. Kennedy, J. G. MacLellan,
R. E. Mulvey* 3245–3247

Landmark Crystal Structure of an Experimentally Utilized Tetralithium–Tetrapotassium Amide–Alkoxide Superbase

Keywords: alkali metals • alkoxides • amides • structure elucidation • superbases

Crystallizing walls: Following the templated solid-state secondary crystallization of an amorphous mesoporous material the amorphous pore walls of the mesoporous precursor are partially reorganized into a crystalline material, the zeolite ZSM-5, as indicated by the dark-field transmission electron micrograph image shown. The bright spots correspond to the ZSM-5 nanocrystals.



Angew. Chem. **2001**, *113*, 3348–3351

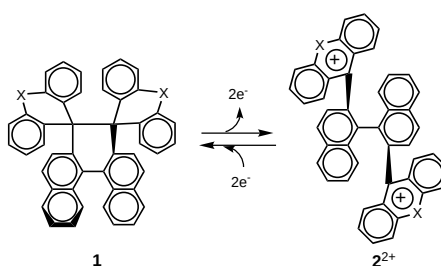
D. Trong On, S. Kaliaguine* 3248–3251

Large-Pore Mesoporous Materials with Semi-Crystalline Zeolitic Frameworks

Keywords: crystal growth • mesoporous materials • microporous materials • template synthesis • zeolites



Helicity and axial chirality are reversibly interconverted in the novel redox pair **1** and **2²⁺**; the compounds exhibit drastic UV/Vis and circular dichroism spectral changes as well as dynamic structural changes upon electron transfer, thus furnishing an unprecedented multi-output response system with high bistability. X = S or O.



J.-i. Nishida, T. Suzuki,* M. Ohkita,
T. Tsuji 3251–3254

A Redox Switch Based on Dihydro[5]helicene: Drastic Chiroptical Response Induced by Reversible C–C Bond Making/Breaking upon Electron Transfer

Keywords: chirality • circular dichroism • electrochemistry • helical structures • redox chemistry

Angew. Chem. **2001**, *113*, 3351–3354



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Eric N. Jacobsen, Andreas Pfaltz,
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Become an expert in the field of carbohydrate chemistry

T. K. Lindhorst,
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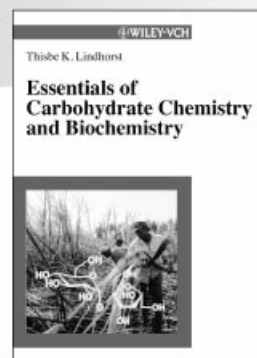
Essentials of Carbohydrate Chemistry and Biochemistry

2000. XIV, 218 pages with 244 figures and 11 tables. Softcover. DM 98.-/ £ 32.50
ISBN 3-527-29543-7

Carbohydrates are probably nature's most common product. Plants and algae biosynthesize millions of tons of them every year. Carbohydrates are stores of energy and structural building blocks; they are versatile enough to serve as encoders of biological information and, last but not least, they are involved in recognition processes at a molecular level. Research into carbohydrate and glycoconjugate functions in cell-to-cell communication processes has even created a new and rapidly developing field of study: glycobiology.

Thisbe K. Lindhorst is one of the leading "next generation" scientists in the area of carbohydrate research. Within her current book she presents a comprehensive introduction to the fascinating world of carbohydrates. In a lucid, explicit language she explains carbohydrate structures and the basic concepts of saccharide chemistry and saccharide biochemistry. With the same clarity she spans the gap to the glycobiological aspects of modern "glycoscience". Sample descriptions of research methods supplement the vital teaching text and open an experienced scientist's bag of tricks required to synthesize and analyze sugar derivatives easily and successfully.

This book offers valuable guidance for students as well as for researchers working in chemistry, biochemistry and biomedicine.



From the Contents:

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- Structure of saccharides
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- Glycoside synthesis
- Important modifications and functionalizations of the sugar ring
- Structure and biosynthesis of glycoconjugates
- Glycobiology
- Analysis of carbohydrates
- The literature of carbohydrate chemistry

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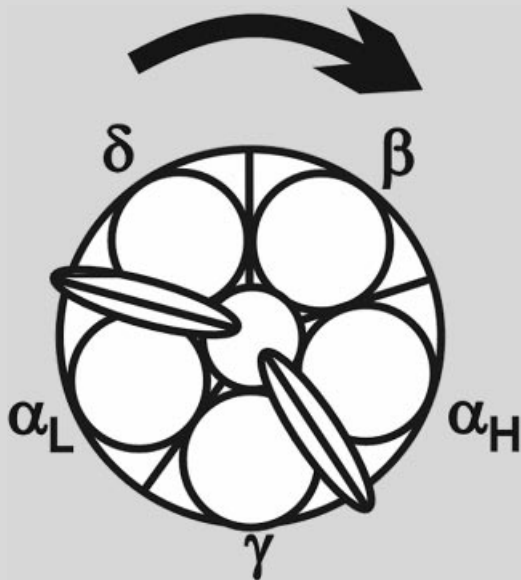
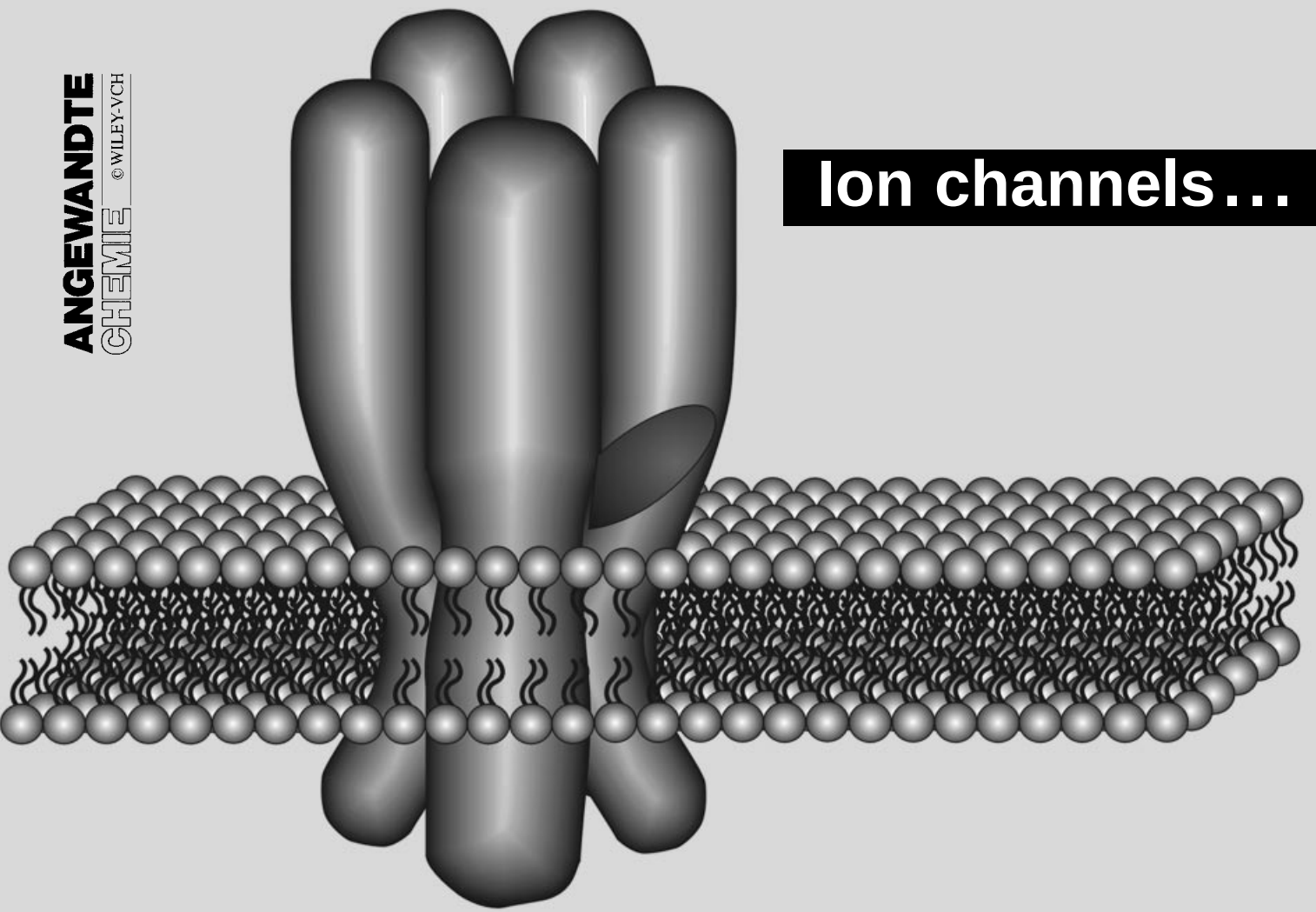


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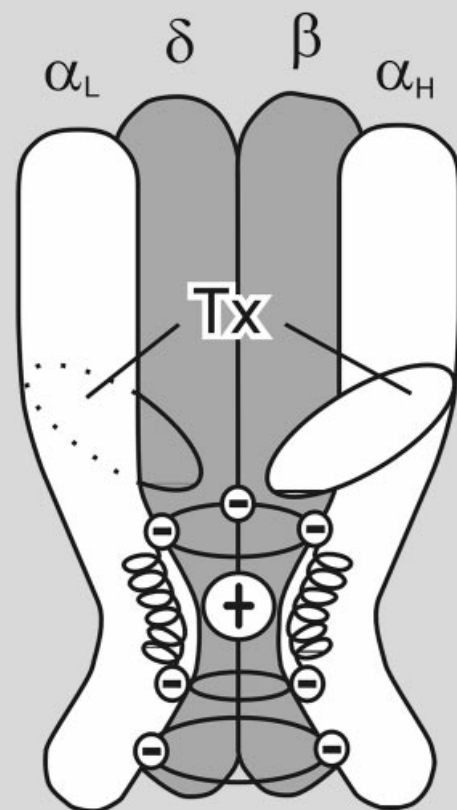
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Ion channels...



...are key information-processing proteins



Ligand-Gated Ion Channels

Ferdinand Hucho* and Christoph Weise

Ion channels are at the heart of many biological processes such as nerve activity and muscle contraction. How are their impressive ion selectivity and highly specialized gating brought about? In recent years, X-ray crystallography and high-resolution electron microscopy, as well as photo-affinity

labeling and site-specific mutagenesis techniques in combination with patch-clamp electrophysiology have provided a detailed picture of some channel proteins. Herein we summarize the main structural and functional properties of channel proteins based on the advances made mainly within the last

decade. We integrate these novel insights into a comprehensive description of the class of ligand-gated ion channels.

Keywords: ion channels • neurotransmitters • proteins • receptors • structure elucidation

1. What are Ion Channels and What Purpose Do They Serve?

Probably all living cells exhibit a membrane potential at their plasma membrane. This diffusion potential is caused by the unequal distribution of ions between the inside and outside of the cell, and by the membrane's semipermeability. The value of the potential is given by the Goldman–Hodgkin–Katz Equation [Eq. (1)], an extended version of the Nernst equation. In Equation 1, R is the gas constant, T is the temperature in Kelvin, F is the Faraday constant, and P_x , $[X]_o$, and $[X]_i$ are the relative permeabilities and the external and the internal concentrations of the respective ions.

$$E = \frac{RT}{F} \ln \frac{P_{Na}[Na^+]_o + P_K[K^+]_o + P_{Cl}[Cl^-]_i}{P_{Na}[Na^+]_i + P_K[K^+]_i + P_{Cl}[Cl^-]_o} \quad (1)$$

Nerve cells (neurons) take advantage of their membrane potential for intracellular and intercellular communication: local changes in the potential, the so-called electrotonic potentials, are the basis for signal processing and triggering of intracellular responses. When they exceed certain threshold values, they also trigger action potentials. These are transient, large depolarizations and repolarizations of excitable membranes which travel over long distances along the neuronal membrane until they reach their target. The nerve ending together with the membrane of the neighboring cell form a synapse. At the nerve terminal, the depolarization releases specific chemicals, the neurotransmitters, which in turn

depolarize the membrane of the postsynaptic cell. There again, local or action potentials may be the result.

Changes in membrane potentials that underlie both local and action potentials are caused by ion currents across the membrane. Since the lipid bilayer that forms the plasma membrane poses an immense energy barrier for charged particles, specialized transmembrane proteins carry the ion currents. These proteins are the ion channels, the topic of this article.

Ion channels have several unusual features, which make them the subject of much biochemical, biophysical, and physiological research. For one, they are highly selective. They discriminate not only between anions and cations, but even between different monovalent and divalent ions, for example, Na^+ , K^+ , and Ca^{2+} . Another important feature is their response to specific signals: at rest they are tightly closed and impermeable, but they are opened (“gated”) either by changes in the membrane potential or by certain ligands such as the neurotransmitters. According to this description, all ion channels are composed of two functional moieties (Figure 1),

- a **selectivity filter (S)**, which determines which types of ions may pass the membrane; and
- a **gate (G)**, which specifies under which conditions the channel is opened.

Channels are subdivided into two major classes according to their gating trigger (L): the voltage-gated channels (VGCs) and the ligand-gated channels (LGCs).

2. Classification of Ligand-Gated Ion Channels

Both classes of channels, the VGCs and the LGCs, are diverse and comprise numerous members. Here it is neither

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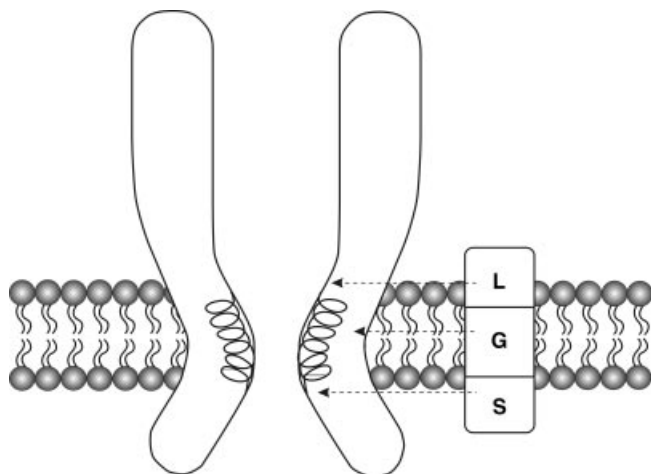


Figure 1. Schematic representation of ligand-gated ion channels. Longitudinal cross section of the membrane-spanning protein depicting the wide extracellular vestibule (top) and the intramembrane structure; the channel is lined with transmembrane helices and contains the channel gate (G) and selectivity filter (S). The ligand-binding site (L), which triggers the gating by an allosteric mechanism, is located on top of G and S.

intended nor possible to give a comprehensive survey covering them all. We rather want to focus on a few general principles of ion-channel structure and function, based on recent crystallographic and molecular biological data. After briefly touching on other channels, we turn to the neurotransmitter receptors as the pharmacologically most important and best-known class of LGCs. By definition, this limits our treatise to LGCs of the animal kingdom, although it should be mentioned that both VGCs and LGCs also occur in microorganisms, in unicellular eukaryotes, and even in plants.

For example, several genes that code for ionotropic glutamate receptors have been discovered in *Arabidopsis*;^[1] they might have been present before plants and animals diverged.^[2]

The term “ionotropic” refers to the ion-channel nature of the receptor and indicates that there is a different type, the metabotropic receptors. These are “seven-transmembrane(7TM)-sequence receptors”. Most neurotransmitters, with the exception of glycine, act through both ionotropic and metabotropic receptors. The 7TM receptors, which signal by coupling to G-proteins and second-messenger-producing enzymes, are functionally and mechanistically completely different and are not discussed further in this review.

A simple classification emerged as a result of the cloning and sequencing of many LGCs. It turned out that several LGCs, although different in function, have similar amino acid sequences. According to this criterion, LGCs can be classified into three superfamilies (Figure 2).

1. The superfamily of receptors that resemble the nicotinic acetylcholine receptors: glycine receptors (GlyR), GABA_A receptors (GABA_AR), nicotinic acetylcholine receptors (nAChR), and some serotonin receptors (5-HT₃R).
2. The superfamily of ionotropic glutamate receptors (GluR).
3. The ATP-gated purino receptors (P2X).

A “family” is defined as receptors that are coded by distinct though similar genes and that react through basically the same mechanism to the same neurotransmitter. A “superfamily” is a set of receptor families that are diverse in function and mechanism, most often reacting to different neurotransmitters, but seem to evolve from a common ancestor. Besides similarities in their sequences, they have conserved several structural features.

Ferdinand Hucho studied Chemistry in Freiburg, where he obtained his Dr. rer. nat. in 1968 with work on enzyme kinetics under the supervision of Kurt Wallenfels. His postdoctoral work was on the regulation of multienzyme complexes at the University of Texas at Austin with Lester Reed. In 1973 he received his habilitation in Biochemistry at the Universität Konstanz. In the same year he changed his field, moving from metabolic regulation to neurochemistry. At the Pasteur Institute in Paris (with Jean-Pierre Changeux) he started working on receptor proteins and signal transduction. In 1979 he became Professor of Biochemistry in Konstanz; in the same year he moved to the Freie Universität Berlin. In 1988/89 he was offered the Chair of Biochemistry in Giessen, Cologne, and Bochum; however, with the collapse of the Berlin Wall he decided to stay in Berlin. He became a member of the Berlin–Brandenburg Academy of Science in 1996.



F. Hucho



C. Weise

Christoph Weise was born in 1956 in Hof, South Germany. He received his Diploma in Biochemistry (1985) and Ph.D. (1991) from the Freie Universität Berlin, where he worked on acetylcholinesterase in the neurochemistry unit under the direction of Ferdinand Hucho. Since then, he has been a research assistant at the Institut für Chemie-Biochemie of the Freie Universität Berlin, where he is in charge of a protein analysis facility and teaches university courses on advanced protein chemistry to biochemistry majors. His research involves the acetylcholine receptor and he has continued his work on acetylcholinesterase during research stays at the Institut Pasteur, Paris and at INRA, Montpellier.

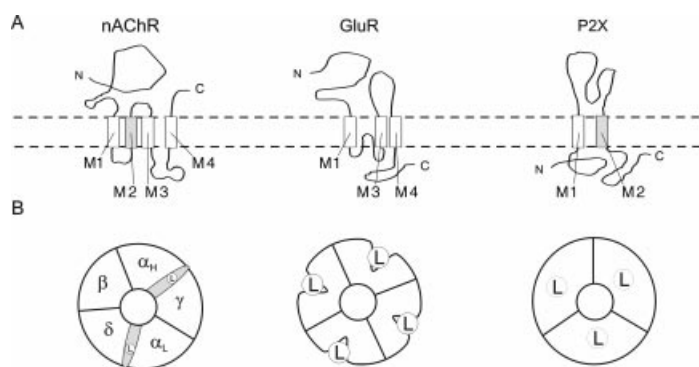


Figure 2. Transmembrane folding (A) and quaternary structure (B) of three representative superfamilies of ligand-gated ion channels. M1 to M4 represent the transmembrane sequences, and N and C represent the amino and carboxy termini of the protein, respectively. L marks the binding pockets for the respective receptor ligands; note that for the nAChR it is depicted as spanning the receptor complex from the outside to the lumen of the channel. The P2X stoichiometry shown is preliminary at this point, as it was determined with a heterologously expressed channel protein.^[5]

The members of the receptor superfamily 1 (Figure 3) all have similar peptide loops formed by disulfide bridges, glycosylation patterns, distribution of proline, and (in one specific domain which forms the channel wall) Ser/Thr residues. Most conspicuously, all the receptors of this superfamily possess four hydrophobic amino-acid sequences, which are long enough to span the plasma membrane. Accordingly, they sometimes are termed “four-transmembrane(4TM)-sequence receptors”.

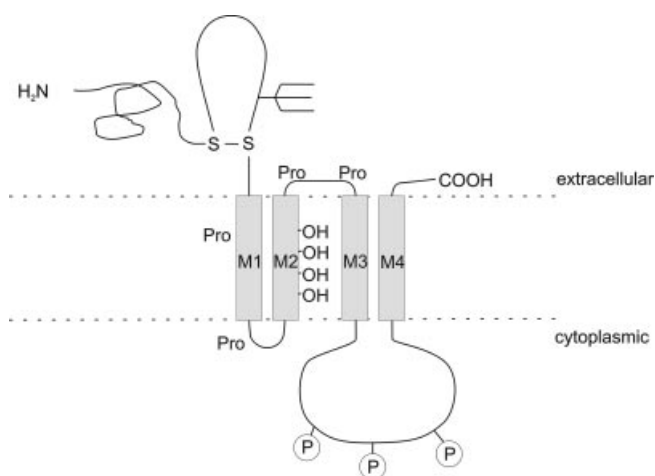


Figure 3. Schematic representation of the transmembrane folding of subunits of the receptors that resemble the nicotinic acetylcholine receptors, showing the position of a characteristic disulfide bridge (-S-S-), proline residues (Pro), and side chains with hydroxy groups (-OH) lining the channel-forming transmembrane helices M2. The trident marks the position of oligosaccharide structures. M3 and M4 are linked by a large cytoplasmic loop which is the target of various protein kinases that introduce phosphate residues (P).

The members of the glutamate receptor superfamily presumably have three transmembrane sequences. A fourth hydrophobic sequence enters and leaves the plasma membrane from the cytoplasmic side, thereby forming a loop (re-entrant loop), which is presumed to line the channel (see

Section 4.1.2). The ATP-binding P2X receptors are thought to contain only two transmembrane (2TM) sequences.

Receptors that belong to the same superfamily are presumed to have similar tertiary and quaternary structures. As shown in Figure 2B, the receptors that resemble the acetylcholine receptors are pentamers,^[3] whereas glutamate receptors are probably tetramers.^[4] It was shown for the P2X receptors that heterologously expressed receptors tend to form stable homotrimers.^[5]

To complete this introductory overview, it should be mentioned that there are other LGCs, such as the cyclic-nucleotide-gated (cNMP) channels (e.g. in the retina of the eye and in the olfactory system) and the calcium-release channels. The latter channels are gated by inositol trisphosphate (IP₃) or by ryanodine, and they regulate the calcium permeabilities of the endoplasmic reticulum and of the t-tubule membrane of skeletal muscle, respectively. The different types of ion channels are related: recently it was shown that TRP channels, a special family of calcium channels that mediate calcium influx into cells, also called store-operated Ca²⁺ channels, may even be gated by another LGC, the IP₃ receptor.^[6, 7] Another family of LGCs are the vanilloid receptors (VR), which are involved in nociception. These VRs are gated by protons (pH < 6.0) and heat (> 42 °C),^[8] and also exhibit little structural and mechanistic similarity to the neurotransmitter-gated receptors discussed herein.

3. Some Features of LGCs

Before we discuss two particularly well-known LGCs in more detail, we would like to summarize some principles of the functions of neurotransmitter receptors:

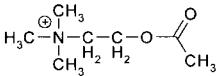
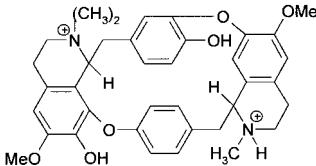
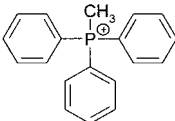
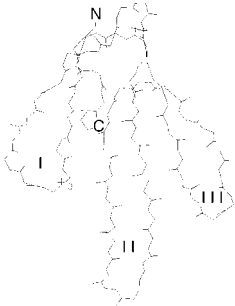
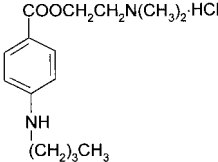
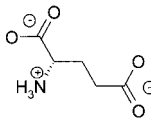
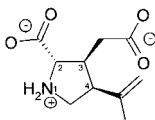
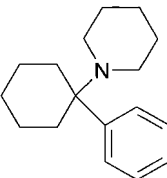
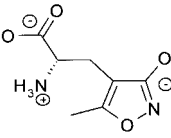
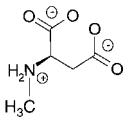
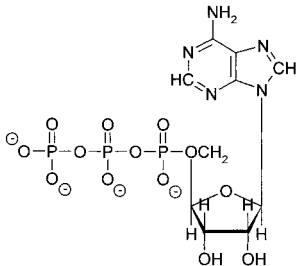
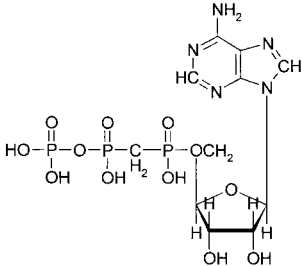
3.1. Sources of Diversity

The functional diversity observed in the central nervous system is not brought about by the use of numerous transmitters; their number is limited to maybe a dozen. Rather, this diversity comes from the multiplicity of receptors. For many of the LGCs, a quaternary structure formed from a number of distinct but homologous subunits has been reported.^[9] The molecular cloning of the neuronal nicotinic receptors has revealed that these receptors are very complex because of the existence of multiple subunits. For each receptor there is a large family of related genes that code for subunits, which assemble in a combinatorial manner to form functionally different receptors. Thus 11 different subunits were found for neuronal nAChRs, 5 for peripheral nAChRs, 16 for GluRs, and at least 17 for GABA_ARs.^[10] The latter family can be subdivided into six classes of subunits (α , β , γ , δ , ϵ , ρ), which have about 20 to 30% sequence identity between and 70% within the classes. Assemblies of most of these subunits have been reported in the vertebrate brain. For the GluRs, even more variants can arise from alternative splicing, and in the case of particular receptor subtypes (see Section 4.1.2), by RNA editing.^[11, 12] The functional importance of this phenomenon will be explained in the chapter on glutamate receptors.

Diverse Receptor Ligands

The specific stimulus for the channel opening of the LGCs is the binding of a specific neurotransmitter, but they can also be influenced by a variety of substances besides their physiological ligand. Some of these compounds are given in Table 1. According to their function, two different groups are distinguished: agonists initiate the same response as the physiological ligand (e.g. nicotine is an agonist of the nAChR) and antagonists oppose the effect of an agonist, normally by occupying the same binding sites with similar affinities as agonists. The reason why agonists and antagonists have such different effects is generally not known. Many naturally

Table 1. A selection of ligands specific for the superfamilies of ligand-gated ion channels.

Super-family	Agonist	Competitive antagonists	Noncompetitive antagonists (NCIs)
(A) nAChR	 Acetylcholine	 (+)-Tubocurarine	 TPMP⁺
		 α-Neurotoxin	 Tetracaine
(B) Glu	 Glutamate	 Kainate	 Phencyclidin
	 AMPA	 NMDA	
(C) P2X	 ATP	 α, β-Methylene-ATP	

occurring plant and animal toxins are antagonists of LGCs. For example, strychnine binds to the GlyR, and (+)-tubocurarine and snake venom α -toxins bind to the nAChR. In many cases, such toxins are valuable tools for biochemical research (reviewed in ref. [13]).

A third group of receptor ligands are the noncompetitive inhibitors (NCIs), a structurally heterogeneous group of compounds that bind to distinct sites on the receptor surface. They either act allosterically or in many cases bind to high-affinity sites within the ion channel, thereby sterically blocking the ion flux (channel blockers). These substances can be used to probe the structure of ion channels. NCIs include drugs such as local anesthetics and neuroleptics as well as naturally occurring alkaloids such as frog toxins.

The picture is further complicated by the existence of a pharmacologically very interesting group of substances called inverse agonists. These compounds act at the same receptor sites as agonists, yet produce an opposite effect. Such compounds have been described for the benzodiazepine-binding site on the GABA_AR. Benzodiazepines themselves are widely used tranquilizers that exert an anxiolytic effect. The esters of β -carbolines act as inverse agonists of benzodiazepines, not only blocking their effect, but provoking opposite behavioral actions, that is, they produce signs of anxiety and cause sleeplessness and seizures.

Allosteric Regulation

In many systems, a separate protein functions as the transducer between the receptor site and the effector site—in the case of the 7TM receptors, the transducers are G-proteins. In the case of the LGCs, however, no such coupling protein exists, and the receptor domain (ligand-binding site) and the effector (ion channel) communicate through conformational changes within the receptor only. The energy necessary to trigger this process comes from the ligand-binding energy.

The LGCs are allosterically regulated proteins, that is, they regulate their activity by shifting the equilibrium between different functional states that correspond to distinct conformational states. For example, the nAChRs show classic allosteric behavior: both ligand binding and channel opening are cooperative processes, evidence for which is provided by sigmoidal dose/response and binding curves (summarized in ref. [14]).

Phosphorylation

Another level of regulation is added by post-translational modifications. The LGCs contain multiple phosphorylation sites that have been mapped to their cytosolic loops; these are phosphorylated by different enzymatic activities.^[15] The best characterized system is the nAChR of the neuromuscular junction: agrin stimulates the phosphorylation of tyrosine residues (reviewed in ref. [16]), whereas phosphorylation of serine residues is stimulated by the neuropeptide CGRP (through protein kinase A) and by acetylcholine (through Ca²⁺-dependent kinases). Although a regulation of the channel activity was observed under artificial conditions—for example, the rate of desensitization of the muscle nAChR

is altered by phosphorylation—an *in vivo* regulation was not convincingly shown. Clearly these modifications have the potential to modulate synaptic efficiency, but their functional significance is far from clear.

Protein–Protein Interaction

LGCs interact with proteins inside the cell. The GlyR is attached to cytoskeletal elements through the peripheral membrane protein gephyrin.^[17] The proteins rapsyn and GABARAP appear to fulfil this task for the nACh and GABA_A receptors, respectively.^[18] Different subtypes of glutamate receptors are linked to specific scaffold proteins of the postsynaptic density, for example, PSD-95 or GRIP.^[19, 20] In this way, extensive protein complexes are formed near the neuronal surface (reviewed in ref. [21]). These interactions seem to be the basis for localized clustering and subcellular targeting of LGCs, thus regulating their activity and post-translational modifications, but raising the possibility of direct signalling into the cell. A signalling complex that involves the NMDA receptor was recently isolated from rats and consists of at least 77 proteins.^[22]

Regulation of Biosynthesis

The biosynthesis of the LGCs is tightly regulated. The process is best understood for the nAChR during synapse formation at the neuromuscular junction (reviewed in ref. [23]). The high local receptor density at subsynaptic locations is brought about by both presynaptic factors (ingrown motoneurons) and postsynaptic factors (electrical activity). First, receptors that are initially diffusely distributed over the entire cell surface are directed to the subsynaptic zone. Subsequently, the transcription of the nAChR genes is enhanced only in those nuclei that are close to the developing synapse by means of a mechanism that involves presynaptically released proteins such as agrin and neuregulin. In contrast, transcription is downregulated in extrasynaptic nuclei in response to depolarization (receptor activation).

3.3. LGCs and Disease

Because of their capacity to modify synaptic function, one can expect that LGCs play an important role in various mental disorders, for example, schizophrenia, epilepsy, or depression, and that drugs that act on the LGCs can be used to treat these. Mutations in LGC genes that underlie a number of inheritable human diseases have been found (listed in ref. [24]), mainly by positional cloning: mutations in the nAChRs of the neuromuscular endplate that compromise normal transmission are the basis for congenital myasthenic syndrome,^[25] whereas mutations in the gene of the neuronal α 4 nAChR subunit cause different conditions such as benign familial neonatal convulsions,^[26] or a partial form of epilepsy called autosomal dominant nocturnal frontal lobe epilepsy.^[27] Hyperekplexia (Startle disease) results from a defect in the

glycine receptor gene GLRA1.^[28] Moreover, anti-nAChR antibodies cause the autoimmune disease *Myasthenia gravis* (severe muscle weakness).^[29]

4. Individual Representatives of LGC Families

We choose two LGCs for a more detailed description: the glutamate receptors (GluR) and the nicotinic acetylcholine receptors (nAChR). Neither has been crystallized yet, but we know enough molecular detail to address the following basic questions:

- What is the molecular basis of the impressive ion selectivity (the selectivity filter)?
- What is the structural correlate of the gating mechanism?

In the case of the LGCs, this question includes the molecular prerequisite of selectivity for the triggering signal, that is, the structure of the neurotransmitter-binding pocket, and the coupling between binding and gating.

4.1. Glutamate Receptors

4.1.1. The “Family”, Function, and Structure

Glutamate is the main excitatory neurotransmitter in the brain. It acts through both metabotropic and cation-selective

ionotropic glutamate receptors. The latter form a large family of ligand-gated ion channels, including the AMPA, kainate, and NMDA receptors (AMPA stands for α -amino-3-hydroxy-5-methyl-4-isoxazole propionate; kainate, a natural product, is an excitotoxic glutamate analogue produced by an algae; NMDA is *N*-methyl-D-aspartate; see Table 1). Although all three subtypes respond to glutamate, they can be distinguished by their response to artificial agonists. Their distribution in the brain, physiological function, and mechanism and kinetics of activation and regulation are very different. We do not intend to duplicate herein the many comprehensive reviews on glutamate receptors,^[30–32] but only to summarize some of the structural information necessary to discuss in detail the two basic questions specified above.

The primary structures of many if not all glutamate receptors, including several splice variants, have been deduced from their cloned and sequenced cDNA. For their nomenclature and evolutionary relation, see Figure 4. As is the case with the receptors that resemble nicotinic acetylcholine receptors, the GluR subunits contain four conspicuous hydrophobic sequences, but in contrast to the 4TM receptors, they are presently presumed to span the membrane only three times (Figure 5). The second hydrophobic sequence was shown to enter and exit the plasma membrane at the cytoplasmic surface, thus forming two antiparallel β -strands that line the channel. Several (probably four) of these polypeptide chains form homo- or hetero-oligomeric quaternary structures.

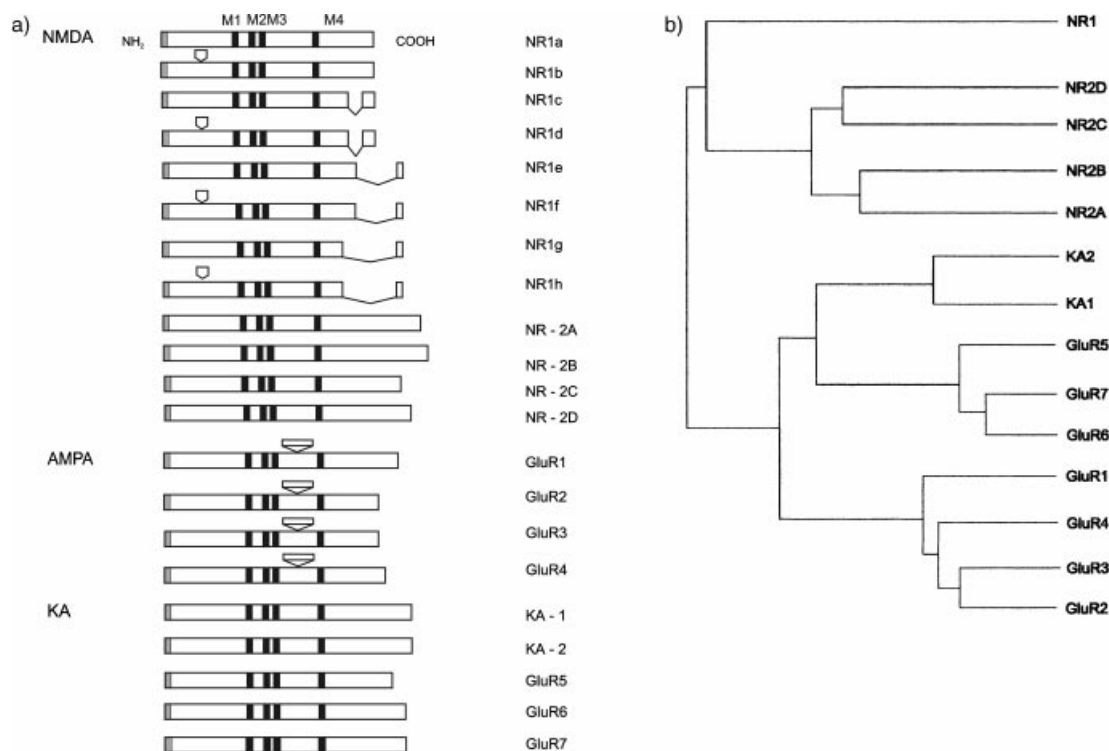


Figure 4. Heterogeneity of glutamate receptors. a) Linear representation of the structure of ionotropic glutamate receptors; GluR stands for the AMPA and kainate subtypes, NR represents the NMDA receptors, and KA represents the kainate-binding proteins. The black boxes represent the hydrophobic sequences M1–M4, and the hatched box at the NH₂ terminus depicts the signal peptide. Variants arise from exonic splicing; the arrows above the diagram indicate splice inserts. The V-shaped lines are for insertions/deletions introduced to obtain an optimal alignment between the sequences. Figure and legend modified from ref. [30]; b) evolutionary tree of glutamate receptors: starting from a common ancestor (left) the actual variety of glutamate receptors (right) with different pharmacological profiles and functional properties has evolved.

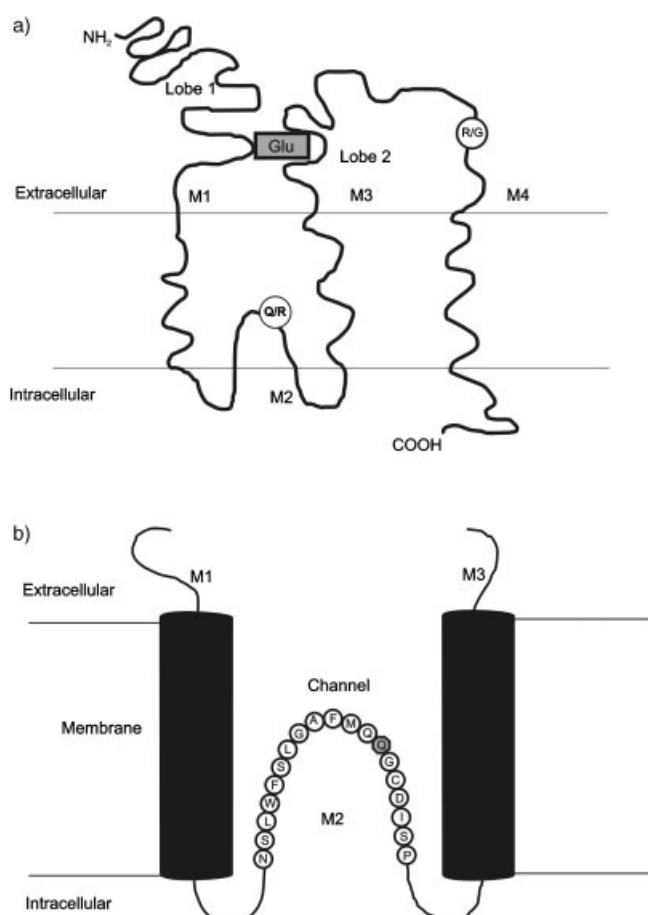


Figure 5. Functional domains of glutamate receptors. a) The agonist-binding site between lobe 1 and lobe 2 of the folded polypeptide chain; M2 represents the re-entrant loop that forms the ion channel; Q/R is the site of modification through RNA editing which affects ion selectivity; b) close-up of M2; the shaded glutamine residue (Q) is replaced by arginine after RNA editing. (Figure modified from ref. [30].)

4.1.2. The Selectivity Filter: Lessons from a Bacterial Channel

Before discussing the channel structure of glutamate receptors, we have to turn to the voltage-gated ion channels, because one of these, the potassium channel KcsA from the bacterium *Streptomyces lividans*, has been crystallized and gives us for the first time an ion channel structure with 3.2-Å resolution (Figure 6).^[33] Some of its features allow us to understand the ion selectivity of voltage- and possibly of ligand-gated ion channels as well, and channel-mediated ion conduction through the membrane lipid phase.

We have good reasons to use this prokaryotic channel as a model for eukaryotic glutamate receptors: an evolutionary missing link was recently detected, the glutamate receptor GluR0 from the cyanobacterium *Synechocystis*.^[34] This protein has a sequence that is similar to eukaryotic glutamate receptors as well as to potassium channels. It is a potassium-selective LGC and contains even the signature sequence TVGYGD characteristic of potassium channels. The KcsA channel is formed by four identical polypeptide chains, each of which contains two-membrane-spanning sequences (2TM) and one re-entrant loop called P. As is the case with

glutamate-receptor channels, the P loop lines the pore and contains the determinants for ion selectivity and conduction.

Potassium channels are slightly permeable to the larger Rb^+ and virtually impermeable to the smaller Na^+ and Li^+ ions. This indicates that channels are not just holes that filter ions of a given radius. On the contrary, they all conduct small ions that have been stripped of their hydration shell, and the selectivity is a result of their ability to remove the water dipoles surrounding the charged particle (Figure 7). The K^+ selectivity filter is lined by carbonyl oxygen atoms. "To compensate for the energetic cost of dehydration, the carbonyl oxygen atoms must take the place of the water oxygen atoms, come in very close contact with the ion, and act like surrogate water. ... a K^+ ion fits in the filter precisely so that the energetic costs and gains are well balanced".^[33] For the smaller Na^+ , the carbonyl oxygen atoms are too far apart to provide enough "energetic gain" to compensate for the cost of dehydration.

This ingenious mechanism poses a major problem: how can a large flux of cations be secured if they are complexed so tightly in the selectivity filter? This problem is overcome by an equally ingenious mechanism: there are two cations simultaneously in the selectivity filter (Figure 7b). Their close proximity and "repulsion overcomes the otherwise strong interaction between ion and protein and allows rapid conduction in the setting of high selectivity".^[33]

We shall recognize the theme of protein oxygen atoms in the selectivity filter of the LGCs discussed in Section 4.2.2. Another feature postulated for the LGCs which was proven by the X-ray crystallographic study of the microbial K^+ channel is a wide water-filled entrance to the channel. In the case of the K^+ channel, a deep largely hydrophobic cavity precedes the selectivity filter (which occupies only 12 Å of the membrane's thickness). This cavity is wide enough to be filled with water and to accept the cation in its hydrated form. Thus the cation can traverse most of the membrane easily; the electrostatic barrier posed by the membrane is lowered, thus ensuring a low-resistance pathway up to the selectivity filter. Experimental proof is simple: TEA (tetraethylammonium) is a selective blocker of potassium channels. It can enter and plug the cavity, because it cannot shed its "ligands" like potassium can do with its hydration shell.

For the channels of GluRs, we have to resort to site-directed mutation experiments in combination with patch-clamp electrophysiology instead of the more direct evidence given by an X-ray crystallographic map. These types of experiments have shown that a re-entrant loop similar to the potassium channel's P loop lines the channel and forms the selectivity filter (Figure 5).^[35] Interestingly, one amino acid residue in this loop determines whether or not the channel is permeable to calcium ions. One specific asparagine (N) residue in the P loop of the NMDA receptors is responsible for their calcium permeability. Nature takes advantage of this key residue: the homologous position in AMPA receptors is occupied by a glutamine (Q) residue; if this residue is converted into an arginine (R) group (specifically in the GluR2), the channel becomes virtually impermeable to calcium ions. This Q/R conversion is brought about by a rare mechanism, namely RNA editing (reviewed in ref. [36]): in the pre-mRNA, the



Figure 6. Stereoview of the X-ray structure of the potassium channel KcsA.^[33] Top: the fold of the KcsA tetramer viewed from the extracellular side. Bottom: the channel seen from a perpendicular perspective. (Figure courtesy of Roderick MacKinnon; reproduced with permission of *Science* and the authors).

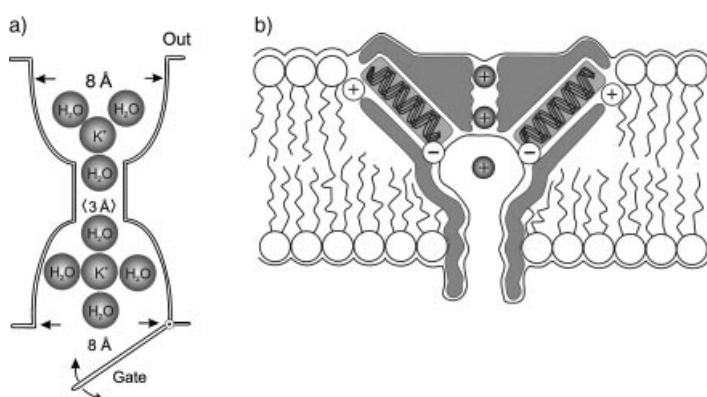


Figure 7. The ion pathway through voltage-gated ion channels with structural features similar to the ligand-gated ion channels. a) Historical representation of potassium channels which indicate the diameter of the intracellular and extracellular vestibule and the selectivity filter, which allows the passage only of partially dehydrated cations; b) the modern view deduced from X-ray crystallographic analysis (see text). (Figure adapted from ref. [33].)

codon CAG for glutamine is deaminated enzymatically to CIG (adenosine to inosine, catalyzed by an adenosine desaminase). One would like to see the consequences of this Q/R conversion on a protein map with atomic resolution, but unfortunately a crystal structure is not available yet.

To understand the gating mechanism, we had to resort to KcsA as a model for LGCs. With the glutamate receptor's ligand-binding site, which confers the specificity of the signal and the triggering mechanism for the channel gating, we are better off, since a 3D structure of this domain of the transmitter receptor itself is available. The glutamate receptor's ligand-binding domain has been crystallized and its structure, when complexed with the toxic agonist kainate, has been solved with high resolution.^[37] Previously, site-directed mutagenesis experiments had shown that the ligand-binding domain of GluRs is formed by two noncontinuous sequences, S1 and S2.^[38, 39] S1 includes about 150 N-terminal amino acids, ending with TM1, and S2 links TM3 with TM4. The fusion of S1 to S2 with a short peptide by means of recombinant DNA techniques yields a water-soluble protein, which was expressed in bacteria in large quantities.^[40, 41] This artificial binding domain has ligand-binding and pharmacological properties very similar to the natural NMDA receptor. An X-ray crystallographic study of this expression product yielded an overall

structure similar to a Venus flytrap (Figures 6 (top) and 9): the two lobes come close together to trap the ligand (kainate). Interestingly, this Venus flytrap model closely resembles the glutamine-binding protein QBP from bacteria, which shows only little sequence similarity to GluRs.^[42]

Amino acid residues of both lobes participate in ligand binding. Kainate binds deep down in a gorge formed by the two lobes. Each lobe points two helices with its amino terminus (the positive end of their dipoles) towards the negatively charged carboxy group of the agonist. Binding pulls the lobes together thereby closing the trap. Together with previous mutagenesis experiments, the residues that determine the binding affinity and specificity can now be identified (Figure 8).^[43–45] For example, the 2-carboxy group of kainate binds to an arginine residue (R485 in GluR2) and to the NH group of a threonine residue (T480) in the polypeptide backbone.

More recent experiments in which a similar GluR construct was cocrystallized with various ligands have shown for the

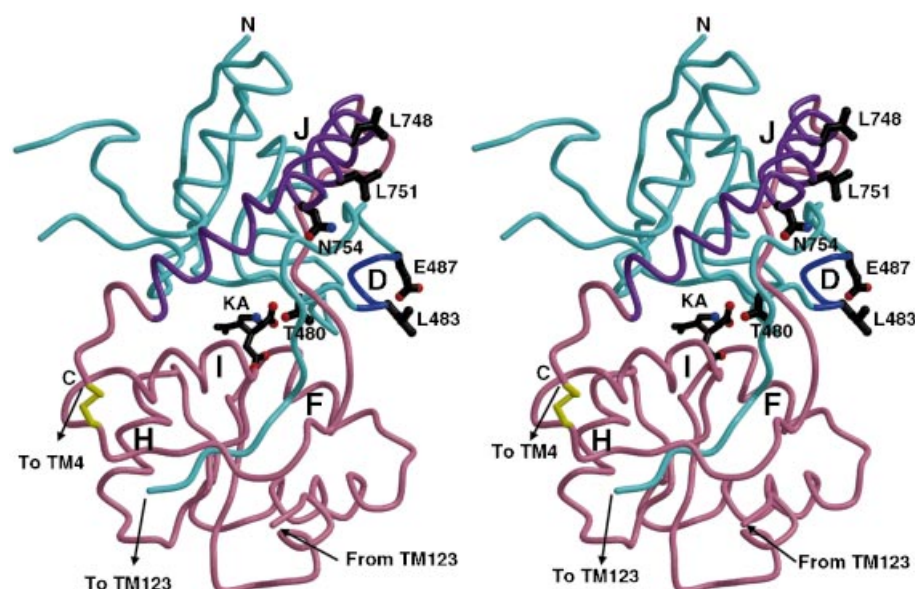


Figure 8. Stereoview of the high-resolution structure of the ligand-binding site of the AMPA-subtype glutamate receptor, cocrystallized with kainate (KA). The upper lobe (represented in turquoise and violet) together with the lower lobe (mauve) form the Venus flytrap structure. (Reproduced courtesy of Eric Gouaux.)

first time the mechanism underlying activation and antagonism: the extent of ligand-induced activation depends on the degree of domain closure, and the degree to which the two domains in the flytrap model are contracted depends on the nature of the ligand.^[46] Agonists cause domain closure, which eventually leads to channel activation. The binding of partial agonists results in correspondingly smaller conformational changes, whereas antagonists act by freezing the ligand-binding core in an open-cleft conformation.

4.1.3. The Gating Mechanism

Once again we resort to the microbial KcsA to learn how channel gating occurs, although its physiological relevance is not clear and one should be aware that it might act very differently from LGCs. The KcsA potassium channel of *Streptomyces lividans* can be stabilized in the open state at low extracellular pH values. Protons seem to be the “ligands” that trigger the gating. The transmembrane helices TM1 and TM2 of the four channel subunits form the pore. The selectivity filter composed of the four P loops is located at the upper (extracellular) end, but where do we look for the gate? Based on a combination of ion-flux experiments, and CD, FTIR, and EPR spectroscopic investigations of wild-type and mutated channels, including inward-rectifier channels from eukaryotes (summarized in a series of reviews, see ref. [47]), the following model has been proposed: during gating, conformational changes along TM2 occur. They are not caused by large changes of secondary structure, nor are they restricted to the intracellular C-terminal ends of the helices (where the gate of voltage-gated K⁺ channels is localized). Both TM1 and TM2 as a whole tilt slightly and rotate counterclockwise. This “rigid-body movement” causes a widening of the gate, as depicted schematically in Figure 9.^[48] The helices behave as

rods that open and close the pore. The “signature sequences” (the selectivity filter) experience no significant movement. As we will discuss in Section 4.2.2, similar minute movements may underlie the gating of LGCs. This mechanism is very different from the situation observed in other voltage-gated potassium channels (discussed in ref. [49]), in which, for example, a channel-occluding plug is removed upon activation (“ball-and-chain” mechanism).

It should be pointed out that much of the dynamics of channel gating discussed in this section is based on indirect biophysical evidence and on static X-ray crystallographic images; therefore it should be considered speculative and needs further experimental support.

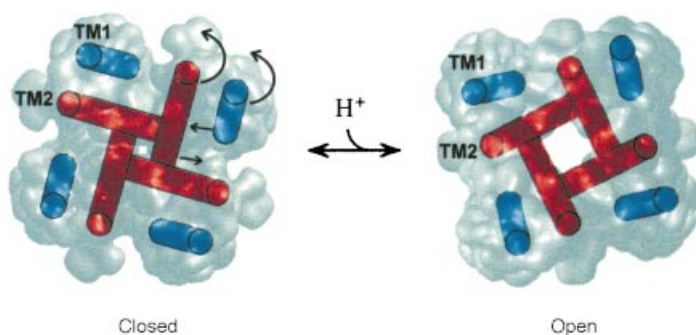


Figure 9. Proposed model of a molecular activation mechanism for the gating of a voltage-dependent potassium channel by a rigid-body movement; view of the KcsA channel (see text) from the extracellular side. Upon activation, the counterclockwise movement of the transmembrane helix TM2 away from the permeation pathway opens the channel. For illustration purposes, the movements depicted are larger than expected and constitute a simplified representation. (Reprinted from ref. [48] with kind permission of *Science* and the authors.)

4.1.4. Crystallographic Proof of the Reliability of Biochemical and More Indirect Methods of Structure Determination

The first X-ray crystallographic analysis of an important ligand-gated channel domain proves above all one important point in this whole field of research: crystallography and mutation research are not only complementary methods; the vast amount of information collected by biochemical and biophysical research, including recombinant DNA technologies, obviously gives reliable information about structure–function relationships. As we experienced in the 1950s and 1960s with many enzymes whose structures were later solved, X-ray crystallographic studies have not led us to correct our 3D models obtained by classic approaches. Rather, our

models have been confirmed and more important details have been added.

And the power of yet another method is confirmed by studies on LGCs: homology modelling. The agonist-binding domain of the glutamate receptor has a low-level sequence identity to various soluble bacterial periplasmic substrate-binding proteins for which high-resolution structures are available.^[50] These were used as a template to calculate the structure of the glutamate-receptor-binding domain, and to propose 3D features of the agonist-binding site. Now, with the first high-resolution structure of a glutamate-receptor binding domain in hand,^[37] we can see that despite the low degree of sequence similarity this approach yielded very reasonable results,^[51] and therefore we can be confident that the predictions made for other proteins also are essentially correct.

What is the use of such detailed knowledge? More than 50% of all synapses in our brain use glutamate as a transmitter. GluRs participate in brain development, and they are implicated in many neuropathies, ranging from epilepsy and strokes to Alzheimer's, Huntington's, and Parkinson's diseases. The treatment of these diseases requires a knowledge of the exact steric, chemical, and physical properties of the ligand-binding site to facilitate the design of drugs that target the glutamate receptors.

4.2. The Nicotinic Acetylcholine Receptors (nAChR)

4.2.1. General Features of the Family

The second LGC to be discussed herein is the nicotinic acetylcholine receptor (nAChR) as the best-characterized LGC and a prototypical structure for the class of 4TM receptors. Because of their homology, the other members of this superfamily are likely to share its structural features, in particular its transmembrane topology. A comprehensive collection of sequences and references regarding this super-

family is found at the web-site <http://www.pasteur.fr/LGIC/LGIC.html> (Table 2).^[52]

nAChRs are found mainly in postsynaptic membranes of central nervous system synapses and of the neuromuscular endplate. The physiological signal to which nAChR responds is the neurotransmitter acetylcholine, but it is also activated by nicotine, one of the most widely consumed drugs.

The nAChR ion channel is cation selective (other members of this superfamily such as the glycine receptor have anion-selective channels). In the case of the peripheral receptors, it is selective for Na⁺, K⁺, and, to a much lesser extent, for Ca²⁺, whereas some of the neuronal nAChR ion channels are mainly calcium channels. Receptor activation leads to depolarization of the plasma membrane; in neuronal cells, Ca²⁺ influx triggers Ca²⁺-mediated signalling processes.

No structure at atomic resolution is available for any of the receptors of this superfamily. For the nAChR, a three-dimensional picture is emerging from combined electron-microscopic, chemical-labeling, mutagenesis, and patch-clamp investigations (for a detailed review see ref. [53]). This structural information has been accumulated for the peripheral receptors, since nature has provided an extremely rich source in the form of the electric organs of electric rays and eels, from which the nAChRs can be easily purified in large amounts. The nAChR from the electric ray *Torpedo*, which serves as a model for the muscle-type receptors of higher vertebrates, has a molecular mass of about 290 kDa, about 20 kDa of which are glycosyl residues. It is a heteropentameric complex, composed of four different polypeptide chains with a stoichiometry of $\alpha_2\beta\gamma\delta$. The five subunits are arranged radially around the central ion channel in the order α_H - γ - α_L - δ - β in a clockwise orientation, when viewed from the synaptic cleft (see Figure 10); H and L designate the high- and low-affinity binding sites of the two α subunits, respectively—because of their different neighbours, the two α -subunits differ in their binding properties.^[54]

The other receptors of the superfamily presumably exist also as pentamers; this was directly demonstrated for

Table 2. Selected internet resources for navigating the field of ligand-gated ion channels.^[a]

Keyword	Short description	Internet address
General information on ion channels	A gateway to all the resources containing relevant information on ion channels	http://phy025.lubb.ttuhsu.edu/Neely/ionchann.htm
General information on ion channels	A resource page for the ion channel community with many interesting links to ion-channel-related sites	http://members.nbci.com/IonChannel/ion.htm
Ligand-Gated Ion Channel (LGIC) Sequence Database (Le Novère and Changeux, 1999)	A well-maintained database containing the complete set (more than 300) of known nucleic-acid and amino-acid sequences of extracellularly activated transmitter-gated ion channels; sequence alignments and phylogenetic trees	http://www.pasteur.fr/LGIC/LGIC.html
GABA receptor database	GABAagent: a compilation of data on GABA receptors	http://gaba.ust.hk/Agent.html
Channel structure	A review of the structure of the KcsA channel with many figures of the pore	http://members.nbci.com/IonChannel/KcsA.htm
Permeation models	Different models on ion selectivity and conductance	http://www.ugrad.cs.jhu.edu/~imran/kcsa/
Interview with Roderick MacKinnon who solved the first channel structure (Doyle et al., 1988)	Mini-lecture on the fascinating intricacies of ion channel structures and the hard labor necessary to identify such a structure	http://www.sciam.com/explorations/2000/050100MacKinnon/index.html
Ion Channels and Disease	Highlights the molecular background of diseases linked to ion channels	http://www.neuro.wustl.edu/neuromuscular/mother/chan.html

[a] A more comprehensive list, including more general resources, is found in ref. [109].

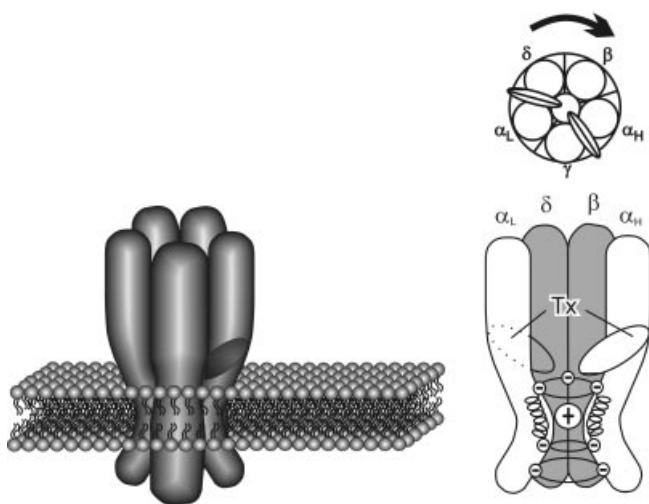


Figure 10. The interface gorge model of the nAChR. This figure summarizes our knowledge of the structure of the nAChR. Left: global layout of the receptor in its membrane surrounding; Right (top): arrangement of the receptor subunits around the central pore, viewed from the synaptic cleft. Right (bottom): the longitudinal section shows that the ligand-binding site spans the receptor wall. The ion channel is formed by transmembrane helices; the channel's gate is a restriction towards the cytoplasmic side of the membrane. Tx refers to α -neurotoxin, the peptide toxin from poisonous snakes, which was used to determine the ligand-binding topography.^[87] The ellipsoid represents the toxin-binding site; since the α -neurotoxins are competitive antagonists, it is also thought to form (at least partially) the agonist-binding site.

GABA_AR and 5HT₃R by using electron microscopy,^[55, 56] and indirectly for the GlyR by using biochemical techniques.^[57, 58] A heteromeric assembly of different subunits is the rule and, as explained above, is the basis for receptor diversity, but a homomeric assembly has also been described, for example, for the 5HT₃R and for certain neuronal nicotinic and GABA_AR subtypes.^[59, 60]

The four nAChR subunits display homologous sequences with 30 to 40% identity of amino-acid residues. Hydropathy plots of the primary structures reveal that they each contain four hydrophobic stretches, which are thought to form membrane-spanning segments (M1–M4). All members of the family share this pattern. They all have extracellular N and C termini, a large extracellular glycosylated N-terminal domain (about 200 residues long), and a long cytosolic connecting loop of 100 to 150 residues between the membrane passages M3 and M4 which contains several phosphorylation sites.

A further characteristic motif of this receptor superfamily is a loop formed by a pair of cysteine residues at a 15-residue spacing in the N-terminal domain (Figure 3). The two cysteine residues are connected by a disulphide bridge,^[61] and the residues between them are highly conserved. This Cys loop, for which a β -folded amphipathic structure is predicted,^[62] occurs independently of the ion selectivity and ligand-binding properties of the receptor at a fixed position relative to the first membrane passage, and might play an important role as a core in protein folding. As this Cys loop forms a typical signature for this superfamily, it has been proposed to call this superfamily “Cys loop receptors”.^[63]

The overall dimension of the pentameric nAChR complex, as deduced from electron micrographs (obtained by using cryo-electron microscopy of tubular crystals grown from membranes isolated from the *Torpedo* electric organ) is about 12.5×8 nm.^[64] One can estimate that about 35% of the protein is immersed in the membrane and about 50% is extracellular.

4.2.2. The Ion Channel—Selectivity Filter and Gate

In the electron micrographs discussed in the previous section, one also readily discerns a central hole in the receptor “doughnut”, which is about 20 to 25 Å wide.^[65] This structure is interpreted as the entrance to the ion channel. Thus, very much like the potassium channel discussed above, the nAChR channel appears to have a wide water-filled extramembrane vestibule, which is followed by a funnel-shaped portion inside the membrane (Figure 10). Its diameter is 11.5 Å for the middle part (determined by the size of channel blockers used to label specific residues of the channel wall^[66]) and about 7 Å in the open state at the narrowest part towards the cytosolic end (predicted by computer modelling^[67]). This value corresponds well to the diameter of the largest permeating cation (6.4 Å).^[68] At the cytoplasmic end, the pore widens again to about 15 Å. Electron micrographs show that underneath the pore, a basketlike structure formed by the cytoplasmic loops between M3 and M4 protrudes about 40 Å from the cytoplasmic membrane surface. It was suggested that ions are prevented by this structure from exiting directly into the cytoplasm; instead they must pass through two narrow transverse openings.^[69]

The wall of the ion channel is formed by the second transmembrane segment (M2) of each receptor subunit.^[66] This was concluded mainly from affinity-labeling studies of substances that directly occlude the ion channel (channel blockers);^[66, 70] this finding was confirmed by mutational analysis.^[71, 72] From the pattern of labeled amino-acid residues, we know that this segment adopts, at least partially, an α -helix conformation, a conclusion largely confirmed by chemical accessibility studies.^[73] Parts of the first transmembrane segment M1 also contribute at least transiently to the channel lining in the wider upper (extracellular) part.^[74]

The five homologous subunits each contribute the corresponding segment for the construction of the ion channel. An interesting stratification of the channel wall results from this construction principle (Figure 11): rings of amino-acid side chains with similar physical properties are formed which determine the characteristic features of the channel—most conspicuously three negatively charged rings (Asp/Glu), three hydrophobic rings (Leu/Val), and two rings of polar residues (Ser/Thr).^[75] The ion conductance properties, for example, are explained by three rings of negatively charged amino-acid side chains.^[76] These rings are located at the cytoplasmic N-terminal and the extracellular C-terminal end of the channel and midway between the two. The intermediate ring, in which glutamate side chains protrude into the channel lumen, forms the constriction that determines which ions can pass. Gating—the opening and closing of the permeation pathway—could be brought about by moving these side

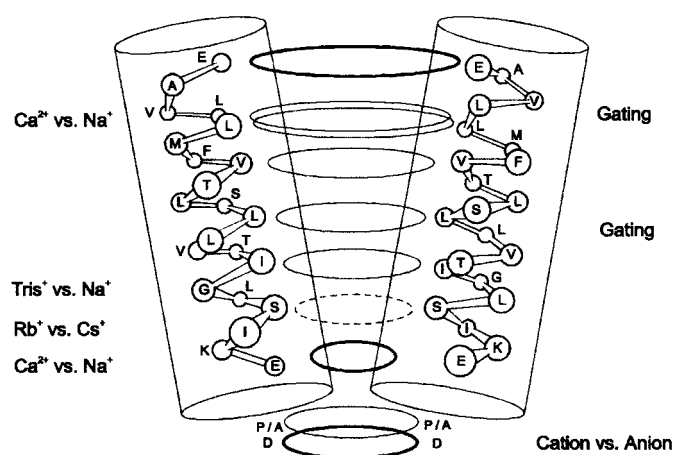


Figure 11. Stratification model of the ion channel of the nAChR. Homologous side chains of the five receptor subunits form rings, which determine the properties of the channel (ion selectivity and gating) as elucidated by mutagenesis experiments (simplified from ref. [75]). Depicted are the M2 sequences from the neuronal $\alpha 7$ subunits. (Tris = tris(hydroxymethyl)aminomethane)

chains out of the channel lumen,^[67] perhaps by a similar “rigid-body movement” of the helices as was postulated for the gating of the voltage-dependent potassium channels.^[48] Other experiments showed that one of the hydrophobic rings formed by a conserved leucine residue at the approximate midpoint of the M2 transmembrane segment (Leu9') contributes to channel gating. It was suggested that these leucine residues from the different subunits participate in a symmetric structural transition between the closed and open states.^[77]

The role of the selectivity filter, in which large ions are selected according to their dehydrated size, was assigned more specifically to the amino-acid residue α T244 that is situated one helix turn above this intermediate ring.^[78] The segment α 238–242 just below this ring was shown to contribute directly to the charge selectivity filter.^[79] This segment is at the cytoplasmic border of the M2 membrane span and is predicted to adopt a coiled conformation, reminiscent of the P loop described above.^[80]

By using the substituted-cysteine accessibility method^[81]—the residues are individually mutated to cysteine, the accessibility of which is then assessed with small sulfhydryl-specific reagents—the gate was located in the same region (between α G240 and α T244) in the vicinity of the intermediate ring.^[82] Thus the gate and selectivity filter seem to be intimately linked.

The construction principle defined in this “helix-M2 model”^[66] (Figure 10) for the cation-selective nAChR channel is also thought to be valid for the other members of this protein superfamily, for example, the anion-selective channel of the GlyR. A comparison between the amino acid residues at the critical positions of these two channels demonstrates that the conversion of the cation-selective nAChR channel into an anion-selective channel can be brought about by introducing mutations at only three positions.^[83] By the inverse mutations, the anion-selective channel of the GlyR can be transformed into a cation-selective channel.^[84] It is mainly the substitution of the intermediate negatively charged

ring by a neutral ring that is relevant for cation versus anion discrimination.^[79] This type of manipulation proves that our concept on how the ion selectivity of the AChR ion channel is regulated is essentially correct.

4.2.3. The Ligand-Binding Site: An Intersubunit Gorge

Now let's have a closer look at the ligand-binding site of the nAChR (“L” in Figure 1). In contrast to the glutamate receptors reviewed in Section 4.1.2, the extracellular domain of the nAChR involved in ligand binding has not yet been crystallized and no high-resolution X-ray structure is available. The binding site itself, however, has been characterized in some detail by using molecular biology and biochemical methods so that a spatial model of the binding pocket was obtained. The region that contains amino acids 190–200 on the two identical α -subunits as well as several functionally important aromatic residues was shown to contribute to the binding site by affinity labeling using various agonists and antagonists. However, there is more to the story: some amino-acid residues of the α -subunits outside this region are also labeled, and even portions of the neighboring subunits (that is, the γ - and the δ -subunits) located close to the interfaces contribute to the binding site.^[85] From these findings, a model of the α -subunit binding site for low-molecular-mass ligands was proposed (Figure 12) which states that the binding site is

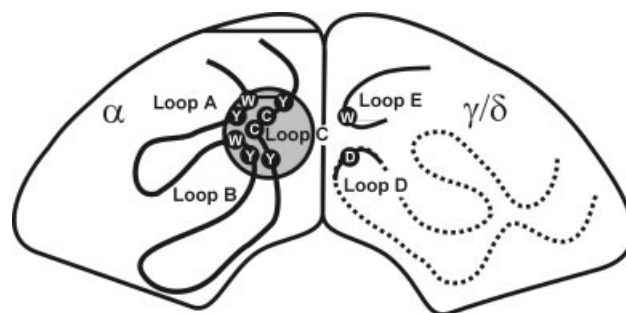


Figure 12. Model of the nAChR agonist-binding site. Three loops of the α -subunit contribute to the binding site, which is located at the subunit interface. Note that additional loops extend from the neighboring subunits.

not confined to a contiguous stretch of the primary structure of the ligand-binding α -subunit, but is built from three noncontiguous loops of the α -subunit.^[86] Additional loops come in from the neighboring subunits. This finding also provides a rational basis for the nonequivalence of the two ligand-binding sites for certain ligands such as d-tubocurarine, which has a high-affinity site at the α/γ interface and a low affinity site at the α/δ interface.

Studies of snake-venom α -neurotoxins as affinity reagents led to the hypothesis that the toxin binds in relative proximity (1.5 nm or closer) to the membrane layer close to the beginning of the funnel structure formed by the M2 spans.^[87] It was shown experimentally that the toxin gains access to this site through a gorge spanning the wall of the ring-shaped receptor, which contains the aromatic residues of the ligand-binding site (“aromatic interface gorge”; Figure 10).^[53, 88] In contrast, tunnels about 10 to 15 Å long which open only to the

vestibule and are framed by twisted β -sheet strands were resolved recently in the extracellular portion of the α -subunits by using electron microscopy.^[69] They connect cavities (interpreted as the acetylcholine-binding sites) to the water-filled vestibule of the channel and are believed to represent the primary access route of agonists to the binding site.

Such a design would parallel the situation in the ACh-degrading enzyme acetylcholinesterase in which the acetylcholine-binding site is located at the bottom of a 20-Å deep aromatic gorge.^[89] Perhaps the analogy with the situation in AChE can even be extended to the binding mode: In both cases binding of the cholinium moiety is achieved by cation- π interaction, in which the face of an aromatic ring provides a region of negative electrostatic potential that can bind cations with considerable strength.^[90] With the ACh esterase it has been shown by site-directed affinity labelling that the binding side chain is the tryptophan residue Trp84.^[91] A combination of theoretical chemistry with molecular biology experiments suggest that α Trp149 is the primary cation- π binding site for AChR.^[92]

Thus the nAChR's ligand-binding site appears to be an intersubunit gorge close to the membrane surface and also close to the channel entrance, made up of at least three different loops of the α -subunits containing numerous aromatic residues as well as negatively charged portions of the neighboring γ - and the δ -subunits.

Operationally, receptors can be described as a triune entity, composed of 1) a signal-recognition moiety (receptor, "R"), 2) an effector function ("E"), and 3) a transducing component ("T"), which couples R to E.^[53] In the nAChR, the sites for agonist binding ("R"), conformational signal transfer to the ion channel ("T"), and the ion channel itself ("E") are confined within a rather restricted area of only about 2 nm which would form a sort of "active center".^[53] The transducing mechanism in this case is the allosteric transition that occurs upon neurotransmitter binding.

Despite this spatial integration, it seems that the extracellular domain and the channel fold independently from each other, since a chimeric molecule could be constructed from two different receptor types (neuronal $\alpha 7$ nAChR and serotonin 5HT₃R) which combined the binding properties of one and the channel properties of the other partner.^[93]

Interestingly, the high-resolution structure (3.5 Å) of a mechanosensitive ion channel from *Mycobacterium tuberculosis* reveals several features that are very similar to the nAChR (Figure 13).^[94] as in the case of the nAChR, the structure is pentameric and a wide water-filled vestibule of about 18 Å in diameter leads from the extracellular side into a pore, which narrows towards the cytosolic side. In both channels, the walls are formed by helices lined with polar residues such as threonine and serine. In both cases, hydrophobic residues are present at the constriction that may act as the channel gate. The structure formed intracellularly in the mechanosensitive channel is reminiscent of the "basket" observed in electron micrographs of the nAChR.^[69]

Despite our detailed knowledge of the nAChR, several key questions remain open and probably will be answered only when a high-resolution structure becomes available. So the

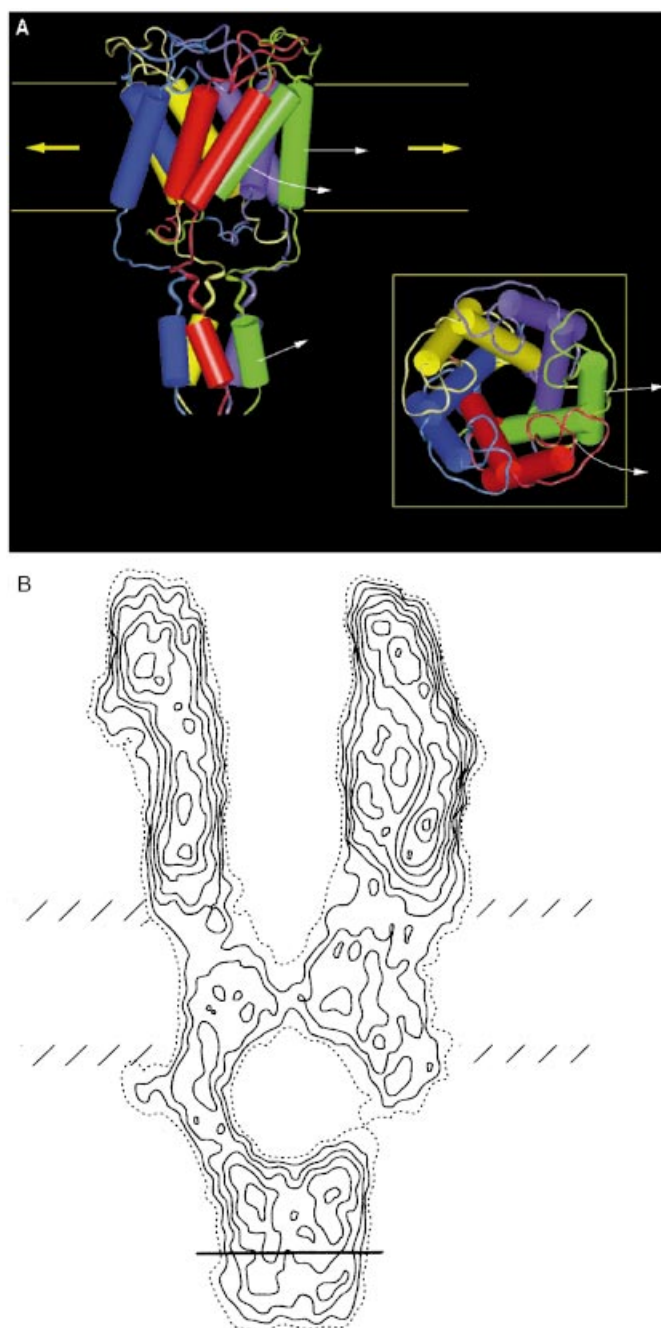


Figure 13. Similarities in the overall structures of a mechanosensitive channel (A) and nAChR (B). A) X-ray structure of a mechanosensitive ion channel from *Mycobacterium tuberculosis*.^[94] B) Electron micrograph (4.6-Å resolution) of the nAChR from *Torpedo californica*.^[69] Both pentameric structures have a wide water-filled vestibule that leads into the channel pore and an intracellular basket-like structure underneath the channel exit. Courtesy of Douglas Rees, reproduced with permission of *Science* and the author(A), and courtesy of Nigel Unwin (B).

precise coupling mechanism by which the detection of a chemical signal (agonist binding) is translated into the opening of the ion permeation pathway (channel opening) remains to be described in detail. Furthermore, the pharmacologically fundamental functional difference between agonists and antagonists, both of which bind to the same site, remains elusive.

4.3. The Purinoceptor Family P2X

The last class of ligand-gated ion channels to be discussed herein are the P2X receptors gated by extracellular ATP (as opposed to P2Y receptors which belong to the G-protein-coupled “metabotropic” receptors mentioned in the introduction). They are cation-selective channels that open in the millisecond range upon binding of extracellular ATP. To date, seven P2X isoforms, referred to as P2X_{1–7}, have been cloned (reviewed in ref. [95]). The deduced proteins display sequence similarity between themselves (38 to 48% amino-acid identity when pairs are compared), but no homology with any of the other ligand-gated ion channels.^[96] Although no high-resolution structure is available, it is clear that some of their structural features are fundamentally different from the two classes of LGCs discussed above. Most importantly, they have a remarkably different predicted transmembrane arrangement: each subunit—about 400 amino acids in length and with both termini inside the cell—is thought to contain only two membrane-spanning segments (M1 and M2), connected by a large (270 residues) glycosylated extracellular loop. The extracellular domain contains ten cysteine residues, which may form five intrachain disulfide bonds.^[97] These are believed to be important for the overall structure, since they are perfectly conserved in all family members.^[96] The precise structure of the agonist-binding site is still not known. Mutational studies showed that a ATP-binding pocket that contains some positively charged residues forms close to the channel vestibule.^[98, 99]

P2X receptors are nonselectively permeable to cations, including Ca²⁺; the effective pore diameter is somewhat larger than that of the nAChR.^[100] Studies on the accessibility of engineered cysteine residues show that the M2 segment contributes to the ion permeation pathway.^[101] The results imply that at least part of the pore would be in an extended rather than in a helical conformation, but how exactly the pore is organized remains unclear. A conserved glycine residue (Gly 342 of P2X₂) may be part of the gate.^[102]

An intriguing feature observed in some ion channels is their dynamic selectivity filter (discussed in ref. [103]). For several P2X subtypes, a progressive dilation of the pore is observed upon prolonged activation by ATP so that large molecules can permeate.^[104] How this dynamic behavior is brought about and whether and how ionotropic receptors use this mechanism to alter neuronal function remains to be shown. Another interesting aspect of P2X channels is that some of them seem to interact functionally with the structurally unrelated nAChRs, thus establishing a cross-talk between differently gated channels.^[105]

Similarly, the oligomeric organization of P2X receptors seems to deviate from the architecture observed in GluR and AChR. The precise quaternary structure of functionally active P2X receptors remains to be established. Although they share their transmembrane topology with a variety of other ion channels such as the epithelial Na⁺ channels or the inwardly rectifying K⁺ channels, no common oligomeric structure can be deduced.^[106] There are hints that some of these receptors share a similar architecture, with trimers as the common denominator. For the P2X receptors, electrophoresis studies

and cross-linking experiments indicate that they occur as homotrimers, or at least that trimeric complexes of identical subunits constitute an essential element.^[5] As with the other LGC classes, hetero-oligomeric assembly is possible and might underlie functional diversity.^[107]

5. Conclusions and Overview

Thanks to the availability of high-resolution X-ray structures of voltage-gated ion channels and receptor domains, we have learnt a lot about the structural basis of the gating and selectivity in LGCs. Clearly, many questions are still unanswered and can only be answered when the Å-resolution structures of LGCs become available. Recently, the first structure of a G-protein-coupled receptor (rhodopsin) was solved.^[108] This indicates that the techniques for crystallizing membrane proteins have reached a stage where crystallization of LGCs should also become feasible in the near future.

Addendum

After the submission of this manuscript, two important relevant articles were published:^[110] An acetylcholine-binding protein (AChBP) from the mollusc *Lymnaea stagnalis* has been shown to have significant structural homology with the extracellular ligand-binding domain of the neuronal nAChR. Since AChBP is a water-soluble protein, it has been crystallized. The high-resolution (2.7 Å) X-ray structure can be seen as a model of the binding domain of the LGC nAChR (Figure 14).

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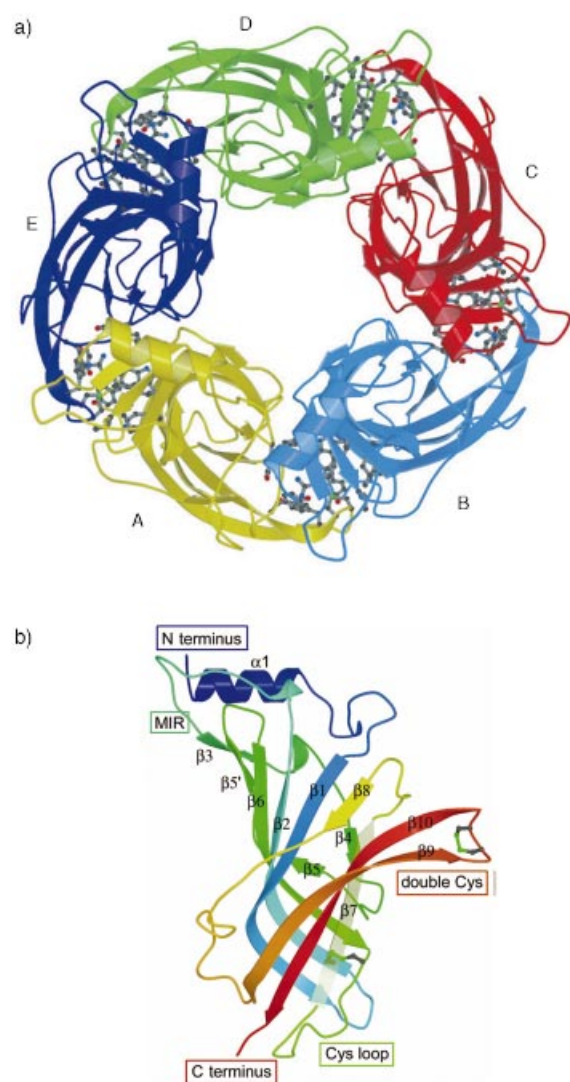
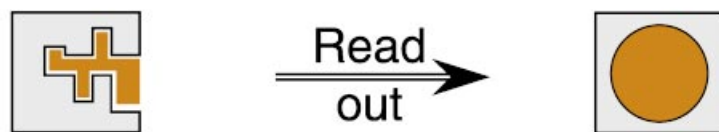


Figure 14. The pentameric structure of AChBP. a) In this representation, each promoter has a different color; subunits are labelled anticlockwise. The ligand-binding sites at the subunit interfaces are shown as ball-and-stick representations. b) Overview of the AChBP promoter structure as viewed from outside the pentameric ring. Rainbow representation colored as a gradient, from blue (N terminus) to red (C terminus); disulfide bridges are shown as green ball-and-stick representations. MIR stands for main immunogenic region. For more details on the Cys loop and double Cys, see text. In a complete LGIC, the N terminus would point towards the synaptic cleft and the C terminus would enter the membrane at the bottom, contributing to the first transmembrane segment. (Modified from ref. [110], with kind permission from the authors.)

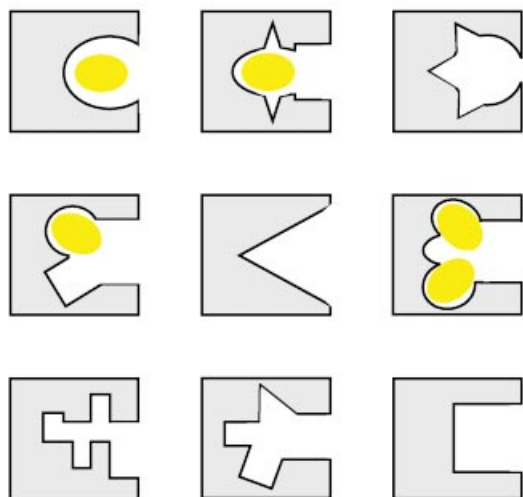
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Lock and Key Sensor

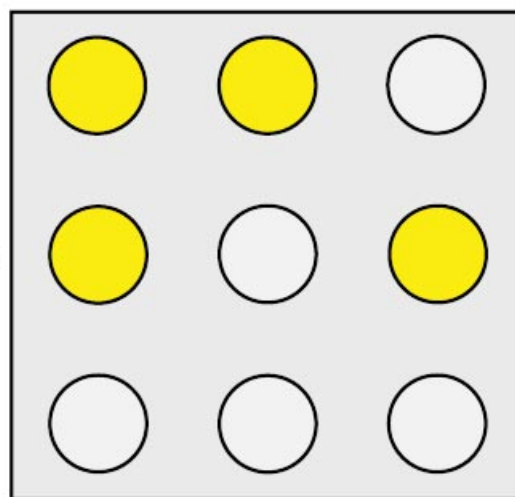


Differential Receptors

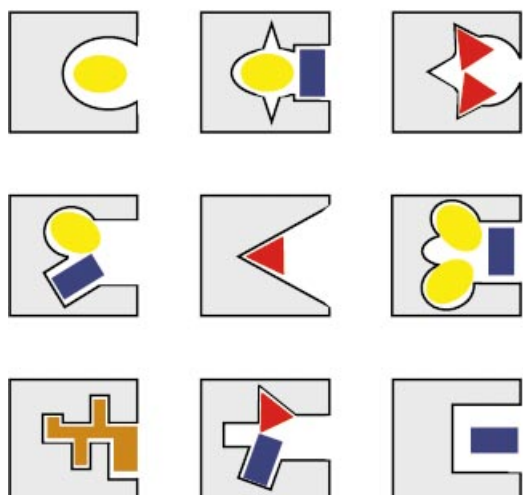


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Single-Analyte Pattern

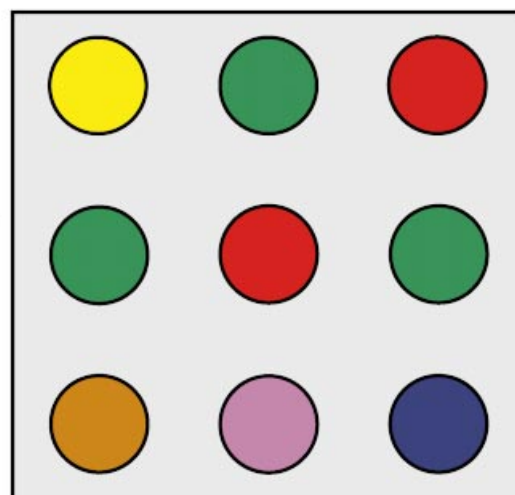


Differential Receptors



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Multi-Analyte Pattern



Sensing A Paradigm Shift in the Field of Molecular Recognition: From Selective to Differential Receptors

John J. Lavigne and Eric V. Anslyn*

Molecular recognition has evolved from a science designed to understand biological systems into a much more diverse area of research. While work continues to elucidate “nature’s tricks” with respect to intermolecular interactions, much attention has turned to the perspective that molecular recognition, by design, can lead to new technologies. Applications ranging from molecular sensing to information storage and even working molecular machines have been envisioned. This review will highlight a few historical hallmarks of molecular recognition oriented at studying the basic science of intermolecular interactions, but then detail recent advances in molecular recognition aimed towards applications in the field of molecular sensing. Rational design can be used to create synthetic receptors with a good deal of

predictability and selectivity, and many signal transduction mechanisms exist for converting these receptors into sensors. This is the first topic discussed. The concept of “differential” or “generalized” sensing is then presented, where one uses an array of sensors that do not necessarily conform to the “lock and key” principle. This approach to sensing is inspired by the mammalian senses of taste and smell, which we briefly describe. To mimic senses of taste and smell, one is naturally led to the use of combinatorial libraries, a direction of research that has seen continued growth over the past few years. We summarize the current state of the art in synthetic combinatorial receptors/sensors, and then predict a future direction that the field of molecular recognition will possibly take. The review is not meant

for the specialist, but instead for a general audience. It does not present a highly detailed analysis of each individual topic: synthetic receptors, sensors, olfaction/gustation, and combinatorial receptors/sensors. Instead, this review shows how all these fields complement each other and fit together to create sensing devices. Our conclusion is that specific analyte sensing, differential sensing, and combinatorial chemistry can and will be combined to create sensor arrays, and give the sub-field of molecular recognition that uses synthetic systems a bright future in this type of sensing scenario.

Keywords: analytical methods • combinatorial chemistry • molecular recognition • receptors • sensors

1. Introduction

The field of molecular recognition has reached a stage where one can confidently design and synthesize a receptor with a good degree of predictability and selectivity for many kinds of small to medium-sized molecules. The quest to obtain this goal has been primarily driven by a desire to understand and mimic nature’s exquisitely specific binding interactions that are exemplified by the “lock and key” analogy (Figure 1A).^[1] Yet, there is an aspect to nature’s methods of molecular recognition that has largely been unexplored by synthetic chemists, and only recently has begun to be

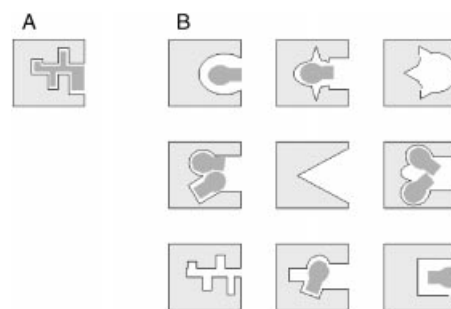


Figure 1. Schematic representation of host–guest interactions. A) A specific binding event (the lock and key paradigm), which exhibits a large degree of complementarity between the host and guest. B) An array of generalized receptors interacting with one analyte. The identification results from the pattern of responses obtained from the entire array.

examined, that of “differential” binding. By differential, rather than specific or selective, we refer to receptors that have different binding characteristics, none of which are

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necessarily specific or even very selective. These receptors are “generalized” rather than “specialized”. This approach requires that an array of sensors be created (Figure 1 B) and the composite signal evaluated and interpreted by pattern-recognition protocols. This is the binding scenario used in the mammalian senses of taste and smell. In the following sections, selective synthetic receptors will be briefly reviewed, with an emphasis on representative successes, and then contrasted to this new area of differential receptors. Applications of synthetic chemistry to the creation of differential receptors are discussed, especially focusing upon combinatorial chemistry. Correlations between the specific and differential approaches to sensors are made, which leads us to conclude that there is a very bright future for the field of molecular recognition in arrayed sensors.

2. Lock and Key Inspired Synthetic Receptors

Hallmarks of specific binding from nature are enzyme–substrate, antigen–antibody, and complementary DNA annealing. Historical hallmarks for synthetic receptors include cryptand–ion,^[2] cyclophane–aromatic,^[3] boronic acid–sugar,^[4] and guanidinium–carboxylate interactions,^[5] while more recent molecular recognition studies target much broader chemical structures.^[6] Scheme 1 shows a few representative synthetic hosts that have been studied to elucidate their selectivity for various target guests over other similar guests. In general, the design of such receptors involved an identification of the proper recognition entities to be incorporated into the receptor in order to bind each particular epitope on the guest. Following this, identification of a proper spacer to pre-organize the recognition entities to complement the display of epitopes on the guest provides the final host design. Molecular-recognition forces including solvophobic effects, hydrogen bonding, and ion pairing can all be exploited with such synthetic systems. Significant rational design and computer modeling, as well as trial and error testing, take place to optimize the ability of the receptor to recognize the

guest. Through this process it is becoming more and more routine to produce synthetic receptors with good selectivity for many classes of small and medium-sized guests, especially those which possess a reasonable number of sites for binding interactions.

2.1. Combinatorial Approach to Receptors

Another approach for targeting small and medium-sized molecules is the creation of a combinatorial library of receptors. This process allows for the creation of several different compounds through the combination of rapid parallel and combinatorial syntheses.^[11] The subsequent screening of the new materials can provide compounds for many applications. Typically, combinatorial chemistry has been used to develop the substrate rather than the host, and is best exemplified with respect to drug discovery, where the final product is often an inhibitor of a biological pathway.

This is not to say that combinatorial approaches have not been used to create effective host compounds. These have usually been formed from oligomeric structures, such as peptides^[12] and nucleotides (aptamers).^[13] Recently unnatural biopolymers formed from linking groups such as ureas,^[14] peptoids,^[15] and guanidiniums^[16] have been created from which useful receptors may be expected quite soon. Encoding schemes can be employed to decipher the structure of a successful receptor derived from an oligomer.^[17]

For example, Still and co-workers have taken this approach to create a library of peptidosteroidal receptors (Scheme 2 A).^[18] The driving force for this work was to mimic nature's ability to bind large oligomeric substrates with high selectivity (for example, antibodies). The library consisted of a conserved steroidal core derivatized with two different tripeptide arms on rings A and B (aa1-aa2-aa3, aa4-aa5-aa6) to generate approximately 10⁴ different compounds. The resin-bound library was screened against a series of labeled

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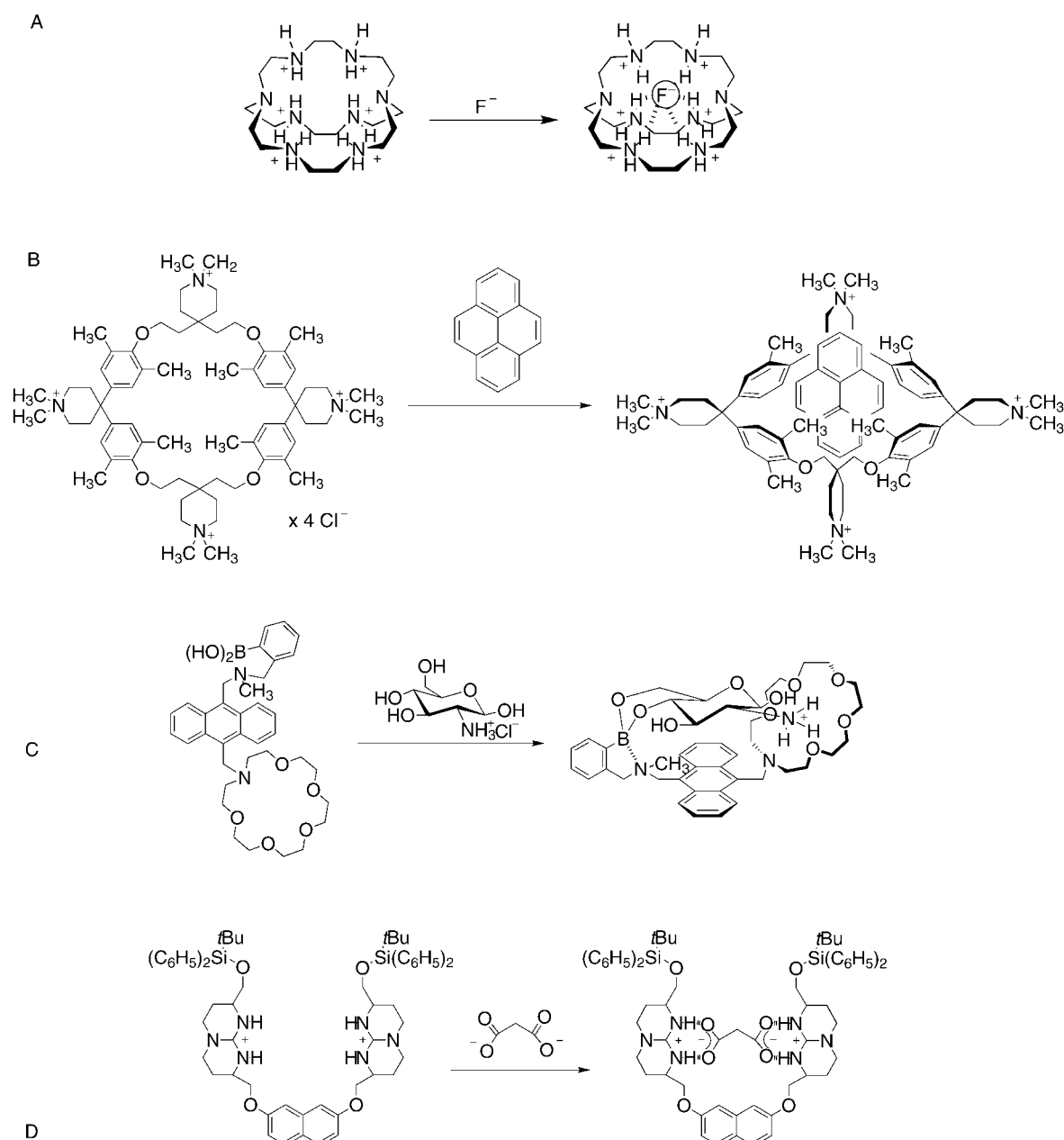
John J. Lavigne was born in 1971. He received his BS from St. Lawrence University in 1993 and his Masters of Education from St. Lawrence University in 1995. He received his Ph.D. from the University of Texas at Austin in 2000 for developing single-analyte and multi-analyte sensing systems under the direction of Dr. Eric Anslyn. Dr. Lavigne is currently a Senior Scientist at Labnetics, an array sensor firm.



E. V. Anslyn



J. J. Lavigne



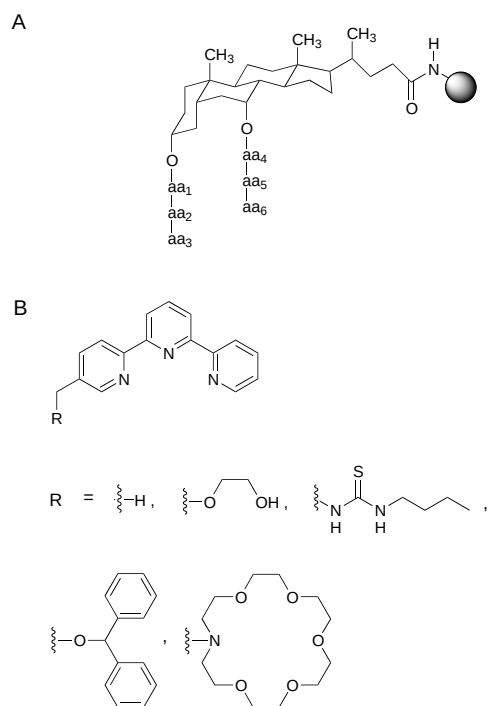
Scheme 1. Representative hallmarks in molecular recognition: A) cryptand–anion recognition,^[7] B) cyclophane–aromatic complexation,^[8] C) interaction of boronic acid with a polyol,^[9] and D) guanidinium–carboxylate interactions.^[10]

pentapeptides. Differentiation of oligomers varying by only one amino acid was accomplished, thus demonstrating the feasibility of the approach. Similarly, Hamilton and co-workers have taken a more targeted approach to create a small molecule library (15 compounds). The compounds were again based on an antibody structure and incorporated a constant template region (a terpyridine) with a variable binding region (Scheme 2B).^[19] The recognition capabilities of the compounds followed expected trends, with cationic binding groups recognizing anions and so on.

A unique approach results in polymeric receptors generated from combinatorial molecular imprinting techniques.^[20] A semi-automatic system has been developed to combinatorially generate libraries of molecular imprinted polymers (MIP)

and screen these compounds for selectivity and affinity towards the original templating species. Receptors for triazine herbicides were prepared by using varying ratios of two functional monomers (methacrylic acid and 2-(trifluoromethyl)acrylic acid). The results suggest that different herbicides prefer different monomer ratios.

One of the most recent advances in the field of combinatorial chemistry is the use of dynamic combinatorial libraries for the creation of receptors.^[21] Herein, a series of monomers are used to create libraries of aggregated structures that are in equilibrium with one another, each of which could act as a host. Introduction of a guest shifts the equilibrium to the host that binds that guest. Covalent capture of the assembled aggregate leads to receptors complementary to the added



Scheme 2. Molecular receptors derived from combinatorial libraries: A) the generic structure for a receptor based on a peptidosteroidal core which were found to be able to differentiate between single amino acid differences in pentapeptides, B) the terpyridine backbone (constant region) and the variable sidearms attached to create a library of dimeric compounds.

guest.^[22] This very ingenious method for the creation of receptors is definitely applicable to the array sensor technologies discussed in Section 2.2.

Recently, combinatorial chemistry has been exploited for the creation of libraries of compounds intended to act as new catalysts.^[23] The size of the library can be readily increased by simply varying process and reaction conditions, such as solvent, temperature, and additives, which are held constant in traditional assays to eliminate assay variability. Materials exhibiting vastly different characteristics have also been generated with combinatorial approaches.^[24] Certainly the use of chemical libraries for creating receptors from synthetic compounds will be seeing an explosion of growth in the near future, and as we explore herein, this method is ideal for the creation of differential or generalized receptors.

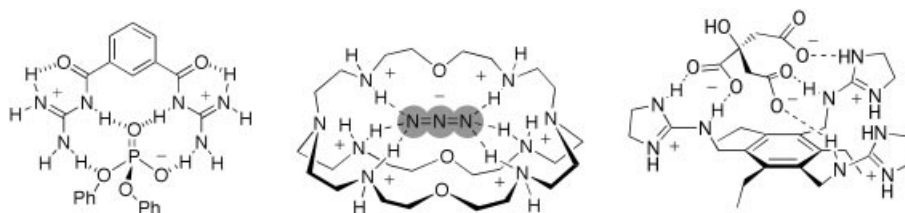
2.2. Single-Analyte Chemosensors

Although much of the driving force for the development of the synthetic receptors discussed above derives from a desire to mimic and understand natural systems, these receptors can also be used for practical applications. Sensing is one area where the synthetic systems have found great utility and promise. For example, it is more practical to sense small

cations such as sodium,^[25] potassium,^[25] calcium,^[26] mercury,^[27] and lead^[28] using a synthetic system (a chemosensor) rather than an antibody or aptamer. Similarly, receptors for small anions, such as phosphate^[29] and phosphodiester,^[30] azide,^[31] and even medium-sized anions, such as citrate,^[32] are readily developed using a synthetic system rather than a large biopolymer (Scheme 3). In addition, synthetic receptors can be successfully used in devices, such as chemically modified field-effect transistors.^[33]

One of the first analytes to be measured using a synthetic sensor was probably the hydronium ion. Derivatization of these pH reporters with chelating arms analogous to those found in ethylenediaminetetraacetic acid (EDTA) leads to metal ion sensors commonly referred to as the complexones.^[34] These derivatives display a response to pH much like their precursors, however, there is a change in the pK_a value of the indicator in the presence of certain ions such that the color change occurs at a different pH value (Scheme 4A). Crown ethers have undergone a similar transformation. Sensors have been developed from these rather simple structures by covalently attaching a chromophore or fluorophore into or onto the ring skeleton (Scheme 4B).^[35]

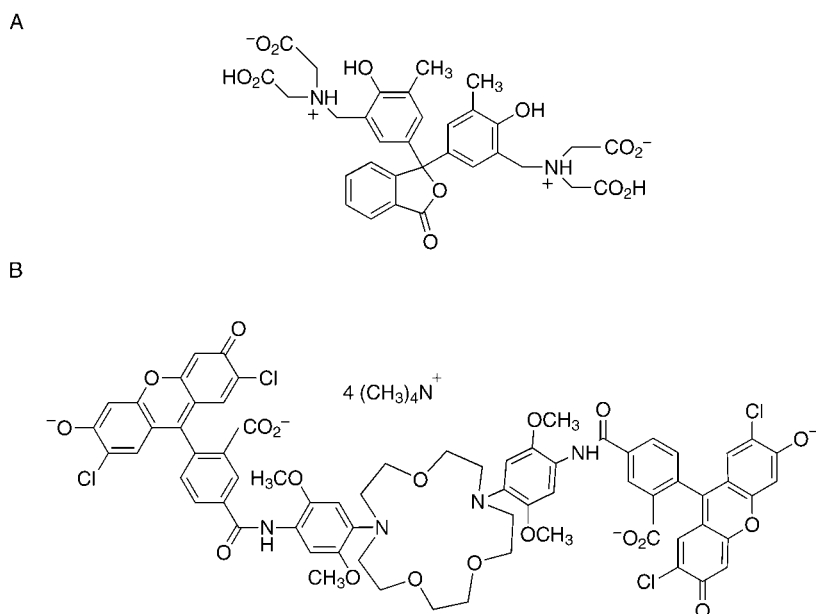
Several more intricate molecular sensors have been developed recently, again with a focus upon selective binding coupled with signal modulation. Most of these sensors have a chromophore or fluorophore covalently attached to the recognition unit. The microenvironment of the sensor is perturbed sufficiently upon binding of analyte to modify the optical signal. Fluorescence resonance energy transfer



Scheme 3. Receptors for small and medium-sized anions.

(FRET) and photoinduced electron transfer (PET) are common mechanisms for transduction. Examples where these processes are utilized include indicators for the recognition of organic cations, anions, and even neutral molecules.^[38] An example illustrating this chemistry is a chemosensor for pyrophosphate (Scheme 5A).^[39] This host displays a thousandfold selectivity for pyrophosphate over phosphate ions. The signaling event is PET based^[38] and is facilitated by a change in the protonation state of the host upon binding the analyte. The PET effect has also been used to signal the presence of sugars. In particular, a bisboronic acid host that exhibits selectivity for glucose has been reported (Scheme 5B).^[40] Metal-containing receptors have also been efficiently used for optical and electrochemical sensing techniques.^[41] All these approaches involve the attachment of the signaling moiety to the recognition unit.

Competition assays using synthetic receptors are also currently being exploited. This methodology takes advantage



Scheme 4. Complexone and crown-type indicators: A) *o*-cresolphthalein complexone,^[36] an EDTA-type cation chelator that incorporates a colorimetric transducer, and B) “sodium green”,^[37] a commercial indicator which can be used to signal the presence of and quantify sodium ions in vivo.

of an optical modulation of a dye molecule being displaced from the binding cavity of a host by an analyte having a higher binding affinity. In this technique, the indicator is used to identify an analyte that it was not originally designed to signal.^[42] The advantage of these approaches is that less covalent bond architecture is required. The disadvantages are

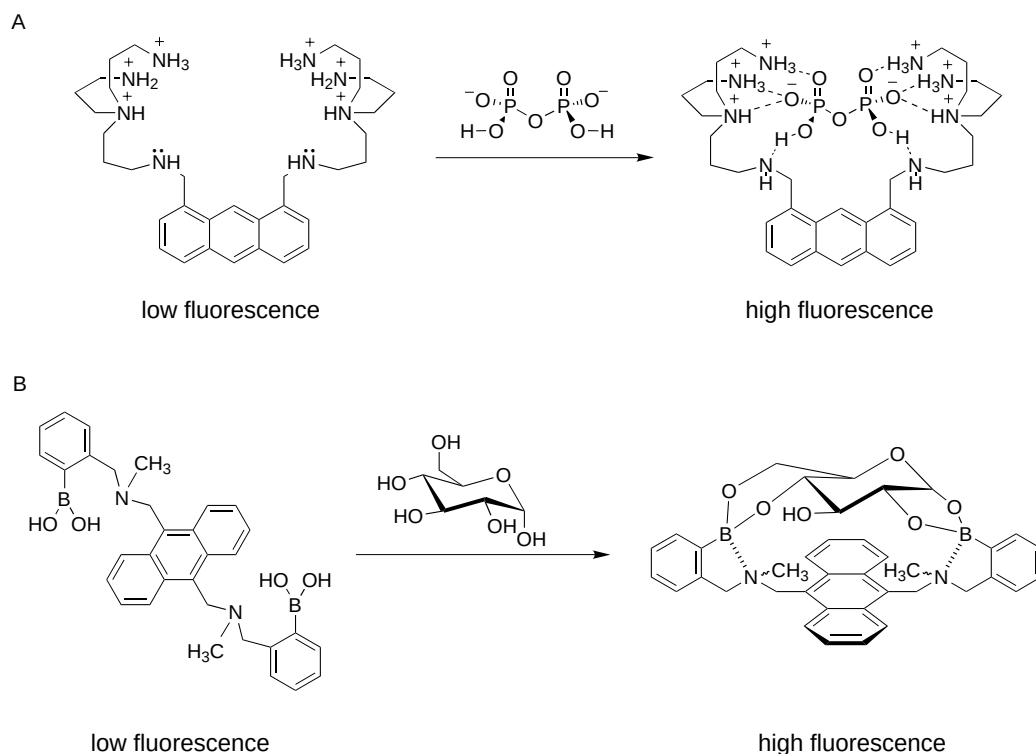
the inability to use the system for applications that require full imaging of a surface or volume, such as whole cell imaging,^[43] and they are single-use systems.

The sensing of hydrophobic analytes using cyclodextrins (CDs) and naphthalene sulfonates is a common example of a competition assay.^[44] The naphthalene sulfonate unit binds within the CD cavity, but is displaced from the binding pocket upon exposure of this ensemble to a hydrophobic guest. The naphthalene sulfonate moiety exhibits solvatochromism by displaying a modulation of its fluorescence intensity in bulk water relative to that in the hydrophobic cavity of the CD.

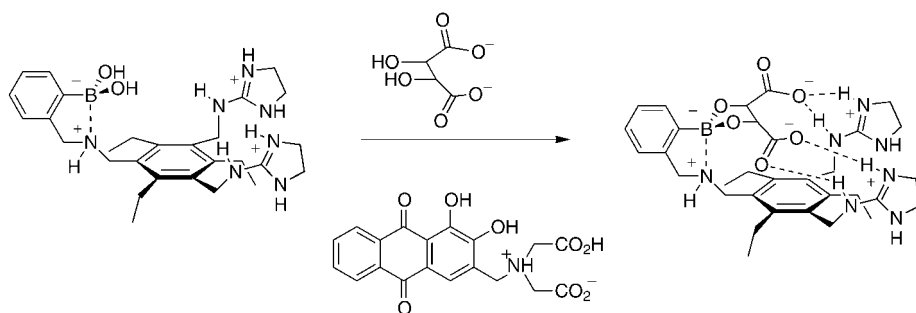
We have shown that even more elaborate receptors can be coupled with a signaling molecule to create a sensing ensemble. For example, tartrate can be readily quantified in common beverages by using a synthetic receptor with selectivity for tartrate, together with alizarin complexone as the signaling molecule (Scheme 6).^[45] This competition technique has been used by other research groups and expanded by us by using common indicators

to encompass chemosensory systems for acetyl choline,^[46] nitrate,^[47] citrate^[48] and inositol trisphosphate (IP₃).^[49]

In summary, it is clear that the “lock and key” inspiration can lead to the creation of synthetic receptors, by design or combinatorial techniques, for simple complexation or sensing applications. Molecular recognition is now sophisticated



Scheme 5. Molecular recognition of pyrophosphate and glucose: A) a 1,8-polyamino-disubstituted anthranil host displays a 2000-fold selectivity for pyrophosphate over phosphate ions, B) glucose is bound by a 9-10-bisboronic acid anthranil host with 6–1000-fold selectivity over other monosaccharides.



Scheme 6. A synthetic host for tartrate. The recognition takes advantage of the guanidinium–carboxylate and boronic acid–diol interactions. A competition between tartrate and alizarin complexone to bind to the host results in a colorimetric assay for tartrate.

enough that selectivity in the recognition process is a common outcome. However, lock and key type selectivity is not the only method used by nature for the development of receptors and sensors. The second method is that used in the senses of taste and smell, where a series of generalized receptors is used. In keeping with the spirit that one goal of the field of molecular recognition is to mimic nature's "tricks", many chemists are beginning to turn their attention in this direction.

3. Differential Receptors: Multi-Analyte Sensing

Interestingly, the sensors used in arrays may be designed to exhibit highly selective association or they may be random, and lack any targeted design. One of the strengths of multicomponent arrays is the fingerprints or pattern generated from the response of all of the sensor elements, which may display specific or nonspecific binding of an analyte. The interpretation of this pattern allows for the analysis of complex mixtures. To this end much work has been done to mimic the senses of smell and taste, thereby leading to vapor-phase (noses) and solution-phase (tongues) sensors.

Of the five primary mammalian senses, namely, vision, hearing, touch, smell, and taste, gustation and olfaction have been designated as the "lower senses" because of the minimal influence they exhibit on behavior relative to auditory, visual, and other sensory stimuli.^[50] However, these senses form the basis of our response to chemical stimuli through chemoreception, and thus are quite amenable to chemists for mimicry.

3.1. Olfaction

The mechanism by which smell functions is still somewhat debated, however, a dominating theme does exist. As odors enter the nose through the nostrils, they reach the mucus of the olfactory mucosa. These odorous molecules enter the mucus according to the partition coefficient of their molecular type. Once dissolved in the mucus, the analytes encounter the olfactory cells, which are nerve cells. It is generally assumed that the primary process of chemoreception takes place at the cell membrane of these sensory neurons. This process involves physical contact between the stimulant and potential or actual

receptor sites which could either be specialists, which react with only one structural class, or generalists, which would react with a multitude of structural classes.^[51] The odor quality of a compound is defined intrinsically by the chemical structure, its solubility, and its diffusion rate. The simultaneous analysis of the multiple components, by potentially thousands of different sensory neurons, leads to a pattern for the odor that is stored in the brain.

Though the understanding of the sense of smell is still somewhat uncertain, it is understood well enough to allow chemists to mimic it using a variety of different transduction schemes.^[52] This area has recently been very thoroughly reviewed by Lewis, Walt, and co-workers.^[53] The methods described include functional sensors based on surface acoustic wave (SAW) crystals,^[54] quartz-crystal microbalances (QCM),^[55] tin oxide sensors,^[56] and carbon black composites.^[57] One example that takes advantage of nonspecific recognition has been called the "electronic nose" and is commercially available as AromaScan.^[58] The technology is designed to identify volatile chemicals based on the adsorption of the odors onto an array of semiconducting organic polymers (Figure 2), much like the

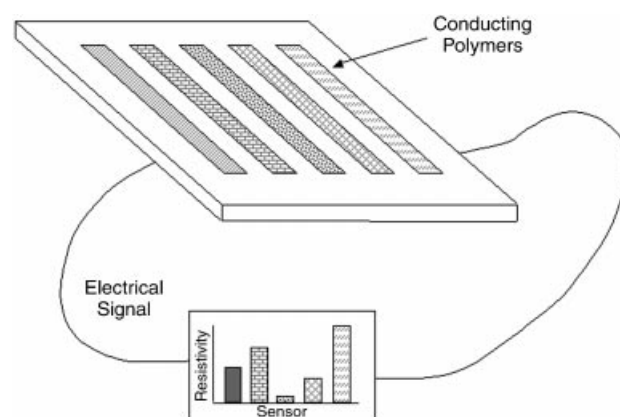


Figure 2. Schematic representation of an electronic nose based on semiconducting organic polymers. The resistance of each polymer is effected by absorption of volatiles into the polymer matrix.

partitioning into olfactory mucosa. The adsorption is dynamic, reversible, and is a function of the size of the voids within the individual polymer sensors. It takes only a few seconds for the system to reach an equilibrium, at which time the changes in electrical resistance across each polymer are measured and a fingerprint pattern created for the particular aroma. This pattern can be stored in a computer and used to identify and quantify further exposures of the array to complex mixtures.

An advantage of this system is that very little chemical design need go into the creation of the conducting polymers. For example, there are no designed receptors appended to the polymers—natural or otherwise—and there are no individual specific binding sites designed into the polymers. The lock and

key principle has little if any influence on the choice of the conducting polymers. The method simply relies on the natural voids within the individual polymers.

The important point is that the polymers are differential, and respond differently to the various analytes in the vapor. Each polymer responds to a multitude of different vapors, similar to the generalized olfactory receptors in the mammalian olfactory mucosa. The identification of the vapor by the electronic nose comes from a composite pattern of the change in resistivity of all the conducting polymers responding simultaneously.

3.2. Gustation

Taste, as part of the chemical senses, is used for food selection and evaluation, including avoidance of potentially harmful substances such as bitter tasting poisons. The surface of the tongue is the major focus of taste transduction in mammals. Clusters of taste buds are located in various regions of the tongue. Taste buds contain taste cells and are located in small depressions in the tongue called taste pores. Some foods are acted upon by enzymes in the saliva which results in components that may be recognized at the cell surface. The taste pores are thought to prevent the diffusion of taste stimuli into the extracellular fluids and restrict the site of interaction to the taste cells.

Similar to the sense of smell, the mechanism of taste is still not clear. Cell surface receptors for particular taste stimuli have not yet been isolated. Traditionally, taste has been divided into four fundamental classes: sweet, salty, sour, and bitter, with each taste bud responding to only one class. Recent findings, however, suggest that most nerve fibers respond to more than one taste stimulus, with only a few responding to stimuli representing one basic taste. In fact most of the nerve fibers react to stimuli from two or three modalities.^[59] This observation has led to the development of additional taste classifications. The Japanese, for example, distinguish a fifth taste, umami (savory or delicious taste), which is elicited by monosodium glutamate (MSG), disodium inosinate, and disodium guanylate.^[59] Further, there is electrophysiological, biochemical, and behavioral evidence for the presence of at least two different sweet receptors. Therefore, in actuality, taste cells respond to a myriad of chemical stimuli including those that lead to the perception of classical taste qualities. It appears from the available data that these taste cells use several signal transduction mechanisms, even within a particular taste class.^[59] Importantly, although there are specific taste sensations, they are elicited from a variety of different tastants. Hence, the taste receptors themselves are not specific, but instead are generalized and differential, similar to the sensors used with smell.

Just as with the sense of smell, the development of human-made arrays to mimic the sense of taste has drawn much attention of late. These approaches include acoustic-wave devices,^[60] arrays based on polyaniline,^[61] polymerized crystalline colloidal arrays (PCCA),^[62] and microelectrodes.^[63] Work by Toko et al. focuses on an array composed of lipid membranes that can detect tastes in a manner similar to the

human gustation sensation (Figure 3).^[64] The output of the sensor is not the amount of taste substances present, but the taste quality and magnitude. The output patterns obtained for different classes of tastants are different, while those obtained

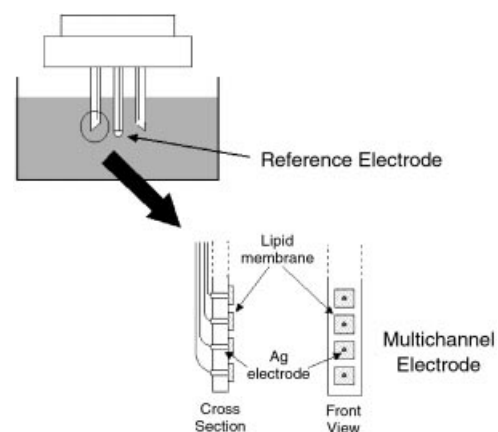


Figure 3. Schematic representation of a taste sensor. This sensor array is based on a multichannel electrode where each individual electrode contact is covered by a lipid membrane. The membranes are designed to function much like the cell surfaces of taste cells.

from analytes within the same class are similar, for example, sodium chloride, potassium chloride, potassium bromide give similar patterns for saltiness. The array was not based on the concept of targeting selective binding to detect specific chemical substances, nor was the design to measure the amount of each chemical substance present. Rather it was designed to measure the taste itself and express it quantitatively.

Though this method has had great success in mimicking the human sense of taste, it seems beneficial to be able to quantitate the amount of analyte in a liquid sample and to be able to assay for specific chemical species, which one may not what to taste, such as toxins, poisons, and bacteria.

In one example the system functions both in the gaseous^[65] and liquid phases.^[66] This work uses specific indicators either covalently linked to or trapped within a permeable polymer matrix on the distal end of a fiber-optic bundle (Figure 4A). The sensor is fabricated by selective photodeposition^[67] of the

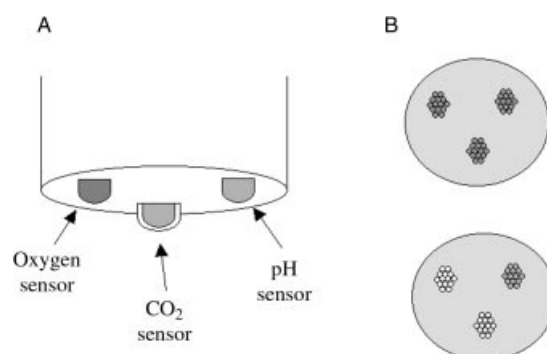


Figure 4. Schematic representation of a fiber-optic-based multi-analyte sensor array. A) This array utilizes indicators incorporated into or trapped within a polymer on the distal end of fiber-optic bundle to assay various analytes in solution. B) The image obtained from fluorescent indicators at the proximal end of the fiber creates the “fingerprint”.

analyte-sensitive polymer matrices. By selectively illuminating only small regions of the bundle at one time, sensors with different specificity may all be placed on one bundle. The signal observed is that transmitted up the fiber bundle from a fluorescent sensing element upon responding to the analyte (Figure 4B). The response is fast, efficient, reversible, and the pattern created may be deconvoluted using standard multi-variant algorithms.^[68] In the early version of this system the receptors used were specific for particular analytes, yet this design is clearly amenable to the utilization of generalized receptors.

An alternate approach is based on an array consisting of fifteen phenolic homopolymers and copolymers.^[69] These sensors are generated using a peroxidase-catalyzed oxidative polymerization of five phenolic monomers. The sensory mechanism is based on the change in the intrinsic fluorescence of the polyphenol upon the addition of metal ions. The array was used to assay for iron, copper, cobalt, and nickel ions as well as various mixtures. The array produced distinct fingerprints for the metal ions that were analyzed. Furthermore, the response from the copolymers varied greatly from that of the homopolymers, which suggests that extreme diversity could be obtained from a limited number of monomers combined in various ratios.

The current method under investigation at the University of Texas attempts to mimic the mammalian sense of taste^[70] by placing “synthetic taste buds” in the recesses of a micro-machined platform (Figure 5). In nature, the taste buds are

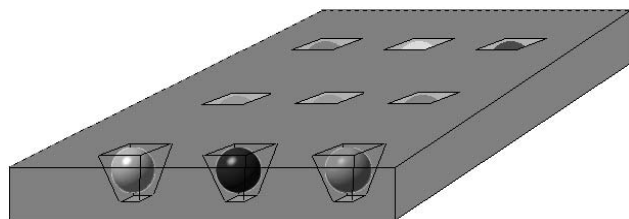


Figure 5. Multicomponent sensor array on a silicon chip. Individual “taste buds” (polyethylene glycol/polystyrene resin beads) are immobilized within cavities on a micromachined silicon “tongue”.

located at the bottom of small depressions in the tongue. Analytes are trapped in these recesses and react with receptors on the taste buds. These synthetic taste buds are polyethylene glycol/polystyrene block copolymer resin beads with the desired sensors covalently attached. Simultaneous exposure of the various taste buds to a solution containing multiple analytes causes changes in the optical spectroscopy of the various beads. This change can be fluorescent or colorimetric. Data streams are collected using a three-chip CCD camera, which creates red, green, and blue (RGB) patterns that are distinct and reproducible for their environments.^[71] Analysis of this data can identify and potentially quantify the analytes present. This method allows for quick and accurate evaluation.

The advantage that all these approaches provide to solution analysis is that complex mixtures of analytes may be identified and quantified from the mathematical interpretation of the detailed pattern, or fingerprint, created by an array of

differentially modified sites. Specific receptors and sensors, such as those discussed in Section 2.2, can be readily used, but adding to the strength of this system is the fact that the sensors do not necessarily need to be specific for the targeted analyte(s). There can be significant “cross-talk” or nonspecific interactions, which actually adds to the success of this method.^[53] Synthetic receptors are naturally not as selective as antibodies and aptamers, and therefore compliment this sensing methodology very well. Most importantly, the receptors do not need to be completely rationally designed to target specific analytes. They simply need to be different, with different.

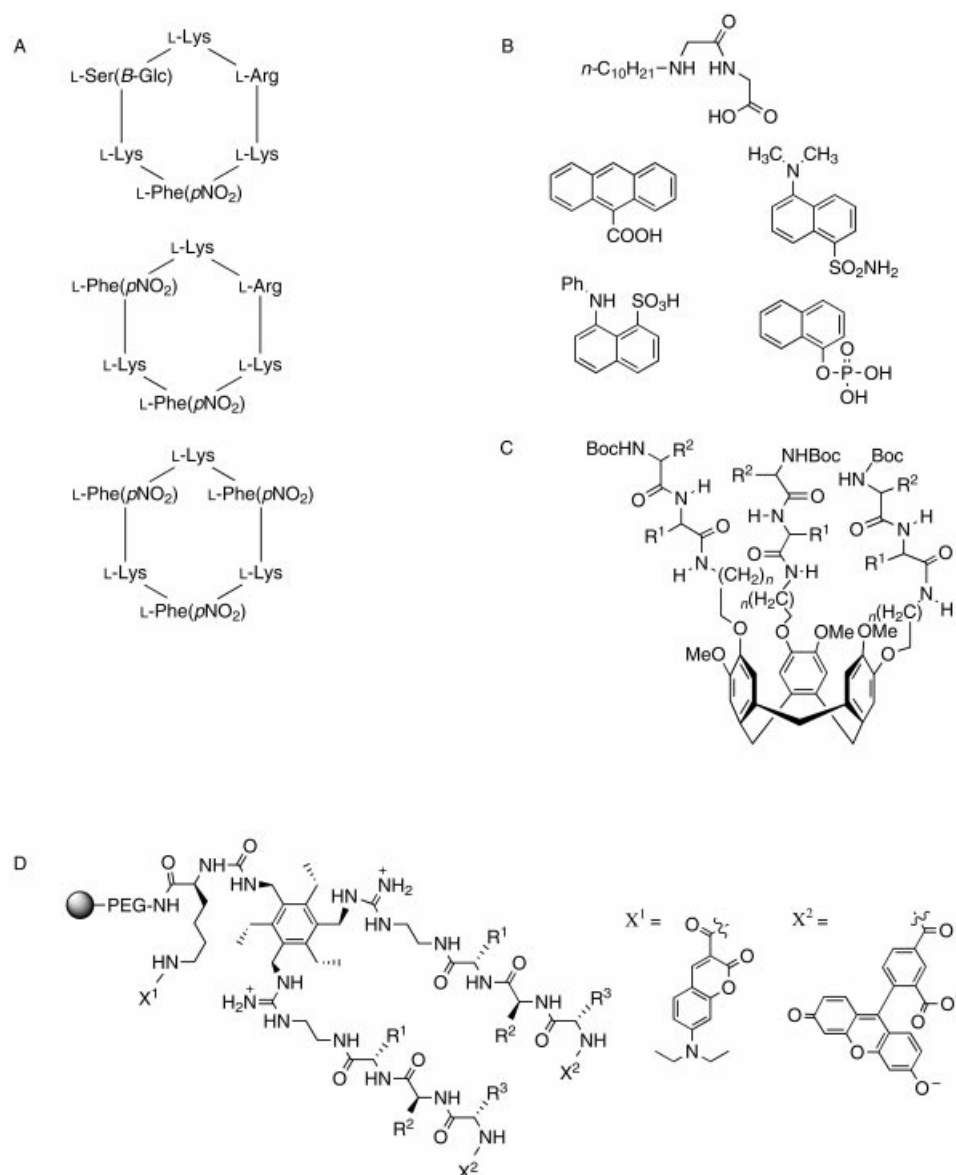
3.3. Combinatorial Approach to Chemosensors

As described in Section 2.1, combinatorial chemistry can create hundreds to thousands of different receptors quickly and efficiently. Hence, it is ideal for the creation of differential receptors. By using the split and pool method^[72] each bead has a single receptor type immobilized, yet each bead is different. Thus, incorporating the use of combinatorial chemistry in multi-analyte sensing, where differential/generalized receptors are useful, is a natural direction for the evolution of the sensing field and the practice of molecular recognition.

One step that must be proven before the use of libraries in arrays is explored is that combinatorial chemistry can lead to receptors with differential responses. Hence, before being incorporated into multicomponent arrays, sensors have been created by a combinatorial approach for the purpose of finding the best sensor for a single analyte, thus demonstrating that receptors with some natural preference for one analyte over another can be created.

Libraries of cyclic hexapeptides have been formed from which selective receptors for amino acids have been isolated.^[73] From this library of just under 6000 compounds, three cyclohexapeptides were identified (Scheme 7A) for their selectivity in binding L-arginine over L-lysine in neutral unbuffered aqueous solution. The interaction between the cyclopeptide and the analyte was examined with reflectometric interference spectroscopy (RIS), thus leading to a sensing scenario. Interestingly, when the compounds were tested in buffered media (phosphate-buffered saline, PBS), the selectivity changed such that L-glutamine was most tightly bound, with all three sensors being able to distinguish between L-glutamine and L-asparagine. This result indicates that further selectivities for other amino acids may be investigated by varying the cyclopeptides and the constitution of the buffer solution.

A similar approach has been taken to develop a chemosensor selective for copper(II) ions.^[74] The methodology involved creating a system in which a receptor and an indicator would spontaneously assemble without the need for any covalent link between them. To realize this, a known copper(II) sensor and various fluorescent dyes (Scheme 7B) were derivatized with long lipophilic tails. The components self-assemble into micelles in aqueous media. Optical modulation of the dye occurs upon complexation of the metal and results in sub-micromolar detection of copper(II) ions.



Scheme 7. A) The amino acid sequences for three cyclohexapeptides used as chemoreceptors showing selectivity for specific amino acids. B) The recognition unit and fluorophores used in a library to determine a self-assembling sensing ensemble for copper(II) ions. C) The general structure of a library based on a cyclo-trivertatrylene scaffold. D) The general structure of a library from which a sensor selective for ATP was obtained. Boc = *tert*-butoxycarbonyl.

van Wageningen and Liskamp have also used this targeted approach to create a 40-member library based on substituted cyclotriveratrylene (CVT).^[75] The desired receptors were obtained by *o*-alkylation of the CTV triol followed by coupling one or two amino acids to these arms (Scheme 7C). Initial studies showed that library members derivatized with dansyl differentially bound N-acylated dipeptides.

An example where combinatorial synthesis has been employed to create diversity in a multicomponent array comes from Walt and co-workers. They demonstrated that polymerization reactions between different combinations of two starting monomers can lead to the creation of new sensors with differing recognition capabilities.^[76] A solvatochromic dye (Nile Red) was entrapped within the polymer matrix to perform vapor-phase sensing. Upon exposure of the sensor to

different organic vapors, a fluorescence modulation was observed that varied with the monomer ratio.

A final example utilizes the rational design of a core structure that targets a particular class of compounds, combined with combinatorial chemistry to provide specificity for certain members. To this end, a core containing cationic guanidinium ions was derivatized with two identical tripeptide arms to recognize organic anions (Scheme 7D).^[77] Multiple sites were available for indicator attachment, such as the ends of the tripeptide arms as well as the side chain of a lysine moiety used in the linkage of the chemosensor to a resin bead. Attempts to monitor fluorescence resonance energy transfer (FRET) between fluorophores at these two sites proved unfruitful. However, attachment of a fluorophore to the end of the tripeptide arms did provide a signal transduction scheme. From a library of just under 5000 possible members, a sensor for adenosine triphosphate (ATP) was derived which showed selectivity for ATP over GTP and AMP.

While this library was screened with the intent of finding an ATP sensor, the creation of a library on resin beads creates the opportunity to create an array and look at

the specific and nonspecific recognition capabilities of several compounds at one time, thereby creating a multicomponent sensor array. The ability to create an array is definitely applicable to the libraries mentioned above for peptide and metal-binding applications, but also a myriad of other classes of analytes. Essentially, all analyte classes are approachable using this technique.

Given these examples of selective sensors derived from combinatorial methods, it is clear that the synthetic libraries themselves inherently possess all the requirements for arrayed sensors. Differential responses from the varying members of the library are present, and significant cross-reactivity is expected. Hence, merging the combinatorial chemistry approach with sensor array technology can be expected to continue for quite some time.

4. Is Design Dead?

Given the concept of arrays of differential receptors, one may ask "Is rational design dead?" within the sensing arena. Our answer is "No". In many settings it will still be necessary to have highly selective host compounds, which can take the form of antibodies, enzymes, aptamers, imprinted polymers, or designed organic receptors. These receptors can target strategically important analytes, whereas the differential receptors can be used to analyze for the rest of the "flotsam and jetsam" of the solution. Furthermore, high affinity is commonly directly related to specificity, and therefore those analytes whose concentrations are exceedingly low are in some cases more easily analyzed for using a highly selective receptor, such as an aptamer or antibody. However, even weak affinity receptors can gain very high avidity as a result of their extremely high effective concentrations when immobilized in matrices such as beads.

Moreover, rational design will facilitate the creation of synthetic combinatorial libraries of functionally differential receptors. The utilization of monomers in the libraries that possess the proper functional groups for binding the analytes of interest is clearly advantageous. One needs to narrow the diversity space to include structures that have a high propensity to lead to the binding interactions desired for a class of analytes. The use of amine and guanidine groups in the combinatorial receptors is the logical choice when targeting multiple carboxylic acid analytes, for example. Similarly, the use of libraries that include boronic acids would be predicted to be successful when analyzing solutions of multiple carbohydrates. Moreover, the creation of cavities is likely to be assisted by monomers that enforce turns and loops. Therefore, incorporation of building blocks with known affinities for certain types of functional groups and that can be used as structural elements, can lead to the creation of differential receptors that target a particular analyte class.^[77]

5. Summary and Outlook

In summary, we predict there will be one clear direction in which the field of molecular recognition will be moving in the coming years. It is becoming apparent that the use of large numbers of differential receptors, coupled with pattern-recognition protocols, can and will be used to analyze for single analytes in mixtures of analytes, as well as in the simultaneous analysis of multiple analytes in a mixture. It will not always be necessary to produce highly selective receptors for each and every individual analyte, but instead one can rely on the pattern of responses derived from several differential receptors to identify and quantitate the analytes. Importantly, the guiding principles of molecular recognition will still be at the heart of the process for creating libraries for the various classes of targets. Therefore, the production of both selective receptors and differential receptors for the large number and variety of sensing applications that have societal benefits presents a clear and compelling case for a continued and bright future for the field of molecular recognition in the arena of molecular sensing.

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Catalysis of the Asymmetric Desymmetrization of Cyclic Anhydrides by Nucleophilic Ring-Opening with Alcohols**

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The asymmetric desymmetrization (ADS) of *meso* compounds by enzymatic^[1] and nonenzymatic^[2] methods has proven to be a versatile and powerful strategy in asymmetric synthesis. ADS of *meso* compounds is particularly valuable because it allows many stereocenters to be established in a single symmetry breaking transformation. "Late-stage" ADS of topologically complex *meso* intermediates prepared by using simultaneous two-directional synthesis illustrates the power of the approach in target-oriented synthesis.^[3] However, although there is aesthetic appeal in simultaneous multiple stereocenter genesis, it is only useful for targets possessing a high degree of latent or real symmetry. In contrast, ADS of relatively simple *meso* compounds can provide highly expedient access to a wide variety of usefully functionalized chiral building blocks for the asymmetric synthesis of innumerable targets.^[4] A good example is the ADS of *meso* cyclic anhydrides by selective ring-opening at one of the enantiotopic carbonyls.^[5] This type of ADS has attracted considerable attention over the years because many *meso* cyclic anhydrides are readily available, for example, through Diels–Alder reactions of maleic anhydride, and because the desymmetrized products, which contain chemically differentiated carboxy functions, provide a plethora of possibilities for further elaboration. In this Highlight we examine recent advances in the development of nonenzymatic catalysts, particularly chiral tertiary amines, for the ADS of *meso* cyclic anhydrides by alcoholytic ring-opening; these advances should pave the way for more widespread exploitation of this powerful approach. Moreover, we discuss some mechanistic aspects of the catalysis that may have implications for other asymmetric processes mediated by tertiary amines.

The ADS of *meso* cyclic anhydrides by direct ring-opening with enantiomerically pure alcohols, amines, and other nucleophiles has been shown to proceed with high levels of diastereoselectivity in a number of instances.^[6] While this strategy is undoubtedly of synthetic value, it is limited in that

stoichiometric quantities of the chiral nucleophile are required. Unless this "chiral auxiliary" is to be incorporated into the target molecule, this is clearly inefficient as additional chemical steps will be required for its removal.

Therefore, processes involving addition of achiral nucleophiles mediated by chiral catalysts are more desirable. The first enzyme-catalyzed processes were described by Oda and co-workers in 1988 who found that the lipase Amano P catalyzed the ADS of several 3-substituted glutaric anhydrides with primary alcohols in toluene or *i*Pr₂O at room temperature over 6–48 hours.^[7] The corresponding hemiesters were obtained in 67–94 % yield and 60–91 % *ee*, which compares favourably with the syntheses of the same products by enzymatic ADS of the corresponding diesters.^[8] Since then a number of related enzymatic protocols for the ADS of *meso* cyclic anhydrides have been reported giving high levels of enantioselectivity in many cases.^[8]

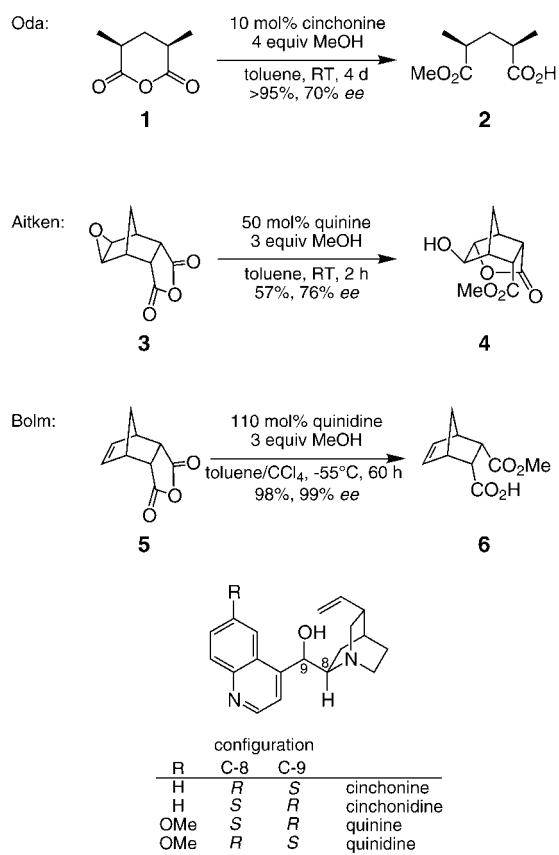
The first nonenzymatic, catalytic process was reported by Oda and co-workers in 1985 who found that cinchona alkaloids catalyzed the methanolytic ADS of the *cis*-dimethylglutaric anhydride **1** with 4–20 equivalents of MeOH in toluene at room temperature over 1–12 days (Scheme 1).^[9] Cinchonine (10 mol %) was the most enantioselective catalyst and gave hemiester **2** in >95 % yield and 70 % *ee*. Cinchonidine, the diastereomer of cinchonine with the opposite enantiomeric configurations at C-8 and C-9, provided the antipodal hemiester in 64 % *ee*.

Shortly thereafter, Aitken and co-workers published details of a similar procedure catalyzed by quinine (50 mol %) in which *meso* epoxy anhydride **3** was converted into lactone **4** in 57 % yield and 76 % *ee* with 3 equivalents of MeOH in toluene at room temperature within 2 hours (Scheme 1).^[10] Under these conditions, the methanolytic ADS is followed by intramolecular epoxide opening to form **4**. In this case, quinidine, the diastereomer of quinine with the opposite enantiomeric configurations at C-8 and C-9, provided the antipodal product with significantly lower enantioselectivity.

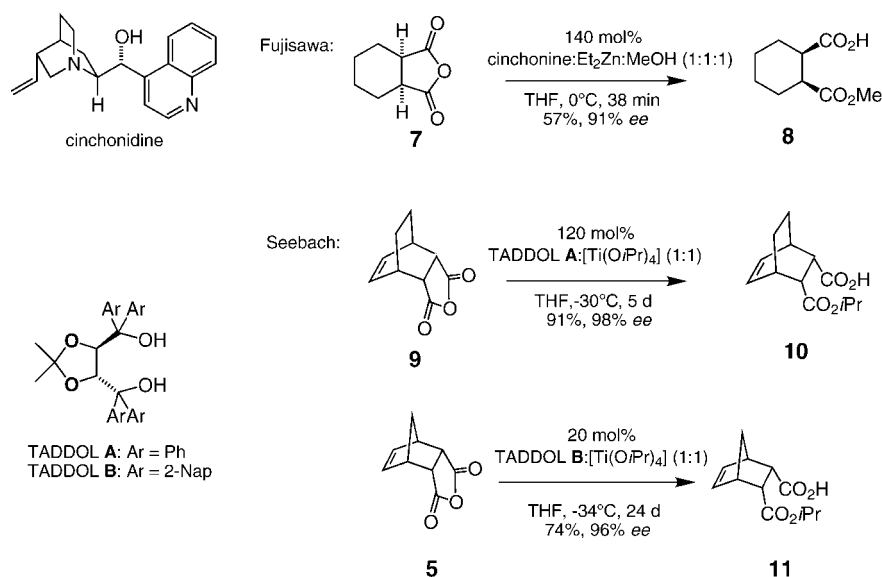
Two chiral Lewis acid-based protocols for the ADS of *meso* cyclic anhydrides were also developed during the 1990s by the groups of Fujisawa^[11] and Seebach,^[12] who utilized a cinchonidine:Et₂Zn complex and diisopropoxytitanium TADDOLates, respectively (Scheme 2). As these are mechanistically distinct from the amine-catalyzed systems, discussion of these lies outside the scope of this Highlight. It is, however, worth

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Scheme 1. Examples of the ADS of *meso* cyclic anhydrides mediated by cinchona alkaloids.



Scheme 2. Examples of the ADS of *meso* cyclic anhydrides mediated by chiral Lewis acid complexes. 2-Nap = 2-naphthyl.

noting that the method of Seebach and co-workers, which is the more selective and extensively investigated of the two, suffers from extended reaction times, particularly in catalytic mode.

In 1999, Bolm and co-workers built upon the findings reported by the groups of Oda and Aitken to develop a more enantioselective methanolysis protocol mediated by quinidine (110 mol %).^[13] A range of *meso* cyclic anhydrides underwent

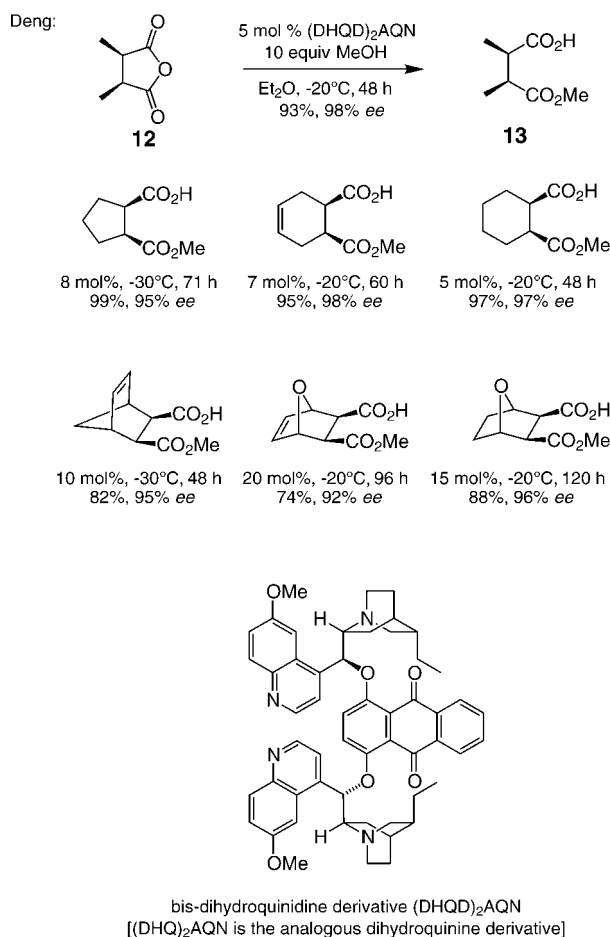
ADS to give methyl hemiesters in 61–99% yield and 85–99% *ee* with 3 equivalents of MeOH in toluene:CCl₄ (1:1) at –55 °C within 60 hours (Scheme 1). Under these conditions, quinine provided the antipodal products with comparable enantioselectivities (75–99% *ee*). The use of just catalytic quantities of the quinidine (10 mol %) resulted in lower yields and dramatic loss of enantioselectivity (for example, 50% yield and 35% *ee* for anhydride 5). However, the catalytic protocol could be improved significantly by the addition of one equivalent of 1,2,2,6,6-pentamethylpiperidine, which allowed the ADS of anhydride 5 in 98% yield and 90% *ee*, albeit after 6 days at –55 °C.

In the light of the above, a recent report by Deng and co-workers describing the methanolysis ADS of various *meso* cyclic anhydrides catalyzed by modified bis-cinchona alkaloids appears to constitute a significant breakthrough in this area.^[14] As the result of screening known bis-cinchona derivatives, developed by Sharpless et al. as chiral ligands for OsO₄-catalyzed asymmetric dihydroxylation reactions, Deng and co-workers found that dihydroquinidine-based catalyst (DHQD)₂AQN (5–20 mol %) mediated the formation of methyl hemiesters in 70–99% yield and 90–98% *ee* with 10 equivalents of MeOH in Et₂O at between –20 °C and –40 °C over 48–120 hours (Scheme 3). The corresponding dihydroquinine-based catalyst (DHQ)₂AQN provided the antipodal products with comparable enantioselectivities (82–98% *ee*). No achiral base is required in the process and the use of highly toxic CCl₄ as solvent is avoided (compare with the protocol of Bolm et al.). Furthermore, the process retains

the ease of catalyst removal/recycling by simple acid extraction that is a feature of all the alkaloid-catalyzed processes (in contrast to, for example, the TADDOL-ates of Seebach and co-workers). The ability to deliver either enantiomer of product simply by switching catalyst diastereomer is also of significant practical importance and constitutes a clear advantage over enzymatic processes.

This is not to say that the process could not be rendered more convenient: Maintaining a reaction at approximately –20 °C for several days is not attractive on process scale. So what are the prospects for the development of more efficient processes? Given that all amine catalysts described so far have been based on cinchona alkaloids it seems likely that screening or rational design (or a combination of the two) could lead to the discovery of better catalysts. Indeed, the first paper describing the use

of noncinchona-based catalysts (a series of chiral hexahydro-1*H*-pyrrolo[1,2-*c*]imidazolones) for ADS of *cis*-hexahydrophthalic anhydride in up to 89% *ee* has recently appeared.^[15] Comparison with the related field of acylative kinetic resolution (KR) of secondary alcohols catalyzed by nucleophilic amines (and phosphines) lends credence to the hypothesis that such efforts should bear fruit. Until just five years



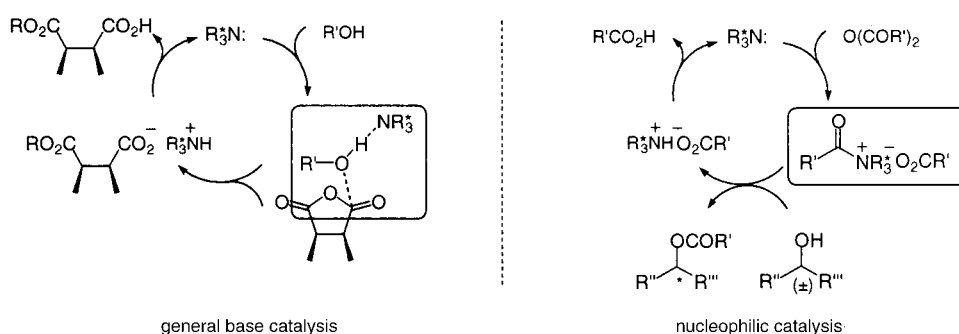
Scheme 3. Examples of the ADS of *meso* cyclic anhydrides mediated by bis-cinchona derivatives. Yields and *ee*'s in parenthesis refer to catalysis by (DHQ)₂AQN to give the opposite enantiomer to that shown.

ago, the alkaloid brucine constituted a state-of-the-art catalyst for these KR processes, but since then spectacular progress has been made by the introduction of “designer” chiral nucleophilic amine/phosphine catalysts, developments pioneered by the groups of Vedejs, Fu, and Oriyama.^[16]

Although acylative KR of secondary alcohols and alcoholytic ADS of *meso* cyclic anhydrides can both be catalyzed by chiral amines, there appear to be significant mechanistic differences between the two modes of catalysis. Amine catalysis of the former almost certainly involves nucleophilic attack by the amine nitrogen on the anhydride to afford a reactive chiral acylammonium salt. Nucleophilic attack on this salt by the secondary alcohol gives the ester product and regenerates the amine (that is, nucleophilic catalysis; Scheme 4). An equimolar amount of an achiral and nonnucleophilic base (for example, Et₃N) is generally present in these reactions to ensure that the chiral amine remains unprotonated to con-

tinue the catalytic cycle. The same mechanism may, as discussed by the groups of Aitken and Bolm, also operate for cyclic anhydride ring-opening. However, neither the quinoline nor the α -substituted quinuclidine nitrogens of cinchona alkaloids are expected to be very nucleophilic, as nucleophilicity is strongly attenuated by steric factors.^[17] Furthermore, for cyclic anhydrides the leaving carboxylate group has a high effective molarity ($EM = 10^4 M$), which makes the equilibrium between anhydride and acylammonium salt very unfavorable towards the latter.^[18] Taken in combination with the fact that in nonpolar solvents, as used in the cinchona-catalyzed reactions, even the position of the equilibrium between the highly nucleophilic amine 4-dimethylaminopyridine (DMAP) and the acyclic anhydride Ac₂O is unfavorable,^[19] this suggests that, at best, an extremely low concentration of acylammonium salt will be present and it is unlikely to be kinetically useful. Oda and co-workers have therefore proposed that cinchona-catalyzed ring-opening of *meso* anhydrides involves the quinuclidine nitrogen acting as a chiral general base rather than a nucleophile.^[9] This is in line with the mechanism proposed by Wynberg for cinchona alkaloid mediated asymmetric conjugate addition reactions of thiols to enones.^[20] In support of general base catalysis Oda and co-workers found that the cinchonine-catalyzed (10 mol %) ring-opening of *cis*-2,4-dimethylglutaric anhydride by methanol (20 equivalents) in toluene displayed a kinetic isotope effect (k_{MeOH}/k_{MeOD}) of 2.3. This value is probably greater than would be expected for nucleophilic catalysis even if proton transfer prior to final C–N bond cleavage were to be rate-determining.^[21] The fact that the nonnucleophilic base Et₃N is an effective achiral catalyst for alcoholytic ring-opening of *meso* anhydrides but not for acylation of secondary alcohols also militates against the former involving nucleophilic catalysis.^[9] The two contrasting mechanisms are shown in Scheme 4 although it should be stressed that, particularly in the case of Deng and co-workers' dimeric catalysts, a synergistic combination of the two mechanisms cannot be ruled out.

Irrespective of the mechanistic details, the catalytic ADS reaction of Deng and co-workers is notable on two accounts: Firstly because (DHQD)₂AQN is a bis-ether derivative, and secondly because no stoichiometric base is employed. Previous workers have noted that ether derivatives impart diminished enantioselectivities relative to the parent alkaloids although no specific role for the hydroxy group, either in



Scheme 4. Comparison of possible mechanisms of amine-catalyzed acylative kinetic resolution of secondary alcohols and alcoholytic ADS of *meso* cyclic anhydrides.

catalysis or in conformational control of the alkaloid, has been suggested. Indeed ab initio calculations suggest that overall the dipole moment, rather than hydrogen-bond interactions, is important in determining the conformation of monomeric cinchona alkaloids and that neither protonation nor the formation of simple ether derivatives perturbs this situation.^[22] It seems likely therefore that in Deng and co-workers' dimeric structures additional π – π ordering interactions are operational at least during catalysis (compare with those implicated as being significant during asymmetric dihydroxylation with this type of dimer as a ligand).^[23] The absence of a stoichiometric achiral base in the protocol of Deng's group (and Oda's) suggests either that both protonated and unprotonated forms of (DHQD)₂AQN mediate enantioselective ring-opening, or that the equilibrium concentration of unprotonated alkaloid is sufficient for the catalysis. The latter scenario seems more likely as Et₃N and quinuclidine, which are monobasic, are also catalytically active.^[9] Indeed it is plausible that NR₃H⁺ is more acidic than RCO₂H in toluene, which would make the unprotonated alkaloid the major species at equilibrium.^[24] Since the groups of Aitken and Bolm have noted that protonated cinchona alkaloids (presumably protonated on the more basic quinuclidine nitrogen) are catalysts but give very poor asymmetric induction, the unprotonated and protonated forms of the catalysts used by Deng's group must show a significant activity differential. Deng and co-workers do not speculate on the mechanism of catalysis beyond describing their catalysts as "chiral Lewis bases" and do not report on the effect of added stoichiometric achiral base.

In line with all workers in this field, Deng and co-workers note that the stereochemical sense of ring-opening is predictable irrespective of the anhydride structure. This implicates the anhydride motif itself as being the primary recognition element for the alkaloid catalyst. The noncovalent interactions responsible for this recognition are clearly critically dependent on the alkaloid configuration at C-8 and C-9 and must lead to a complex in which one of the enantiotopic carbonyl groups is activated to attack. Once the precise form of this activation can be more closely defined (for example, general base/nucleophilic catalysis or some other mechanism) the prospects for rational design of more rapid and selective catalysts will be greatly improved. Given that this type of catalysis has many elements in common with enzyme catalysis and indeed with many "enzyme-like" processes, including a number of other synthetically important asymmetric processes mediated by low molecular weight organic catalysts,^[25] significant further progress can be expected in the not too distant future.

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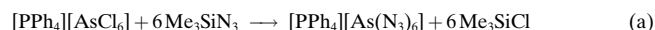
Homoleptic Azidometalates

Andreas Kornath*

Azides were discovered by Curtius more than 100 years ago, and even though they are among the most explosive compounds they are still an interesting object of research.^[1] It is known that azides of ionic composition, such as those of alkaline and alkaline earth metals do not have a tendency to explode, whereas covalently bonded azides are extremely shock-sensitive compounds.^[2] This different behavior is explained by the different bonding situation. In the symmetric azide anion the nitrogen atoms are equally strongly bonded by a double bond. In covalent azides the terminal N–N bond is stronger and thus the exothermic formation of nitrogen inevitable. The driving force is the disproportional increase in the average bond enthalpy for higher bond orders (N–N 159 kJ mol⁻¹; N=N 466 kJ mol⁻¹; N≡N 946 kJ mol⁻¹) which is a fundamental difference between nitrogen and all other elements with the exception of oxygen.

With this in mind it is even more remarkable that recently some very nitrogen-rich, covalent compounds, such as the azidometalates and pentaazonium salts have been reported.^[3–6]

Azidometalates have been known for a long time, however there are very few examples of X-ray structure investigations of these salts. The common preparation of azidometalates is based on the reaction of the corresponding chlorometalates with sodium or silver azide. By this method usually not all chloroligands are exchanged for azidoligands. The use of trimethylsilylazide as an azide-transfer agent is an improvement.^[3,4] Recently by this method the synthesis of salts of the ions [Pt(N₃)₆]²⁻ and [As(N₃)₆]⁻ was possible [Eq. (a)].



For the stability of the salts formed the choice of the cation plays an important role. Beck et al. found that azidometalates are stabilized by large cations, such as [PPh₄]⁺ or [AsPh₄]⁺, and can be handled without danger.^[7] Clearly, in the solid state the anions are separated sufficiently, thus a high activation barrier results for the decomposition. However, such a stabilizing effect is not always observed. Dehnicke et al. showed that [AsPh₄]₂[Pt(N₃)₆] follows this rule, but that [AsPh₄]₂[Pt(N₃)₄] with a lower-azide content is shock-sensitive,^[4] this arises because the azido groups of the anions are adjacent along one crystallographic axis.

The use of large cations reduces, at least in part, the danger and opens the way for structural investigation of these compounds which are otherwise difficult to handle. ¹⁴N NMR spectroscopy of the dissolved salts indicates that the

azide groups are covalently bonded to the central atom. The same conclusion can be derived from vibrational spectroscopy of the solid salts which shows that both the symmetrical and antisymmetrical vibrations are visible in the Raman and infrared spectra. The possibility of growing single crystals opens a way to elucidate the variety of azidometalate structures.

A number of anions with up to six azido groups is known from X-ray diffraction studies. The simplest example is hydrogendiazide ([H(N₃)₂]⁻) with an asymmetric and nearly linear N–H⋯N bridge. The azido groups are arranged with an dihedral angle of 66°. ^[8] The ions [Ag(N₃)₂]⁻ and [I(N₃)₂]⁻ have the expected C₂ symmetry.^[9] In anions with more than two azido groups the central atom has a square planar ([Pt(N₃)₄]²⁻,^[4] [Cu(N₃)₄]²⁻,^[10] [Pd(N₃)₄]²⁻,^[11] [Au(N₃)₄]²⁻,^[12]), tetrahedral ([Zn(N₃)₄]²⁻,^[13] [Mn(N₃)₄]²⁻,^[14]), trigonal bipyramidal ([Fe(N₃)₅]²⁻,^[15] or octahedral ([Sn(N₃)₆]²⁻,^[16] [Pb(N₃)₆]²⁻,^[17] [Pt(N₃)₆]²⁻,^[4] [As(N₃)₆]²⁻,^[3] [Ge(N₃)₆]²⁻,^[5]) coordination geometry with covalently bonded azido ligands which have a bent arrangement around the central atom (Figure 1). The symmetries deviate only slightly from the ideal values.

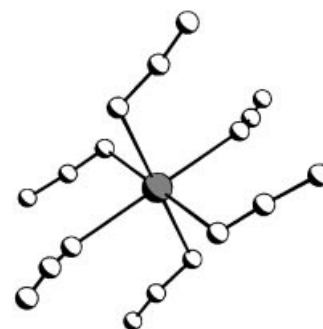
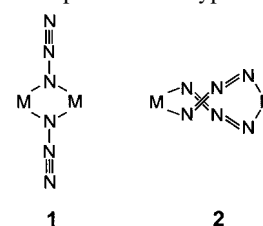


Figure 1. Structure of hexaazidometalates.

Azide groups can act as bridging ligands between metal atoms leading to di- and polynuclear complexes. Two types of connection via azido bridges are known from the chemistry of transition metal azides: type 1 and 2.

Only a few examples of dinuclear azidometalates are described in the literature.

In the ions [Fe₂(N₃)₁₀]⁴⁻,^[18] [Pd₂(N₃)₆]²⁻,^[19] and [Cu₂(N₃)₆]²⁻,^[20] only azido bridging of type 1 is found (Figure 2). In contrast to the metal azides, the dinuclear anions are stable in solutions. The same type of azido bridging is observed for the infinite-chain polynuclear azidometalate ions {[Cd(N₃)₃]_∞}, {[Cd(N₃)₄]_∞}, and {[Cd₃(N₃)₁₄]_∞}.^[21] In contrast the chain structure of the ion {[Cu(N₃)₃]_∞} additionally contains an azido bridging of type 2.^[22] The {[Co₃(N₃)₈]_∞} ion consists of condensed units



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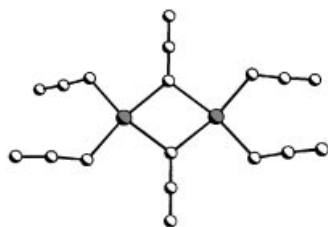


Figure 2. Structure of a dinuclear azidometalate ion; $[\text{Cu}_2(\text{N}_3)_6]^{2-}$.

of type 1 arranged in infinite chains that are connected by 1,3-azido bridge (half type 2) forming infinite layers.^[23] An example for three-dimensional bridging is given by the $[\text{Mn}(\text{N}_3)_3]^-$ ion, that possesses a perovskite-like structure with a 1,3-azido bridge (half type 2) between the Mn atoms.^[22]

From the relatively few examples of homoleptic azidometalates one can assume that a large variety of structures is possible. Moreover the azidometalates are interesting for material science because of their magnetic properties. It is known that azido bridging of type 1 leads to a ferromagnetic coupling whereas type 2 results in an antiferromagnetic coupling. In some cases cooperative magnetic long-range effects are observed (molecular magnets).^[24]

Large cations and improved syntheses, for example, by making use of trimethylsilylazide could facilitate access to new azidometalates. Transferring the stabilizing effect to cationic species by employing large counter anions is also conceivable; recently $[\text{N}_5^+][\text{AsF}_6^-]$ has been prepared by K. O. Christe et al. (Figure 3).^[6] In contrast to the azidometalates, the bent pentaazonium cation is mesomeric stabilized

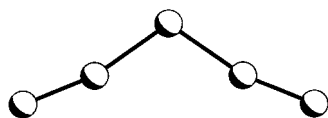
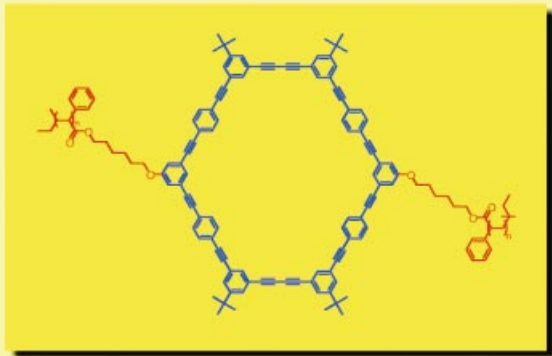


Figure 3. Structure of the pentaazonium cation $[\text{N}_5]^+$.

thus no isolated single N–N bonds are present. Both, the increase of stability gained by using larger anions and the preparation of such an unusual salt as $[\text{N}_5^+][\text{N}_3^-]$ seem feasible, however, one should consider that this hypothetical new modification of nitrogen is a combination of an oxidizing cation and a reductive anion. The examples show that surprising results can be expected from the azide chemistry in near future.

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Coil–Ring–Coil Block Copolymers as Building Blocks for Supramolecular Hollow Cylindrical Brushes

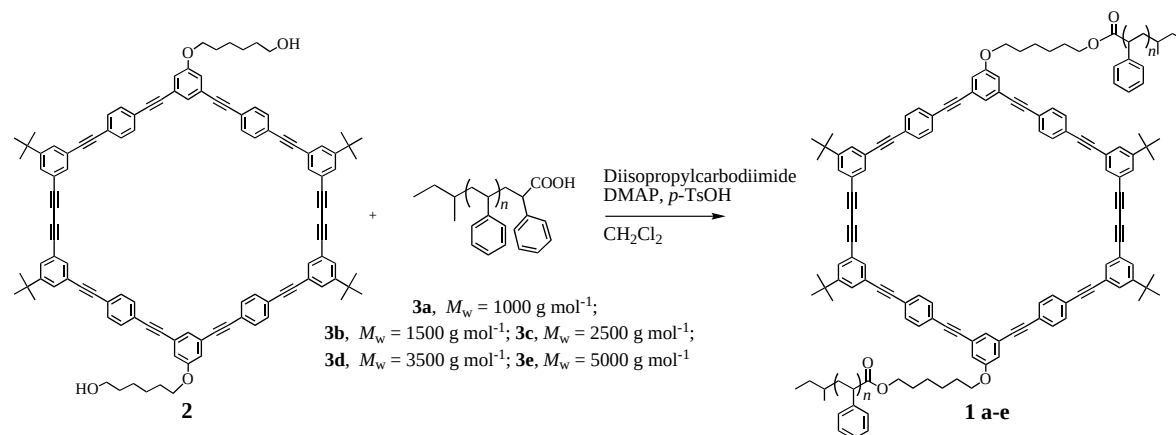
Silvia Rosselli, Anne-Désirée Ramminger, Thomas Wagner, Beate Silier, Simone Wiegand, Wolfgang Häußler, Günter Lieser, Volker Scheumann, and Sigurd Höger*

Dedicated to Professor Fred Wudl on occasion of his 60th birthday

Molecular self-assembly of relatively small, well-defined molecules to complex structures by noncovalent interactions is well known and often found in nature.^[1] Considerable effort has been given not only in the investigation and understanding of the basic principles and driving forces in self-organizing systems but also in the design of structures which imitate the complexity of these systems.^[2] One of the most intensively studied natural supramolecular systems is the hollow cylindrical-shaped tobacco mosaic virus (TMV) formed by self-assembly of the constituent protein subunits and the viral RNA.^[3] Synthetic supramolecular architectures based on gallic acid derivatives have been prepared and indeed show superstructures which are very similar to the natural model.^[4] In addition, cylinders having a lumen, as the virus, have also been described. Based on cyclopeptides or cyclosaccharides, the driving force for the aggregation are specific interactions, such as hydrogen bonding donors and acceptors.^[5]

mentarity). For this purpose, side groups that are incompatible with the cyclic backbone of the ring are attached to the macrocycle to induce (in a suitable solvent) microphase segregation and aggregation into cylindrical objects. Previous work in our group^[6] has shown that the aggregation behavior of oligoalkyl-substituted shape-persistent rings is strongly solvent dependent.^[7] For example, in solvents which act as good solvents for the rigid core and the flexible corona (such as CH₂Cl₂), no aggregation was observed. By adding a solvent which can solubilize only the alkyl corona but not the rigid core (for example *n*-hexane), a solvophobic aggregation of the structures ($K_{\text{dim}} = 130 \text{ M}^{-1}$ in hexane/CH₂Cl₂ (3/1); 790 M^{-1} in hexane/CH₂Cl₂ (6/1)) can be induced. However, with this approach we cannot observe larger aggregation constants by further decreasing the solvent polarity of the solvent mixture, since the connection of twelve dodecyl groups is still not sufficient to keep the materials in solution. Herein, a simple method is described to overcome this solubility problem by attaching short, non-crystallizable oligomeric side groups to the ring.

The central block of the target structures **1** is the macrocycle **2**, which has been obtained in good yield by oxidative Glaser coupling of the appropriate bisacetylene.^[8] Onto the macrocycle, narrowly distributed ($M_w/M_n < 1.1$) polystyrene–carboxylic acid oligomers^[9] (**3a–e**) of different molecular weights ($M_w = 1000$ (**3a**), 1500 (**3b**), 2500 (**3c**), 3500 (**3d**), 5000 g mol^{-1} (**3e**)) have been attached through carbodiimide coupling (Scheme 1) in good yields (70–90 %).^[10]



Scheme 1. Synthesis of coil–(rigid)ring–coil block copolymers **1a–e**. DMAP = 4-dimethylaminopyridine; *p*-TsOH = *p*-toluene sulfonic acid.

Recently, we synthesized functionalized shape-persistent macrocycles, that should also be suitable building blocks for the formation of nanoscale hollow cylinders. An objective of this study is to facilitate aggregate formation through non-specific interactions, opposite to the macrocycles mentioned above (for example, solvophobic effects and shape comple-

The resulting structures can be described as coil–(rigid)–ring–coil block copolymers, a subclass of rod–coil block copolymers, that are known to microphase separate to well-ordered superstructures, even at relatively small block sizes.^[2, 11]

The block copolymers described here are readily soluble in chloroform, dichloromethane, THF, and toluene at room temperature, and **1b–e** in warm cyclohexane.^[12] Upon cooling, **1b** forms a gel at concentrations above 0.5 wt %, **1c** rapidly forms a very viscous solution, and **1d** forms a similar viscous solution after some days. These solutions are also strongly birefringent (Figure 1).^[13] The exceptions are

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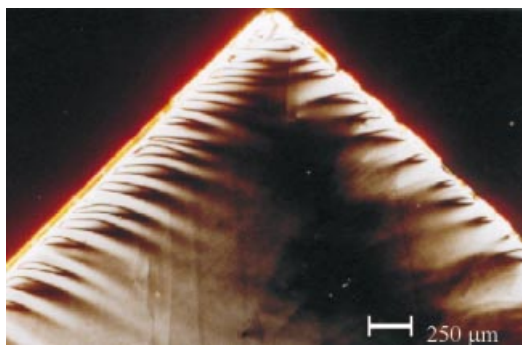


Figure 1. Texture of **1c** in cyclohexane between crossed polarizers (2 wt %; at the edges of the cover glass).

solutions of **1e**, which are neither very viscous nor birefringent.

These observations can be explained by the different solubility of the rigid cyclic block and the flexible polystyrene (PS) blocks of the copolymer. Cyclohexane is a solvent for PS but, in contrast to THF or toluene, a nonsolvent for the unsubstituted macrocycle **2**. The attachment of the polymeric side groups, even of relatively low molecular weight, is sufficient to solubilize the block copolymer in cyclohexane at elevated temperatures. However, upon cooling, the rigid parts of the block copolymer aggregate if the size of the coiled blocks is below a certain level. As expected, extension and aggregation kinetics strongly depend on the size of the PS block. Due to the structure of the block copolymers, the aggregation should lead to the formation of rodlike objects with large aggregate numbers, thereby reducing the contact surface of the rigid cyclic unit with the solvent.^[14] In order to support this assumption, the aggregation behavior of **1c** was investigated in more detail. Dynamic light scattering (DLS) was performed on solutions of **1c** in toluene and cyclohexane. The distribution of the relaxation times in the autocorrelation functions were obtained by CONTIN analysis.^[15] In toluene (0.11 wt %), only one maximum that corresponds to a hydrodynamic radius of approximately 2 nm is found, which correspond to the size of a simple block copolymer molecule **1c**. In contrast, the light scattering data in cyclohexane at the same concentration showed the formation of more complex structures. The elaboration of the measured autocorrelation function resulted in a distribution of relaxation times that contain two maxima, one at a hydrodynamic radius of approximately 2 nm, the other at approximately 60 nm (Figure 2 a).

Concentration-dependent measurements showed that the fraction of the larger species in solution increases with increasing concentration.^[16] The angular dependence of the scattered light intensity of the larger objects (Figure 2 b) suggested the presence of rodlike objects (linear increase of the Kratky plot) with a “virtual” persistence length, a measure of the rigidity, of more than 100 nm.^[17] The total length of the objects varies between 250 and 1300 nm, whereby a hydrodynamic radius of 60 nm corresponds to a rod length of approximately 500 nm.^[18]

The diameter of these cylindrical objects was determined by ultra small angle X-ray scattering (USAXS) and small angle X-ray scattering (SAXS) in cyclohexane at 2 wt % (Figure 3).

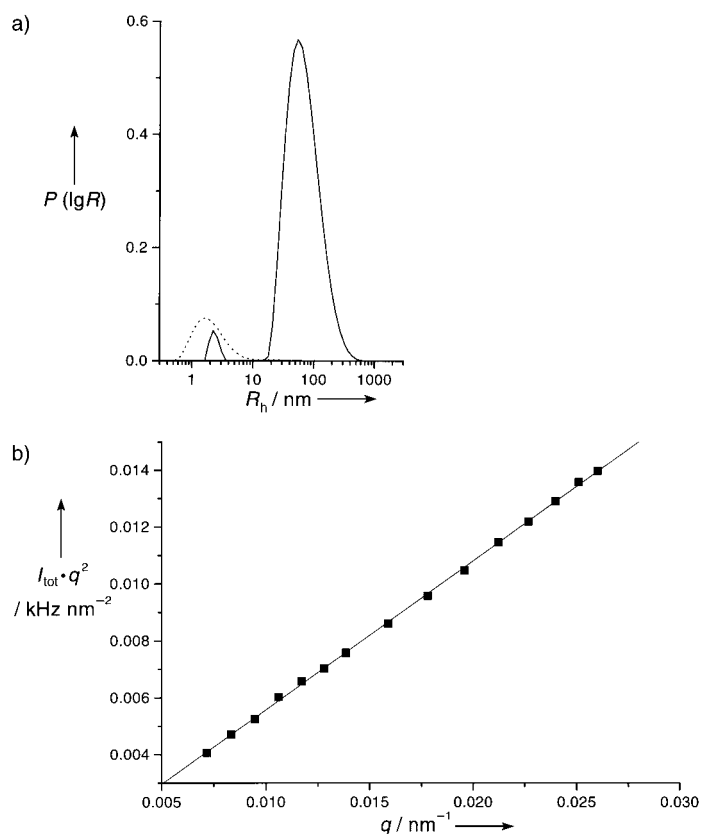


Figure 2. a) CONTIN fit of DLS-derived distributions of **1c** in toluene (---) and in cyclohexane (—) at a concentration of 0.11 wt %; b) Kratky plot for the slow mode of **1c** in cyclohexane (■) at a concentration of 0.09 wt % and linear data fit (—). R_h = hydrodynamic radius; I_{tot} = total intensity; q = scattering vector.

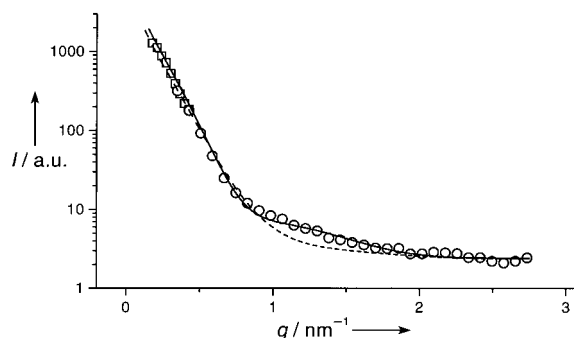


Figure 3. USAXS (□) and SAXS (○) data of a 2.0 wt % cyclohexane solution of **1c**. The lines represent the form factor for a solid cylinder (---) with an external diameter of 10 ± 3.8 nm, and for a hollow cylinder (—) with an external diameter of 10 ± 3.8 nm and an internal diameter of 1.8 ± 0.5 nm. I = total intensity; q = scattering vector.

The description of the measured data was not possible by using a simple solid cylinder model (showing an abrupt radial electron density change) of any length or thickness.^[19] Therefore, a model was used that describes the electron density profile on the outside of the cylinder more accurately. The different length of the oligomeric PS chains and the radial decrease of the cylinder density was taken into account by giving the cylinder a polydispersity in the diameter. Since variations in the cylinder length do not change the form factor

at all, it was set to 500 nm in accordance with the average object length determined by the light scattering. The calculated form factor of a solid cylinder of mean polydisperse diameter 10 nm ($d_{\text{out}} = 10$ nm, polydispersity 3.8 nm, half width at half maximum of a Gaussian distribution) agrees with the observed data at low q values. However, the observed scattering intensity is remarkably higher than the theoretical prediction in the q range between 1 and 2 nm⁻¹ and it was not possible to adjust the theoretical form factor to the experimental data, neither by variation of the cylinder diameter nor its polydispersity. Only the use of a radial density profile with a reduced electron density inside the cylinder resolved this discrepancy. The calculated form factor of a cylinder with the same electron density profile on the outside as described before and with an additional reduced electron density on the inside (hollow diameter $d_{\text{inner}} = 1.8$ nm, polydispersity 0.5 nm, half width at half maximum of a Gaussian distribution) fits with the observed data over the whole q range.^[20] The scattering data undoubtedly show that the coil–ring–coil block copolymers aggregate in solution into hollow cylinder-shaped objects with a high virtual persistence length.

Further evidence of the existence of these supramolecular cylinders was obtained by imaging methods applied to solid samples prepared under nonequilibrium conditions.^[21] The transmission electron micrograph (TEM) of a sample obtained by freeze drying a cyclohexane solution (Pt/C shadowed film) shows ribbons of different width at the sample surface, the narrowest in the range of approximately 15 nm (Figure 4A).

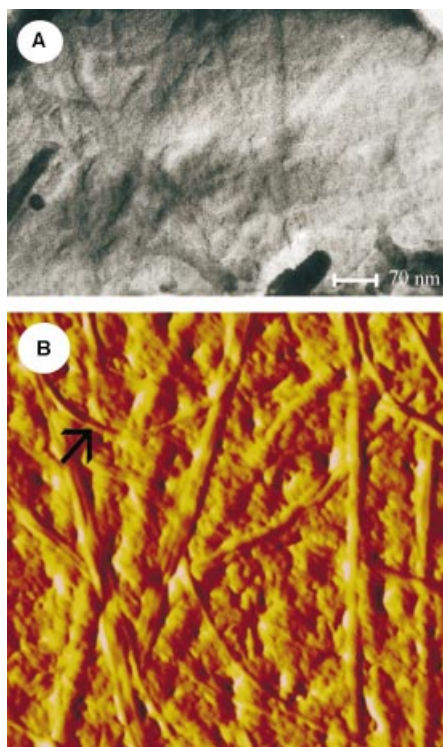


Figure 4. A) TEM: C/Pt shadowed film obtained by freeze drying a 0.15 wt % cyclohexane solution of **1c**; B) AFM (amplitude picture, $1.5 \times 1.5 \mu\text{m}^2$): Film obtained by dipping mica into a 0.15 wt % cyclohexane solution of **1c**.

Atomic force microscopy (AFM) images of a block copolymer film obtained by dipping mica into a cyclohexane solution of **1c** show long bundles of two or three cylindrical aggregates, together with individual aggregates (Figure 4B).^[22] Taking in consideration the width of the AFM tip, the cylindrical units have a diameter of approximately 10–20 nm. The dimensions of the aggregates obtained by TEM and AFM correspond well with the molecular dimensions obtained by the scattering methods and are in accordance with the molecular dimensions of the molecular building blocks.^[23] Even the most curved cylindrical objects found in the AFM images (Figure 4B, black arrow) show a large radius of curvature indicating their rigid nature.

The correspondence of our aggregates with covalently linked polymer brushes^[24] suggests a description of them as supramolecular hollow cylindrical brushes (Figure 5).

The results shown here demonstrate that coil–ring–coil block copolymers can aggregate into hollow cylinderlike objects by a combination of shape complementarity and demixing of rigid and flexible polymer parts (namely by nonspecific interactions). This is opposite to most of the



Figure 5. Schematic representation of the aggregation of the block copolymers into hollow cylindrical brushes. The PS block is the corona (orange) and the rigid ring is located in the core (blue) (not in scale).

previously reported tubular superstructures, where attractive forces induce the self-assembly. Since this approach bears the possibility to organize functionalized macrocycles while leaving the functional groups of the ring “free”, it opens a new entry to intra-annular functionalized rods. In other words, the functional groups do not have to be used to organize the macrocycles, but the formation of the superstructure is used to organize the functional groups.

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$$\int_0^{\frac{\pi}{2}} \int_0^{\frac{R_g + R_i}{2}} \int_{\frac{R_g + R_i}{2}}^{\infty} \{4r_a j_1(qr_a \sin(\alpha)) - 4r_i j_1(qr_i \sin(\alpha))\} \sin(ql \cos(\alpha/2)) / \{q \sin(\alpha) q l \cos(\alpha) (r_a^2 - r_i^2)\}^2 \sin(\alpha) \exp[-(r_a - R_a)^2 / (2\Delta r_a^2) - (r_i - R_i)^2 / (2\Delta r_i^2)] dr_a dr_i d\alpha \quad (1)$$

A better fit is obtained by a more sophisticated electron density profile in the range $2 < d < 4$ nm. However, a precise analysis of the electron density requires more accurate scattering data.

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From Structure to Function: A New Approach to Detect Functional Similarity among Proteins Independent from Sequence and Fold Homology

Stefan Schmitt, Manfred Hendlich, and Gerhard Klebe*

The explosion in information regarding protein sequence and structure demands new computational tools for the direct inference of protein function. The molecular function of proteins is almost invariably linked with the specific recognition of substrates and endogenous ligands in given binding pockets; proteins of related function should therefore share comparable recognition pockets. We have developed a new approach that is based on the placement of physicochemical descriptors assigned to the exposed binding-site residues. The deduced property descriptors can be used to retrieve common sub-structures and, thereby, related binding pockets. The solutions obtained are scored by comparing similarly exposed surface patches assigned to the same property, thus allowing detection of functional relationships among proteins independent of a particular sequence or fold homology.

The sequencing of the human genome represents only the first step toward understanding the functional and structural interplay of proteins in biological systems. Through methodological developments in "structural genomics", such as high-throughput X-ray crystallography,^[1] we will be increasingly confronted with 3D structures of proteins whose actual biochemical function has yet to be assigned. In addition, structure prediction and homology modeling techniques might mature to a state where the overall protein geometry can be predicted correctly from sequence.^[2] Thus, methods to infer protein function from 3D structure are desperately required. Since protein function is not necessarily confined to a particular fold or vice versa,^[3] this assignment is by no means straightforward.

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Protein function is often intimately connected with the recognition and/or chemical modification of endogenous ligands or substrates.^[4, 5] Recognition usually occurs in well-characterized clefts or cavities of the protein surfaces.^[6] In enzymes, for example, elementary steps along chemical pathways require a strictly defined spatial arrangement of the reaction partners. This, in turn, demands a corresponding highly conserved spatial arrangement of molecular recognition determinants in the protein to accommodate and spatially arrest them. The idea that a molecular recognition pattern may be conserved between binding sites of proteins of similar function motivated us to develop a new approach to comparing protein structures. Rather than considering complete proteins in terms of sequences, we search for similarities of surface-exposed physicochemical properties within binding pockets.

Several methods have been described to locate depressions on protein surfaces as putative binding sites.^[7–11] We used the program LIGSITE^[12] and implemented its algorithm into the protein–ligand database RELIBASE^[13, 14] to detect and retrieve binding cavities from the entire body of resolved crystal structures. In the analysis, each protein considered is embedded in a regularly spaced grid. Any lattice intersection coinciding with a protein atom, or within its van der Waals radius, is discarded and the remaining lattice points are scored according to their degree of burial in the surface depressions. Adjacent lattice points that are deeply buried are clustered together to reveal contiguous cavities, which are deposited in the new object-oriented database CAVBASE. For each cavity the surface is approximated by assigning the surface-contacting intersections of the initially embedded lattice that was generated in the cavity-extraction step. The atomic coordinates of the amino acids flanking the cavity are reduced to a set of generic pseudocenters, which are classified according to five properties essential for molecular recognition: hydrogen-bond donor, hydrogen-bond acceptor, mixed donor/acceptor (for example, hydroxyl groups or side-chain nitrogen atoms on histidine), hydrophobic aliphatic, and aromatic contacting group.^[15] These pseudocenters are further examined for their surface exposure and assigned to the nearest lattice surface

intersections (Figure 1). The cavity shape, the set of assigned descriptors of exposed recognition properties, and the corresponding surface patches are stored in CAVBASE together with information on the individual cavity occupants. The data stored are fully integrated with RELIBASE by using common pointers for the protein and ligand features (for example, atoms, residues) to interlink with corresponding objects in RELIBASE. A sample of 31 441 cavities was retrieved from 8308 protein structures. The new database is equipped for interactive visualization of cavity information and of results from a cavity comparison.

The stored information allows for fast and efficient comparisons within large data sets. A clique algorithm is applied to detect common sub-graphs generated by nodes corresponding to pairs of pseudocenters of equivalent properties and similar mutual distances.^[16] Appropriate tolerances have been incorporated to consider structural variations resulting from conformational flexibility of the protein (for example, ligand-induced fit, domain mobility) and inherent limitations of the accuracy of determining protein structures. The multiple solutions generated are ranked by scoring their corresponding matches in terms of the assigned surface-exposed physicochemical properties.

The direct comparison of two mean-sized cavities (ca. 800 Å³) requires about 100 s CPU time on a state-of-the-art single processor.^[17] The approach is easy to parallelize. Thus, our method is capable of scanning a cavity of interest (“query cavity”) against a sample of several thousand binding pockets. To reduce the original sample set in CAVBASE to a manageable size of 5000–6000 entries we filtered the data by criteria such as average cavity size and resolution of the input proteins. In the searches described below, we further discarded any cavities of proteins sharing high sequence identity with the query cavity protein to exclude trivial solutions. As a result, the top-ranked solutions exhibit both, local-surface similarity and shared binding motifs with the query cavity.

As this approach is entirely based on physicochemical properties exposed at the surface of a binding pocket it allows detection of relationships independent of a particular sequence or fold homology; it is entirely based on physico-

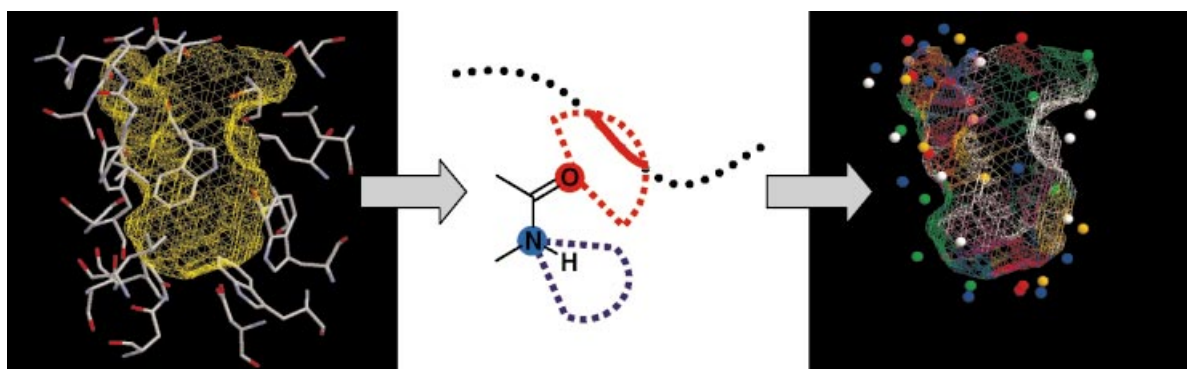


Figure 1. An extracted binding-site cavity is represented by a set of well-placed pseudocenters describing the physicochemical properties of the exposed binding-site residues flanking the cavity (H-bond donor (blue), H-bond acceptor (red), ambivalent donor/acceptor (green), hydrophobic aliphatic (white), and aromatic (orange) property; left). Only those centers that expose their property towards the cavity surface are considered (shown for the acceptor and donor properties of a peptide binding; here the donor group cannot produce a pseudocenter, because it is not exposed to the surface; center). The individual surface patches that describe the accessibility of the pseudocenters are stored and subsequently used to score the similarity among binding pockets. According to this protocol, the entire cavity is transformed into a set of property-labeled pseudocenters and associated surface patches (same color coding for pseudocenters (spheres) and the surface patches (net); right).

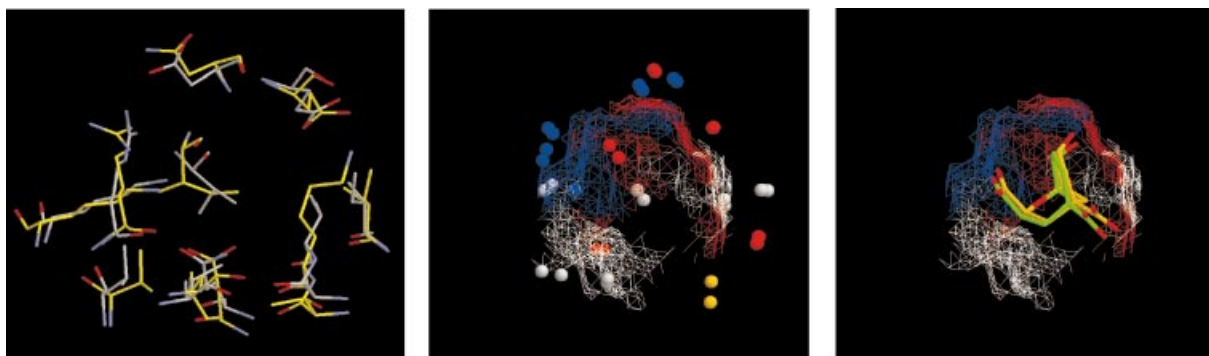


Figure 2. Binding pockets of chorismate mutase from *S. cerevisiae* (4csm) and *E. coli* (1ecm) showing less than 20 % sequence homology. Bound ligands are recognized mostly through interactions experienced by side-chain residues (left). The pocket from *E. coli* was screened against a set of 5445 different pockets using our associated pseudocenter description (center). On the right the superimposed cavities are shown together with the matched common surface patches and the bound bicyclic transition-state-analogue inhibitors.

chemical properties rather than amino acid complementarity. This is in contrast to other methods described^[18–23] that are dependent on information merely retrieved directly or indirectly from the protein fold. Recently, Stahl et al.^[24] reported the analysis of a preselected set of 176 zinc-containing metalloproteinases that had been clustered using a self-organizing neural network by means of solvent-accessible surface patches assigned to physicochemical properties. The active sites could be discriminated from other depressions on the surfaces of these enzymes with their approach.

In our first example we compared the binding pockets extracted from two chorismate mutases originating from the species *Saccharomyces cerevisiae* and *Escherichia coli*.^[25, 26] This example was also studied by Rosen et al.^[19] by using sparse critical points^[27] derived from the Connolly surface of manually extracted binding pockets. Although they show sequence identity below 20 %, both adopt a similar fold. The

bound ligand, a bicyclic transition-state-analogue inhibitor, is recognized mostly through side-chain interactions. A sample set of 5445 cavities containing the chorismate mutase cavity from *E. coli* was screened, but excluding that from *S. cerevisiae*, which instead was used as the query cavity. The cavity from the *E. coli* structure was found as the best-scored solution; Figure 2 shows the matching pseudocenters and corresponding surface patches. Although no information on the bound ligand was used, the surface match generates a transformation that results in a virtually perfect superposition of the bound inhibitors.

In a second example we probed the binding pocket from trypsin against a set of 5248 cavities taken from proteins with sequence identities below 35 % of the parent structure. The best cavities came from other members of the trypsin family, which exhibited steadily decreasing homology with trypsin. At rank 114, thus among the first 3 % of the data sample

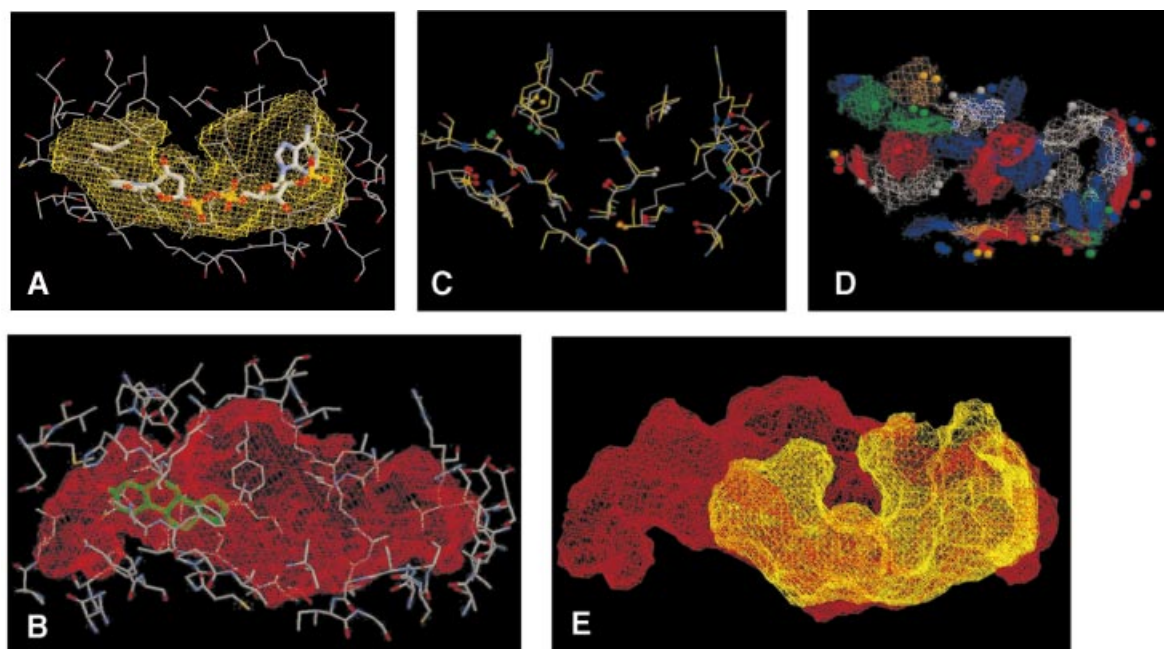


Figure 3. The NADPH cofactor binding pocket of a carbonyl reductase (1cyd; A) has been screened against a set of 5377 cavities (C and D illustrate the equivalent amino acids, pseudocenters, and surface patches, respectively). The algorithm detects a similar pocket present in a NADPH-dependent steroid dehydrogenase at rank 41 (1fds; B). The crystal structure of the steroid dehydrogenase has been determined without the bound cofactor; however, the cofactor cavity observed in the carbonyl reductase (A, yellow) matches well with a large unoccupied pocket in the steroid dehydrogenase (B, red) overlay (E).

considered, a binding pocket from the structurally unrelated subtilisin family was detected. Further examples from this family followed together with other members of the trypsin family. The trypsin and subtilisin superfamily are representatives of the major class of serine proteases, that is, they share functional similarity without sequence and fold homology.

The entire NADPH cofactor binding pocket of a carbonyl reductase^[28] was taken as the query and screened against a set of 5377 cavities extracted from proteins with no significant sequence homology. The top-scoring cavities also accommodate NADPH; subsequent cavities host ligands with decreasing similarity, but which contain parts of the NADPH skeleton. The large cavity of an NADPH-dependent steroid dehydrogenase^[29] is detected at rank 41 (Figure 3), although the crystal structure had been determined in the absence of the bound co-factor. A cavity from a phenol hydrolase is found at rank 116 which accommodates FAD as a co-factor. In this case, the adenine recognition site is shared with the original NADPH query pocket.

Surprisingly, in another search we detected a surface patch of an adenine-binding pocket that was similar to an unoccupied binding-site region in HIV protease. In the proteinase structure, a macrocyclic peptidomimetic is bound to the active site,^[30] and leaves a binding-site region that is similar to a patch in the catalytic subunit of protein kinase A unoccupied.^[31] In the latter case, this patch accommodates the adenine portion of adenylylimino diphosphate. This surprising finding is of potential interest for drug design, as an adenylyl moiety might be used to supplement the macrocycle in the unoccupied binding niche in HIV protease. It is likely that a large database of binding-site cavities can be used to generate interesting suggestions for new molecular portions that can be used as potential bioisosters in skeletons of novel leads.

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functional group on a neighboring ligand within a certain angular range. These ranges are calibrated at composite crystal-field environments stored in the program IsoStar (I. J. Bruno, J. Cole, J. Lommerse, R. S. Rowland, R. Taylor, M. L. Verdonk, *J. Comput. Aided Mol. Des.* **1997**, 11, 525–537).

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Unusual Formation of an Azaphospholene from 1,3,4,5-Tetramethylimidazol-2-ylidene and Di(isopropyl)aminophosphaalkyne**

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During recent studies of the reactivity of N-heterocyclic carbenes^[1] towards phosphoalkynes, we found that the anellated compound *N,N'*-bis(2,2-dimethylpropyl)benzimid-

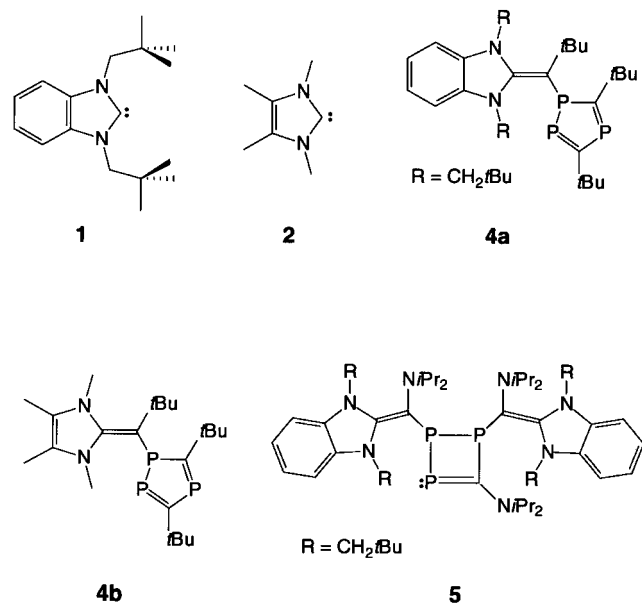
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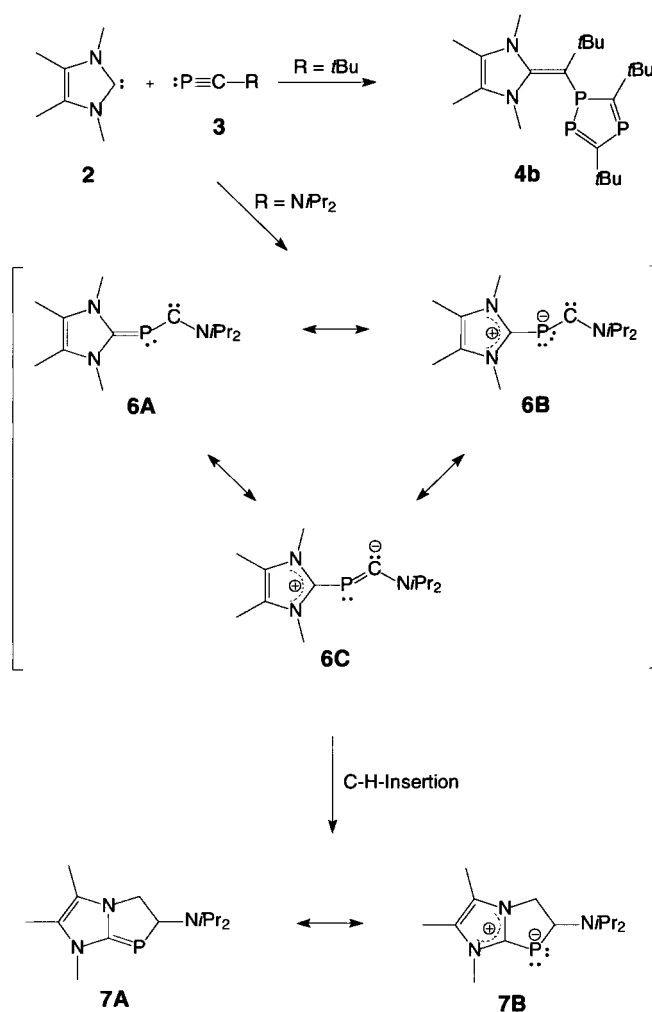
azolin-2-ylidene (**1**)^[2] reacts quantitatively with $\text{P}\equiv\text{CtBu}$ (**3a**), to form the triphosphole **4a**, and with $\text{P}\equiv\text{CNiPr}_2$ (**3b**), to yield the 1,2,3-triphosphetene **5**.^[3] Similarly, Nixon et al.^[4] have synthesized the triphosphole derivative **4b** by treating the Arduengo carbene 1,3,4,5-tetramethylimidazol-2-ylidene (**2**) with the 2,4,6-tri-*tert*-butyl-1,3,5-triphospha benzene.



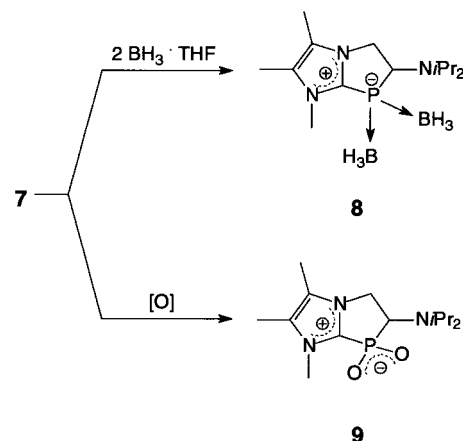
results demonstrate that the bond formation between the unsaturated carbon of the P–C multiple-bond system and the carbene carbon atom is favored. The reaction of the Bertrand phosphanylsilyl carbene with the phosphaaalkyne **3a** proceeds in the same manner. The primarily formed 2-phosphanyl-2*H*-phosphirene is stabilized by ring opening under preservation of the C–C_{carbene} bond, and breaking of the P–C_{carbene} bond to yield the 1λ⁵,2λ³-diphosphetene.^[5] Herein we report the synthesis of an isolable 1:1 adduct between the N-heterocyclic carbene **2** and the phosphaaalkyne **3b**, which results in the formation of a P–C_{carbene} bond, and we propose a mechanism for this novel reaction on the basis of quantum-chemical calculations.

The main product of the reaction of the carbene **2** with **3a** (carried out in toluene at room temperature) is the triphosphole **4b**^[4] (ca. 70 %). However, when **2** is treated with the aminophosphaaalkyne **3b**, the bicyclic compound **7** is formed in almost quantitative yield (Scheme 1).

Compound **7** was isolated as pale yellow, air and moisture sensitive microcrystals. The ¹³C NMR spectrum shows the resonance signal of the carbene carbon at $\delta = 178.9$ (¹J(P,C) = 81.5 Hz), which is strongly shifted to higher field when compared to **2** ($\Delta\delta = 33.8$). The ³¹P{¹H} NMR signal is observed as a singlet at $\delta = -64.8$ which is in the region characteristic for C-diaminophosphaalkenes.^[6] The bonding situation in the new azaphosphole **7** is best described by the two resonance structures **7A** and **7B**, in which **7B** represents an inversely polarized phosphaaalkene with a π electron-density distribution of $\text{P}^{\delta-}=\text{C}^{\delta+}$.^[7] This interpretation is supported by the derivatization of **7** with the Lewis acid $\text{BH}_3 \cdot \text{THF}$, which yields exclusively the complex **8** with two coordinated BH_3 groups on the phosphorus atom (Scheme 2).



Scheme 1. Proposed mechanism for the formation of the azaphosphole **7** from **2** and **3b** (DFT calculations: B3LYP/6-311G(2d)).



Scheme 2. Formation of **8** and **9** through reaction of **7** with $\text{BH}_3 \cdot \text{THF}$ or air.

Cowley et al.^[8] have recently described an analogous diborane complex with an acyclic diaminophosphaalkene.

In further support of these experimental data, we obtained a small amount of the oxidation product **9** (Scheme 2), with two oxygen atoms bound to the phosphorus atom, from a solution of **7**, kept for several weeks in benzene at room temperature (obviously because of the effect of air and moisture).

The ^{31}P NMR spectra of **8** and **9** (δ_{P} (**8**): 2.8; δ_{P} (**9**): 90.5) show, as expected, a strong low-field shift compared to the azaphospholene **7**. The $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum signal of the carbene center of **8** appears as a doublet at $\delta = 151.7$ with a remarkably small $^1J(\text{P}, \text{sp}^2\text{-C})$ coupling of only 9.0 Hz.

The crystal-structure analysis^[9] confirms the mass spectrometry (MS) and NMR data, which demonstrates that both BH_3 groups or oxygen atoms in the bicyclic species **8** and **9** are bonded to the phosphorus atom (Figures 1 and 2). In both cases the phosphorus atom assumes a distorted tetrahedral geometry. The P–B and P–O bond lengths are comparable to those of related acyclic derivatives.^[8, 10] The P–C_{carbene} distances (**8**: 1.818(5); **9**: 1.824(3) Å), shortened C–N bond lengths in comparison to the free carbene **2**,^[1d] and the planar environment of the ring nitrogen atoms suggest a strongly polarized P–C_{carbene} bond with the formal positive charge localized in the imidazole ring.

The mechanism of the surprising formation of compound **7** was studied by quantum-chemical density functional theory (DFT) calculations (B3LYP/6-311G(2d) optimizations and

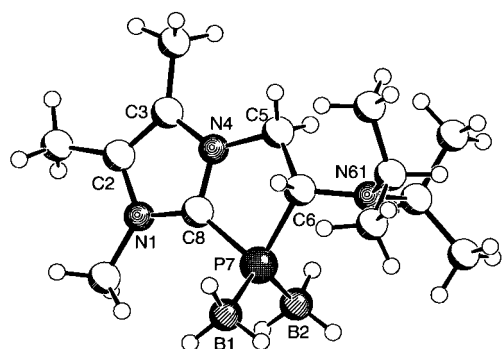


Figure 1. Molecular structure of **8**. Selected bond lengths [Å] and angles [°]: P7–C8 1.818(5), P7–C6 1.899(5), P7–B1 1.934(5), P7–B2 1.953(6), C8–N1 1.337(6), C8–N4 1.330(5), N1–C2 1.385(6), C2–C3 1.356(6), N4–C3 1.388(6), N4–C5 1.462(6), C5–C6 1.546(6); C8–P7–C6 86.8(2), C8–P7–B1 112.2(3), C6–P7–B1 107.8(2), C8–P7–B2 102.7(2), C6–P7–B2 118.5(2), B1–P7–B2 122.6(3), N1–C8–N4 107.3(4), P7–C8–N1 137.7(4), P7–C8–N4 114.6(3), C2–N1–C8 109.1(4), C3–N4–C8 110.4(4), N1–C2–C3 107.7(4), N4–C3–C2 105.5(4).

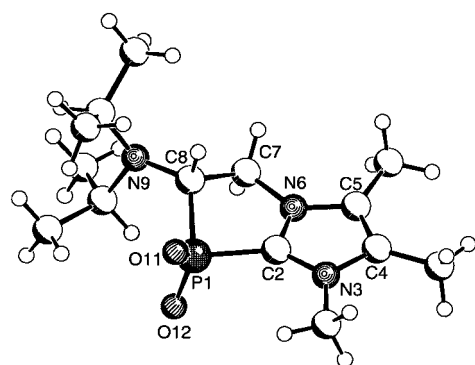


Figure 2. Molecular structure of **9**. Selected bond lengths [Å] and angles [°]: P1–C2 1.824(3), P1–C8A 1.861(4), P1–C8B 1.878(8), P1–O11 1.471(2), P1–O12 1.468(2), C2–N3 1.325(3), C2–N6 1.329(3), C4–C5 1.353(4), N3–C4 1.388(3), N6–C5 1.382(3), N6–C7 1.463(3), C7A–C8A 1.532(5); C2–P1–C8A 86.5(2), C2–P1–C8B 90.5(3), C2–P1–O11 110.64(12), C2–P1–O12 107.32(12), C8A–P1–O11 109.1(2), C8B–P1–O11 126.5(5), C8A–P1–O12 117.3(2), C8B–P1–O12 96.5(5), O11–P1–O12 120.55(13), N3–C2–N6 107.1(2), N6–C5–C4 105.9(2), C5–C4–N3 106.9(2), P1–C2–N3 139.5(2), P1–C2–N6 113.3(2), C2–N6–C7A 117.3(2), N6–C7A–C8A 104.9(2), C7A–C8A–P1 107.7(2).

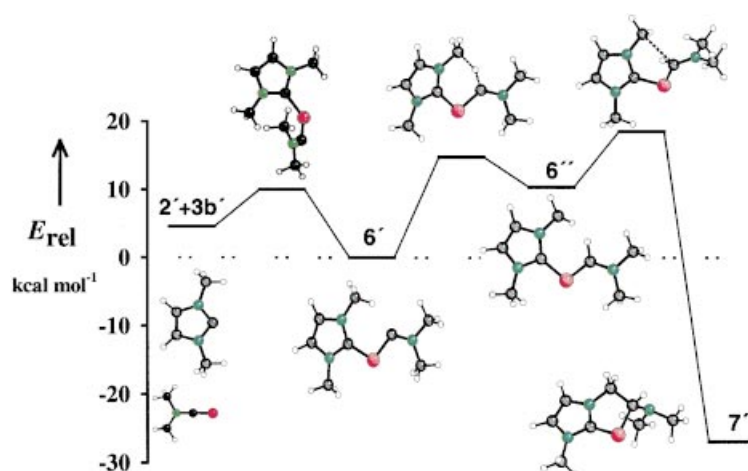


Figure 3. Relative energies for the intermediates in the reaction of the carbene **2'** with the phosphalkyne **3b'** to **7'**.

zero-point energy corrections ΔZPE ; all stationary points were characterized by vibrational frequency calculations, Program GAUSSIAN98;^[11] the methods B3LYP/6-311G(d,p) and B3LYP/6-31G* give similar results). Because of computing-time considerations the slightly simplified model systems **2'** (H instead of CH_3 at $\text{C}=\text{C}$ in **2**) and **3b'** (CH_3 instead of *i*Pr at the nitrogen atom in **3b**) were used. According to the calculations the initial adduct, which is formed by an attack of the phosphorus atom of **3b'** on the carbene **2'**, has structure **6'** with significant bending at both the newly formed carbene center (Scheme 1) and at the phosphorus atom (Figure 3). The bonds from phosphorus to the heterocycle (1.82 Å) and to the former phosphalkyne carbon atom (1.77 Å) are calculated to be unusually long. A natural bond orbital (NBO) analysis of **6'** indicates a substantial negative charge on the carbene carbon atom as in resonance structure **6C** (Scheme 1). According to the calculations the CH-insertion^[12] to produce the bicyclic compound **7'** proceeds in two steps: first, an intramolecular 1,5-H shift takes place, which leads to the 1,5-dipolar intermediate **6''**. The corresponding transition state is quite low in energy (14.8 kcal mol^{−1}) and can be reached at room temperature. The resulting intermediate **6''** is calculated to be 10.4 kcal mol^{−1} higher in energy than **6'**. It is transformed to **7'** in a 1,5 ring-closure reaction by passing over an activation energy barrier of 18.5 kcal mol^{−1}. Compound **7'** is 26.9 kcal mol^{−1} lower in energy than the intermediate **6'**. For **7'** again unusual long P–C bonds are calculated (P–C_{heterocycle}: 1.76, P–CHNMe₂: 1.96 Å (bond–no bond resonance)).

According to the calculations we interpret the attack of the imidazol-2-ylidene **2'** at the phosphorus atom of **3'** as a result of a kinetically controlled reaction. The thermodynamically favored C–C bond formation leading to a vinylphosphinidene (3.7 kcal mol^{−1} lower than **6'**) is not observed. An isomeric 2*H*-phosphirene structure does not correspond to a minimum on the potential-energy surface.

According to the DFT calculations benzimidazol-2-ylidene is a harder nucleophile than **2'** (HOMO energy is 0.25 eV more negative than for imidazol-2-ylidenes). It undergoes exclusively the thermodynamically favored P–C attack.^[3] Specific solvation effects may additionally contribute to the remarkable selectivity observed.

The results presented here demonstrate clearly that both the electronic structures of the phosphalkyne (with or without π -donor substituents),^[13] as well as those of the N-heterocyclic carbenes determine the reaction pathway of the adduct formation. In general the formation of three different types of phosphaheterocycles (type **4**, **5**, or **7**) may be expected. By treating a soft carbene (**2**) with an electron-rich phosphalkyne (**3b**) we could direct the course of the reaction towards primary attack of the carbene carbon atom at the phosphorus atom of the phosphalkyne. This allowed the subsequent unusual C–H activation at the nitrogen substituent of the Arduengo carbene.

Experimental Section

All reactions were carried out under argon using standard Schlenk techniques. Reactions were monitored by using ³¹P NMR spectroscopy.

Preparation of 4b: A solution of **2**^[15] (0.058 g, 0.47 mmol) in [D₈]toluene (1 mL) was added dropwise, at room temperature, to a stirred solution of **3a**^[14a] (0.140 g, 1.40 mmol) in [D₈]toluene (1 mL). The mixture was stirred continuously and afterwards transferred into the NMR tube. ³¹P NMR measurements indicated a complete reaction of **3a** and the formation of **4b** after 2 days. After removal of the solvent in vacuo and recrystallization from pentane, **4b** was obtained as a red powder (0.135 g, 68% yield). **4b** was characterized by comparison of the NMR data with those in the literature.^[4]

7: A solution of **3b**^[14b] (0.100 g, 0.70 mmol) in [D₈]toluene (1 mL) was added dropwise, at room temperature, to a solution of **2** (0.087 g, 0.70 mmol) in [D₈]toluene (1 mL) under stirring. At the beginning of the reaction, the mixture changed from yellow to red. ³¹P NMR measurements indicated a complete reaction of **3b** and a nearly quantitative formation of **7**. After removal of the solvent in vacuo and recrystallization from pentane, **7** was obtained as pale yellow, air and moisture sensitive powder (0.153 g, 82% yield). ¹H NMR (200.1 MHz, [D₈]toluene, 25 °C): δ = 1.04 (d, J (H,H) = 7.0 Hz, 6H, CHCH₃), 1.23 (d, J (H,H) = 6.2 Hz, 6H, CHCH₃), 1.36 (s, 3H, CCH₃), 1.53 (s, 3H, CCH₃), 2.67 (s, 3H, NCH₃), 3.38 (m, 3H, NCH₂ and CHCH₃), 3.52 (dsept, J (H,H) = 7.0, ^4J (P,H) = 3.2 Hz, 1H, CHCH₃), 5.06 (dt, J (H,H) = 8.0, ^2J (P,H) = 7.2 Hz, 1H, PCH); ¹³C{¹H} NMR (50.3 MHz, [D₈]toluene, 25 °C): δ = 8.1 (s, C=CCH₃), 8.8 (s, C=CCH₃), 22.5 (s, CHCH₃), 21.6 (s, CHCH₃), 32.4 (s, NCH₃), 52.0 (d, ^2J (P,C) = 10.7 Hz, NCH₂), 55.2 (s, CHCH₃), 55.3 (s, CHCH₃), 60.0 (d, ^1J (P,C) = 38.7 Hz, PCH), 125.4 (s, C=C), 129.3 (s, C=C) 178.9 (d, ^1J (P,C) = 81.5 Hz, C=P); ³¹P{¹H} NMR (81.0 MHz, [D₈]toluene, 25 °C): δ = –64.2 (s); MS (70 eV): m/z (%): 267 (60) [M^+], 224 (10) [M^+ – CH(CH₃)₂], 194 (11) [M^+ – CH(CH₃)₂ – 2CH₃], 166 (100) [M^+ – iPr₂NH].

8: A 1 M solution of BH₃·THF (1.0 mL, 1.12 mmol) was added dropwise, at room temperature, to a stirred solution of **7** (0.150 g, 0.56 mmol) in [D₈]toluene (1 mL). After 3 h colorless crystals precipitated from the reaction mixture (0.126 g, 76% yield). Their quality was sufficient for a single-crystal X-ray structure analysis. ¹H NMR (200.1 MHz, CDCl₃, 25 °C): δ = 1.03 (d, J (H,H) = 6.5 Hz, 6H, CHCH₃), 1.15 (d, J (H,H) = 7.1 Hz, 6H, CHCH₃), 1.63 (br, 6H, BH₃), 2.20 (s, 3H, CCH₃), 2.22 (s, 3H, CCH₃), 3.46 (sept, J (H,H) = 6.5 Hz, 1H, CHCH₃), 3.79 (s, 3H, NCH₃), 3.46–4.1 (m, 3H, NCH₂ and CHCH₃), 4.53 (dt, J (H,H) = 8.0, ^2J (P,H) = 10.3 Hz, 1H, PCH); ¹³C{¹H} NMR (50.3 MHz, CDCl₃, 25 °C): δ = 8.6 (s, C=CCH₃), 9.0 (s, C=CCH₃), 22.9 (s, CCH₃), 23.3 (s, CCH₃), 32.9 (s, NCH₃), 46.1 (s, CHCH₃), 46.2 (s, CHCH₃), 49.0 (d, ^2J (P,C) = 2.2 Hz, NCH₂), 61.4 (d, ^1J (P,C) = 24.3 Hz, PCH), 124.7 (s, C=C), 129.0 (s, C=C), 151.7 (d, ^1J (P,C) = 9.0 Hz, C=P). ³¹P{¹H} NMR (81.0 MHz, CDCl₃, 25 °C): δ = 2.8 (brs); MS (70 eV): m/z (%): 295 (1) [M^+], 281 (14) [M^+ – BH₃], 267 (100) [M^+ – 2BH₃].

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- [9] a) X-ray structure analysis of **8** (C₁₄H₃₂B₂N₃P): M = 295.02, colorless crystals 0.20 × 0.20 × 0.15 mm, a = 10.394(1), b = 16.959(2), c = 11.189(1) Å, β = 111.52(1)°, V = 1834.8(3) Å³, ρ_{calc} = 1.068 g cm^{–3}, μ = 1.45 cm^{–1}, empirical absorption correction with SORTAV (0.972 ≤ T ≤ 0.979), Z = 4, monoclinic, space group $P2_1/n$ (No. 14), λ = 0.71073 Å, T = 198 K, ω and φ scans, 7778 measured reflections ($\pm h$, $\pm k$, $\pm l$), ($\sin \theta$)/ λ = 0.59 Å^{–1}, 2648 independent (R_{int} = 0.095) and 1689 observed reflections ($I \geq 2\sigma(I)$), 190 refined parameters, R = 0.077, wR^2 = 0.180, max. residual electron density 0.40/–0.26 e Å^{–3}, hydrogen atoms calculated and refined as riding atoms.^[16] b) X-ray structure analysis of **9** (C₁₄H₂₆N₃O₃P·1.5C₆H₆): M = 416.51, yellow crystals 0.35 × 0.15 × 0.05 mm, a = 6.866(1), b = 28.907(1), c = 12.439(1) Å, β = 104.68(1), V = 2388.2(4) Å³, ρ_{calc} = 1.158 g cm^{–3}, μ = 1.37 cm^{–1}, without absorption correction (0.954 ≤ T ≤ 0.993), Z = 4, monoclinic, space group $P2_1/c$ (No. 14), λ = 0.71073 Å, T = 198 K, $\omega/2\theta$ scans, 14554 measured reflections ($\pm h$, $\pm k$, $\pm l$), ($\sin \theta$)/ λ = 0.65 Å^{–1}, 5417 independent (R_{int} = 0.054) and 3943 observed reflections ($I \geq 2\sigma(I)$), 293 refined parameters, R = 0.078, wR^2 = 0.144, max. residual electron density 0.34/–0.31 e Å^{–3}, disorder of the atoms C7, C8, N9 and the *iso*-propyl groups are refined with restraints, hydrogen atoms calculated and refined as riding atoms.^[16]
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and SCHAKAL (E. Keller, Universität Freiburg, 1997) for molecular graphics. 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-155454 (8) and CCDC-155453 (9). 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).

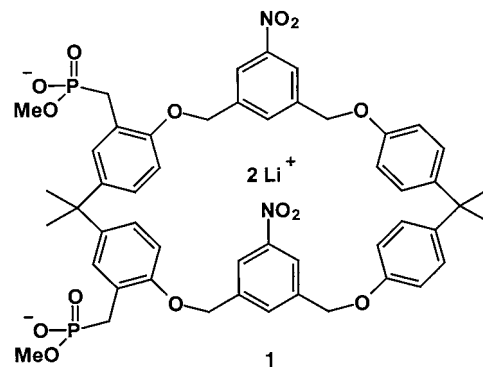
Towards Synthetic Adrenaline Receptors—Shape-Selective Adrenaline Recognition in Water**

Michael Herm, Oliver Molt, and Thomas Schrader*

*Dedicated to Professor Günter Wulff
on occasion of his 66th birthday*

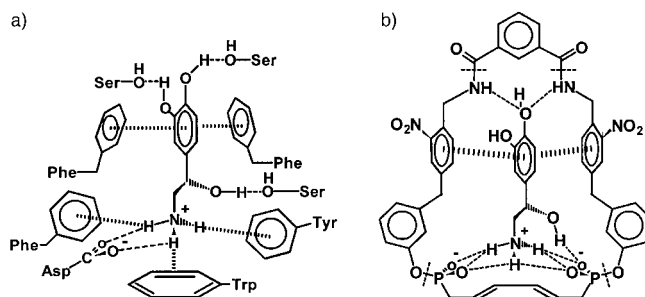
The adrenergic receptor family is one of the most intensively investigated receptor types. Its G-protein-coupled signal transduction influences a broad range of vital body functions—from respiration to blood pressure.^[1] Every year about 3000 research articles appear dealing with biochemical, medicinal, and pharmaceutical aspects of this important class of receptors. However, mainly because of the lack of X-ray crystal structures of these integral membrane proteins, their tertiary structure and mechanism of action have not been fully elucidated to date.^[2] A synthetic model which imitates the postulated receptor–ligand interactions could shed new light on the efficiency of the specific combination of selected weak attractive forces. Such a model could also become a new prototype for artificial adrenaline sensors. Many attempts have been made to create synthetic receptor molecules for catecholamines. Most of these are monotopic: in some recent developments dopamine selectivity has been achieved with a pyrazol-containing podand, a homocalix[3]arene triether, as well as with a sol–gel process.^[3] Enantioselective 3,4-dihydroxyphenylalanine (DOPA) recognition has been achieved by a peptide–bipyridinium cyclophane.^[4] Boronic acids have been used in newer ditopic receptors for molecular recognition of the catechol ring.^[5a,b] In an alternative design the catechol has been bound by a symmetric hydrophobic cavity with peripheral carboxylate groups for dopamine recognition.^[5c] All these artificial host molecules, however, are far from biomimetic and not selective for catecholamino alcohols.

Recently, we introduced macrocyclic receptor molecule **1** with a hydrophobic cavity for the strong binding of adrenaline derivatives in methanol.^[6] However, the amphiphilic host molecule undergoes strong self-association in water and is not



selective for adrenaline derivatives. We felt, that in order to imitate the natural binding site, an artificial biomimetic adrenaline host should be able to provide—at least after an induced-fit process—a microenvironment with a shape complementary to the geometrical form of its guest. A high number of van der Waals contacts would help desolvation in water and lead to a strong hydrophobic attraction.^[7] In addition, the functional groups of the artificial receptor molecule should be positioned much more precisely, so that multiple noncovalent interactions could become effective in a cooperative fashion simultaneously after docking of the substrate. Extensive modeling experiments suggested a new approach, namely incorporation of the xylylene bisphosphonate moiety in a macrocycle, which should also be able to form the sandwich arrangement found in the natural example, as well as provide binding sites for the catechol hydroxyl groups at its opposite end.

Scheme 1 shows our solution to this problem: the nitro-arene groups in the macrocyclic receptor molecule **2** can undergo double π -stacking interactions with the catechol ring of adrenaline without producing any significant ring strain in the receptor molecule, while the isophthalic amide group is ideally preoriented to form hydrogen bonds to the phenolic OH groups. Molecular mechanics calculations predict high binding enthalpies resulting from the interplay of noncovalent interactions operating simultaneously.^[8] Monte–Carlo simu-



Scheme 1. a) Noncovalent interactions between noradrenaline and the β -adrenergic receptor; b) energy-minimized structure of the complex formed between noradrenaline and macrocyclic bisphosphonate **2**. The dashed lines indicate retrosynthetic cuts.

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lations in water reproducibly find energy-minimum structures very close to the postulated conformation of the complex formed between noradrenaline and **2** in Scheme 1 (Figure 1). Even the geometry of the free host is confirmed as an open conformation ready to receive adrenaline-type guests. In the absence of a metal cation the phosphonates are rotated more or less outwards to avoid electrostatic repulsion and to gain solvation energy.

In the retrosynthetic analysis (Scheme 1b) two apparent cuts lead to an activated *p*-xylylene bisphosphonate moiety and an isophthalic acid derivative. The remaining unit is the

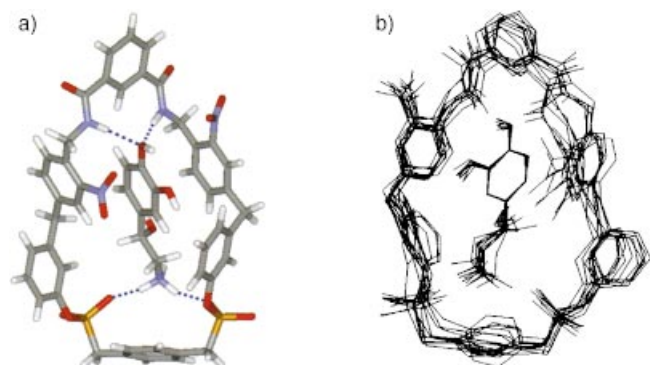


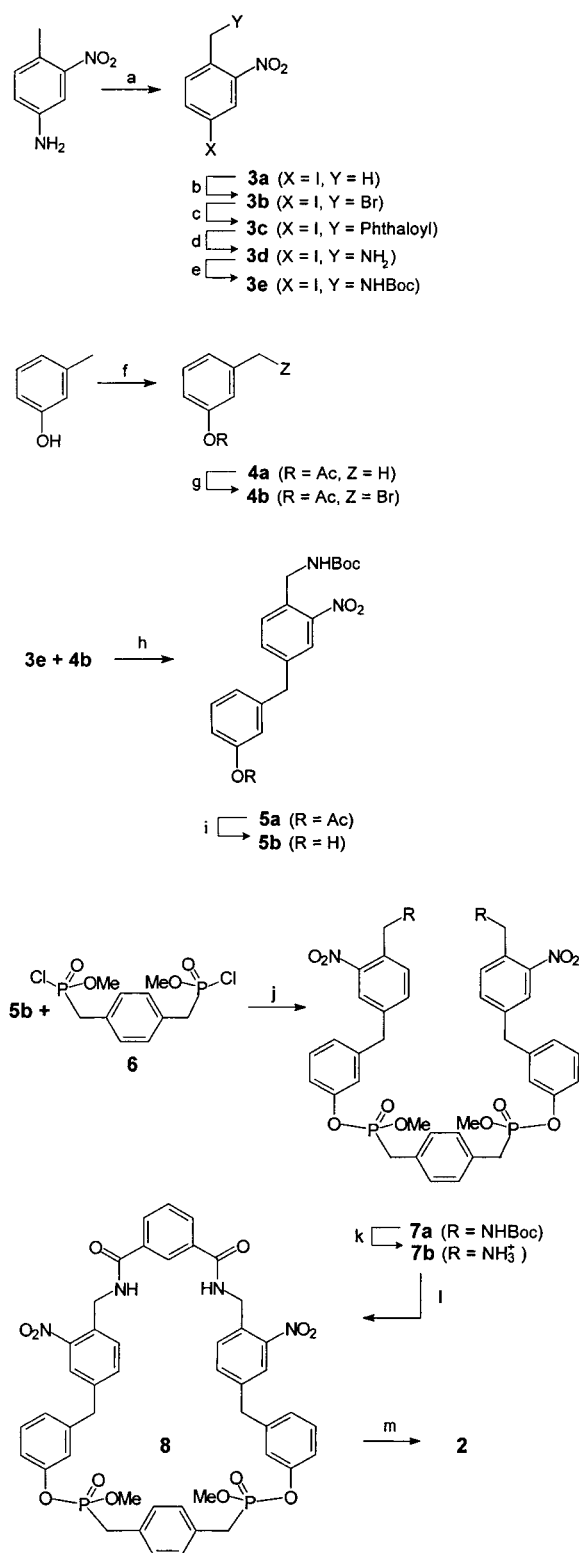
Figure 1. a) Structure of the complex formed between noradrenaline and macrocyclic bisphosphonate **2** according to Monte-Carlo simulations in water; b) superimposed snapshots of the subsequent molecular dynamics calculation.

central highly functionalized diphenylmethane building block with an unsymmetrical substitution pattern.

This target molecule can be effectively synthesized by a modified Negishi coupling, that is, a Pd-catalyzed cross-coupling reaction of aromatic iodide **3e** with the benzylzinc reagent obtained from **4b** (Scheme 2).^[9] Deprotection of the *O*-acetyl groups in **5a**, followed by treatment with *p*-xylylene bisphosphonic acid ester chloride **6** to form a double ester furnishes the U-type predecessor **7a**. After cleavage of the Boc protecting groups under mild conditions, the critical macrocyclization step is carried out with isophthalic acid dichloride under high dilution conditions. Finally the methyl phosphonate is selectively cleaved with an equimolar amount of LiBr as a mild nucleophile. Receptor molecule **2** is obtained as a colorless hygroscopic solid that is soluble in a wide range of polar solvents (from DMSO to water).

Initial binding experiments were carried out with **2** and noradrenaline hydrochloride in DMSO, methanol, and water. A Job plot (Figure 2a) proved unambiguously that in all solvents a 1:1 complex is formed between host and guest molecule.^[10] The electrospray ionization (ESI) spectrum shows a strong molecular ion peak for the 1:1 complex formed between noradrenaline and **2** (*m/z* 1046). Molecular ion peaks of higher mass could not be detected. For additional structural information NOESY NMR spectra were recorded in DMSO of both individual complexation partners as well as the complex itself.

Intramolecular NOE effects for the guest molecule confirmed that noradrenaline is bound in its thermodynamically favorable, bioactive conformation. Two of the critical flexible



Scheme 2. Modular and convergent synthesis of host **2** from 4-amino-2-nitrotoluene, *m*-cresol, and *p*-xylylene bisphosphonic acid dimethyl ester dichloride; a) 1. NaNO₂, 2. KI (61 %); b) NBS, CCl₄ (46 %); c) potassium phthalimide, [18]crown-6, toluene (94 %); d) N₂H₄, ethanol (65 %); e) Boc₂O, CH₂Cl₂ (95 %); f) Ac₂O (89 %); g) NBS, CCl₄ (47 %); h) 1. Zn, reflux, 2. [Pd(PPh₃)₄]/DIBAL-H (58 %); i) K₂CO₃, methanol, RT (94 %); j) CH₂Cl₂, Et₃N (57 %); k) TFA, CH₂Cl₂, 0 °C (99 %); l) isophthaloyl chloride, Et₃N, THF, benzene, RT (38 %); m) LiBr, acetonitrile, 80 °C >(66 %). NBS = *N*-bromosuccinimide, Boc = *tert*-butoxycarbonyl, DIBAL-H = diisobutylaluminum hydride, RT = room temperature, TFA = trifluoroacetic acid.

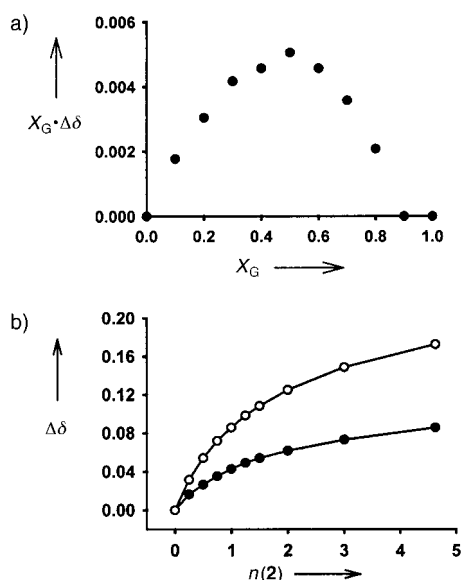
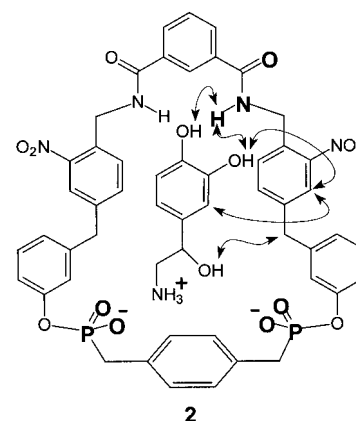


Figure 2. a) Job plot for the formation of a complex between host **2** and the guest (G) noradrenaline hydrochloride (CH-N proton) in D₂O:methanol (1:1); b) ¹H NMR titration curve showing the complexation-induced shifts (CIS) of the CHN (○) and CHO protons (●) on formation of a complex between host **2** (*n* equiv) and noradrenaline hydrochloride in methanol.

host units, namely the *p*-xylylene bisphosphonate and the diphenylmethane centerpiece, show a strong tendency for optimal preorganization, as evidenced from numerous NOE effects observed. In the complex, none of the intramolecular NOE effects of the host and guest are significantly altered, which demonstrates that again there is a good preorientation of the host structure. We were pleased to find a number of intermolecular NOE effects, which indicate the mutual orientation of both complexation partners. The result is exactly the same as predicted by molecular modeling studies (Scheme 3): The amino alcohol binds to the bisphosphonate, the catechol comes close to the nitroarenes, and even the catechol phenolic hydroxyl groups show NOE contacts to the isophthaloylamidic protons.^[11]

Another indication for the complex geometry came from the FT-IR spectra. In the complex of **2** with noradrenaline the phosphonate P=O valence bands as well as those of the amide carbonyl groups were shifted towards lower values. Both effects can be explained by strong hydrogen bonds operating in the recognition motif between the amino alcohol and the catechol phenolic hydroxyl groups.

We then performed NMR titrations (Figure 2b) and calculated the association constants by nonlinear regression.^[12] The decrease of binding constants along the series DMSO (ca. 10⁴ M⁻¹) to methanol (ca. 10³ M⁻¹) to water (ca. 10² M⁻¹) is 100-fold smaller than in the case of the open chain *p*-xylylene bisphosphonate receptors, which rely almost exclusively on electrostatic and hydrogen-bond interactions with the substrate. This result is a clear indication of the presence of additional attractive forces operating especially effectively in water. The weak self-

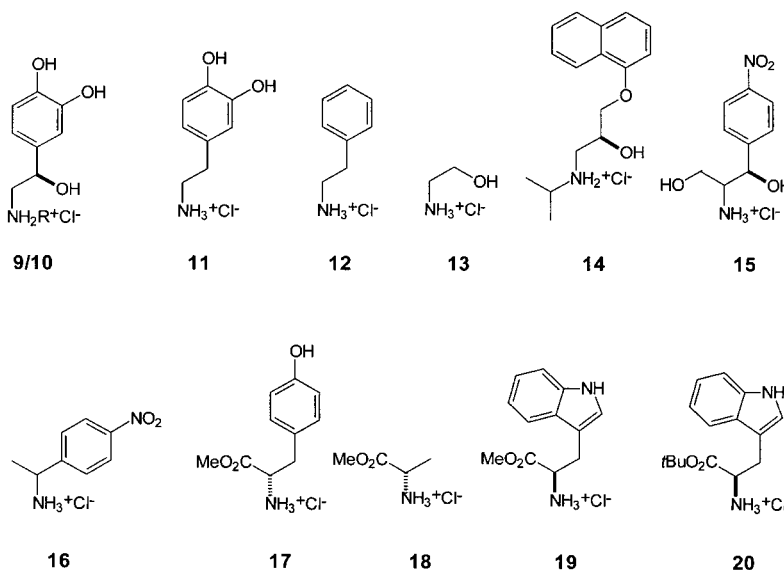


Scheme 3. Intermolecular NOE interactions in the complex formed between host **2** and noradrenaline. The carbon-bound hydrogen atoms have been omitted for clarity. Bold characters show IR-sensitive functional groups involved in hydrogen-bond formation.

association of the free host in pure water (270 M⁻¹) could be completely suppressed in a 1:1 mixture of water and methanol, which was used for almost all of the following investigations.

We examined a number of closely related biogenic amines and amino alcohols to check the selectivity of the new host molecule. We systematically varied the guest structure by deleting selected functional groups from the target molecule adrenaline; this should allow us to estimate their contribution to the overall free binding enthalpy. The investigated guest molecules are presented in Scheme 4; the respective binding constants and ΔG values are summarized in Table 1.

The most striking effect is the small *K_a* value of ethanolamine which is half an order of magnitude below that of noradrenaline: the receptor molecule clearly recognizes the hormone's catechol ring (**13** versus **10**). This effect is also evidenced by the large upfield shift of the aromatic protons in many guest molecules with a 2-phenylethylamine skeleton (up to almost 0.5 ppm). Such aromatic upfield shifts are usually



Scheme 4. Guest molecules for binding experiments with **2**. The structure of adrenaline has been systematically truncated to establish the contribution of specific noncovalent interactions.

Table 1. Binding constants in complexes formed between host **2** and various guest molecules without (**9–15**) and with an alkyl or aryl substituent α to the N atom (**16–20**) as determined by NMR titrations in D₂O:MeOD (1:1).

Guest ^[a]	K_a (1:1) [M ⁻¹] ^[b]	ΔG [kJ mol ⁻¹] ^[b]	$\Delta\delta_{\text{sat}}$ [b,c]	Stoichiom. ^[d]
adrenaline 9 (R = Me)	153 ± 14 %	12.5	0.17 ± 10 %	1:1
noradrenaline 10 (R = H)	215 ± 12 %	13.3	0.12 ± 8 %	1:1
dopamine 11	246 ± 38 %	13.6	0.20 ± 26 %	1:1
2-phenylethylamine 12	102 ± 14 %	11.5	0.41 ± 11 %	1:1
ethanolamine 13	54 ± 45 %	9.9	0.07 ± 34 %	1:1
propranolol 14	204 ± 5 %	13.2	0.23 ± 3 %	1:1
D-threo-2-amino-1-(4-nitrophenyl)-1,3-propanediol (ANP) 15	137 ± 7 %	12.1	0.36 ± 6 %	1:1
α -methyl-4-nitrobenzylamine 16	weak binding ^[e]	–	no saturation	complex
L-tyrosine methyl ester 17	weak binding	–	no saturation	complex
L-alanine methyl ester 18	weak binding	–	no saturation	complex
D-tryptophan methyl ester 19	weak binding	–	no saturation	complex
D-tryptophan <i>tert</i> -butyl ester 20	weak binding	–	no saturation	complex

[a] As hydrochloride salts. [b] Errors [%] are calculated as standard deviations from the nonlinear regression. [c] Bound shift at 100 % complexation, obtained from the curve fitting (selected CH protons). [d] From Job plots and curve-fitting of the titration curves. [e] Maximum observed chemical shifts $\Delta\delta = 0.03$; upper limits for K_a are estimated at $<10 \text{ M}^{-1}$.

indicative of π -stacking interactions, which are necessary for the postulated sandwich-type binding mode of host **2**.

Another interesting feature is the decrease in binding energy when the phenolic hydroxyl groups are deleted from the guest structure (**11** versus **12**). This effect makes **2** selective for catecholamines. Free phenolic OH groups do not significantly enhance the electron-rich character of a benzene ring because of their large negative inductive effect. Hence, the observed decrease in binding energy must be explained by loss of hydrogen bonds between the catechol hydroxyl groups and the isophthalamide head group of the host molecule. A control experiment in DMSO revealed a large downfield shift of one of the hydroxyl proton signals in noradrenaline on formation of a complex with **2**, and is another strong indicator for the formation of hydrogen bonds. The contribution of hydrogen bonds to the catechol hydroxyl groups can be estimated in the 1:1 mixture of water and methanol to be about 2.1 kJ mol^{-1} , whereas formation of π stacks with the catechol arene is worth about 1.6 kJ mol^{-1} .

Table 1 demonstrates a remarkable shape selectivity of the new adrenaline receptor molecule: in general the slim dopamine skeleton is a favorable binding motif for **2** (**9–15**). By contrast, the introduction of an additional substituent α to the ammonium ion, such as in amino acids, leads to weak binding and higher stoichiometries. Clearly, these guests are not included in the interior of the new host (**16–20**). This situation is in sharp contrast to host **1**, which, as a result of its large cavity, could not distinguish between amino acids and adrenaline derivatives. All the effects discussed above confirm that macrocyclic host **2** recognizes adrenaline derivatives in mixtures of water and methanol (1:1) by multiple non-covalent interactions including electrostatic attraction, hydrogen bonds, π stacking, and hydrophobic forces.

In summary, we have designed a shape-selective adrenaline host for dopamine with a binding constant in water which is three orders of magnitude lower than that of the natural example (10^5 M^{-1}). We are currently optimizing the host structure by implementing elements of much higher rigidity to achieve an even more effective preorganization and desolvation.

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From Split-Pool Libraries to Spatially Addressable Microarrays and Its Application to Functional Proteomic Profiling**

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Numerous technologies have been developed to investigate cellular events on a genome-wide scale. Oligonucleotide arrays provide information on changes in mRNA expression levels in response to a variety of physiological stimuli.^[1, 2] Two-dimensional gel electrophoresis,^[3] or other chromatographic separation methods, in conjunction with mass spectroscopy^[4] offer a more direct analysis of proteome function.^[5] Technologies have also been developed for genome-wide analysis of protein structure.^[6] In a more targeted analysis of protein function, maps of protein–protein^[7] and protein–DNA^[8] interactions have been reported as well as preliminary work towards a protein chip.^[9] Methods to monitor the catalytic activity of proteins on a genome-wide scale also provide critical insights into cellular activity.^[10, 11]

Small molecules have long been used to analyze and control the catalytic activity of enzymes as well as to modulate biological networks by acting as agonists or antagonists of receptors.^[12] As such, microarrays of small molecule inhibitors or substrates provide a tool for profiling cellular activity. If one hopes to discriminate between the more than 30000 potential gene products in humans, it is clear that microarrays containing large collections of compounds will be a necessity. High density microarrays of peptides and synthetic oligomers have been reported (40000 compounds cm⁻²),^[13] however the photolithographic techniques used limit the range of accessible molecular diversity. More recently, several small molecules have been printed on a glass slide in an effort to merge robotic printing and split-pool libraries.^[14] While split-pool library synthesis^[15] is far more efficient for the generation of molecular diversity than parallel synthesis,^[16] the identity of each library member is unknown and must be individually decoded for each active library member.^[17, 18] If one wishes to screen such libraries against more than 30000 gene products, the decoding of library members becomes problematic.

Herein we report a method for the preparation of small molecule microarrays using positionally encoded libraries and its application to functional proteomic profiling in a model system. Libraries of small molecules tethered to peptidonu-

cleic acid (PNA)^[19] tags are constructed as shown in Figure 1. The PNA tag serves two purposes, first to encode the synthetic history of the small molecule and second to positionally encode the identity of the small molecule by its location upon

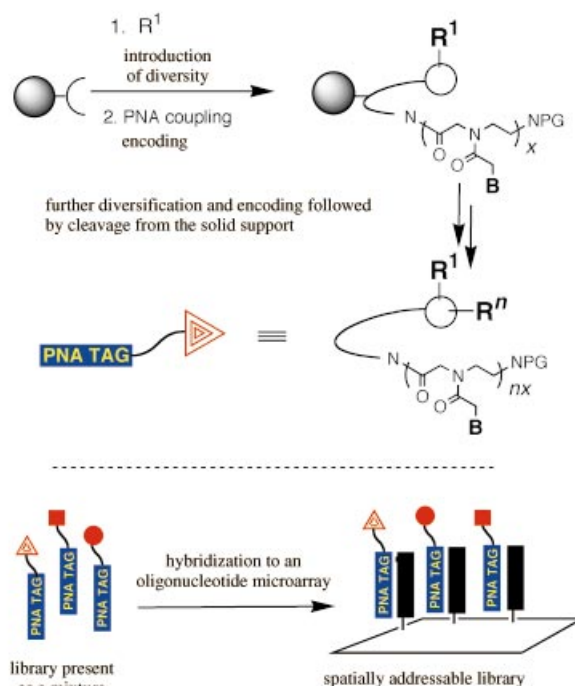


Figure 1. Split-pool synthesis of PNA-encoded combinatorial libraries. Rⁿ = element of diversity present in library, B = base of the peptidonucleic acid, x = number of bases encoding a single element of diversity, n = number of chemical diversity-introducing steps, PG = protecting group.

hybridization to an oligonucleotide microarray. The choice of PNA as the encoding oligonucleotide was based on its desirable hybridization properties, the flexibility of its synthesis, and its chemical robustness.^[20] For library synthesis, the oligomerization of PNAs relies on an amide bond formation, one of the mildest reaction in organic chemistry. Furthermore, the wide array of possible protecting groups for the nitrogen of the PNA's N-terminus should accommodate a wide range of diversity-introducing reactions. Finally, in terms of hybridization properties, the lack of negative charges on the PNA backbone increases its affinity for DNA and reduces the influence of salt concentration on hybridization strength.

PNA-encoded libraries of protein ligands can be screened against several targets simultaneously by incubating the library with the various targets containing different fluorophores. Upon hybridization of the mixture to an oligonucleotide array, fluorescent detection reveals the identity and selectivity of library members for each target. Although attractive for drug discovery, this strategy does not lend itself to profiling biological samples since it is difficult to uniformly label all proteins in a sample of interest. Conversely, the PNA–small molecule conjugate can be synthesized with a fluorophore such that, upon incubation with a sample of interest, the PNA–small molecule conjugate bound to a macromolecule can be separated from the unbound PNA–

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small molecule conjugate by size-exclusion chromatography.^[21] As shown in Figure 2, hybridization of the high molecular weight fraction to a chip will reveal the identity of the small molecules bound to macromolecules, thereby generating a profile of protein function. The correlation between profiles and phenotypes may be rapidly assessed in the biological system using the small molecules identified in the profile while their molecular target(s) can be determined by affinity chromatography.

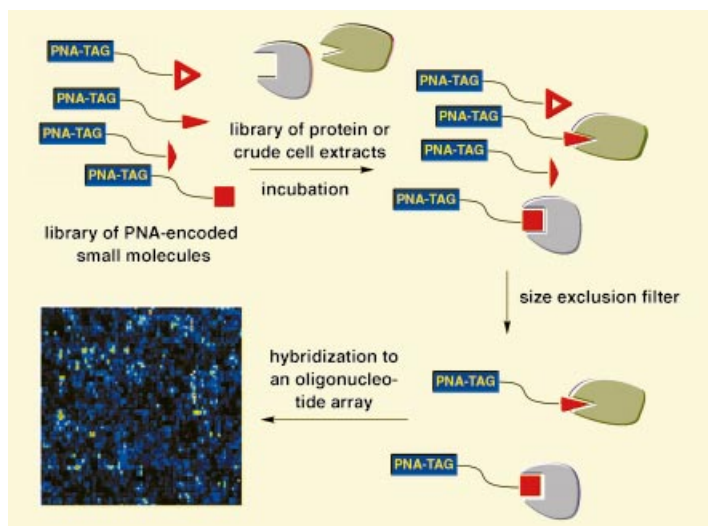
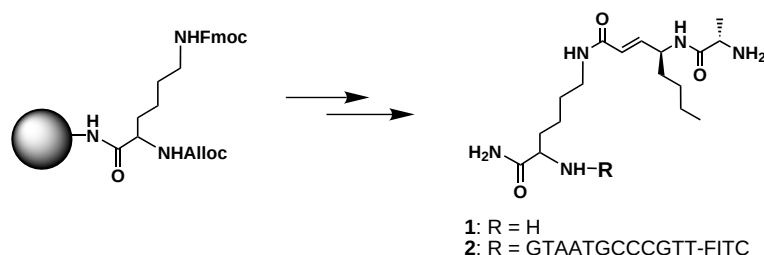


Figure 2. Screening of PNA-encoded libraries.

In order to demonstrate the feasibility of this approach, we focused on mechanism-based cysteine protease inhibitors containing an acrylamide functionality.^[22] Preliminary studies to determine the optimal length of PNA indicated that 12mers have good hybridization properties and allow ample sequence variation to encode very large libraries. The synthesis was carried out on acid-labile Rink resin with mutually compatible Fmoc and Alloc protecting groups for the inhibitor and PNA synthesis, respectively. The side chains and bases were protected with acid-labile groups (Scheme 1).^[23] The design of inhibitors was based on the information gathered from a previously developed method to rapidly assess the substrate specificity of proteases.^[24] A comparison of the activity (Table 1) of compounds **1** and **2** (Scheme 1) against cathepsin C and cathepsin L reveals that the PNA tag does not significantly affect the activity or selectivity of the inhibitor



Scheme 1. Synthesis of the designed cathepsin L inhibitor with (**2**) and without (**1**) PNA tag on a Rink amide resin. Fmoc = 9-fluorenylmethoxycarbonyl, Alloc = allyloxycarbonyl.

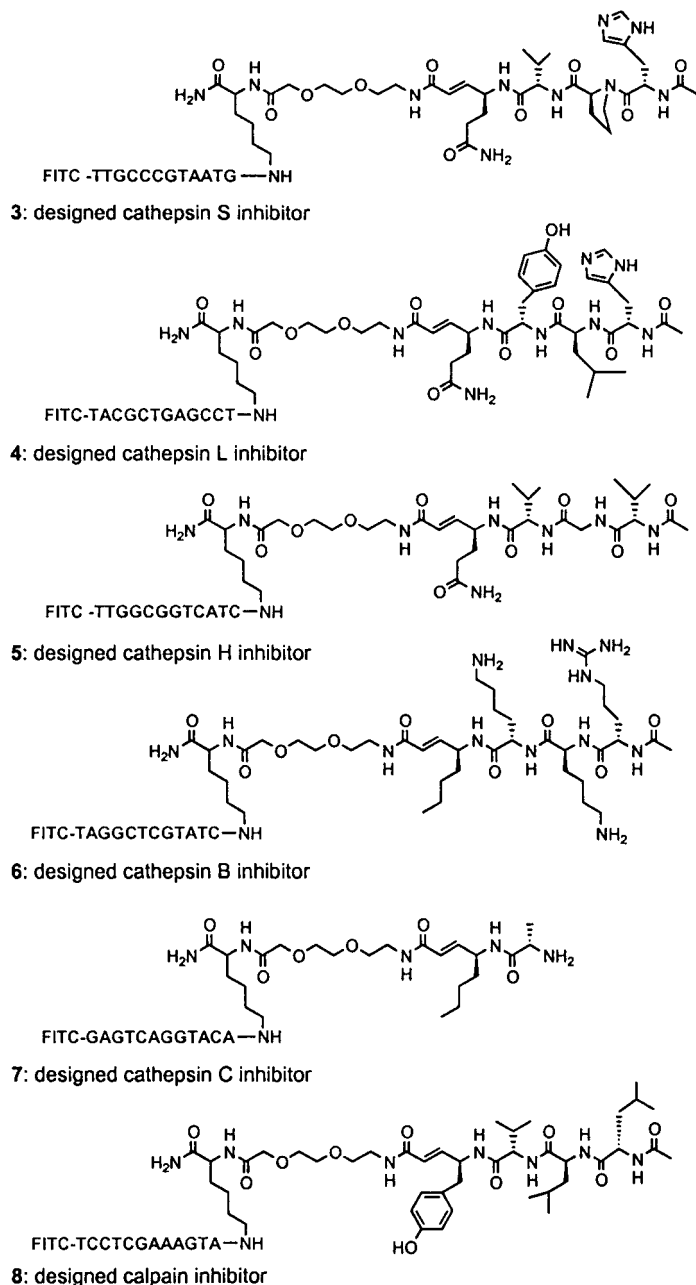
Table 1. Kinetic inhibition of cathepsins by acrylamide inhibitors **1** and **2**.

	Cathepsin C		Cathepsin L	
	IC ₅₀ [μM]	k _{inact} /K _i [M ⁻¹ s ⁻¹]	IC ₅₀ [μM]	k _{inact} /K _i [M ⁻¹ s ⁻¹]
1	17.6	40	> 2000	–
2	14.1	70	> 2000	–

(Table 1). An additional PEG spacer was incorporated in the library synthesis to insure good water solubility.

A series of compounds designed to inhibit cathepsin S, L, H, B, C, and calpain (Scheme 2) were synthesized. The PNA sequences were selected to hybridize to the terminal twelve residues of the 20mer probes of a GenFlex tag array^[25] (arrays of this type are currently available at a density of 400 000 features cm⁻²). The PNA tags only hybridize to a portion of the array probe, and it was expected that each probe would have different hybridization properties. Hybridization of a mixture of the six probes (0.45 pmol of each in 150 μL) afforded the results shown in Figure 3, panel A. The difference in intensity of each array feature reflects the differences in melting temperature of the individual probes. Importantly, despite such differences in melting temperature, 30% changes in probe concentration were reliably detected. An equimolar mixture of the six compounds (**3–8**, 28 pmol) was incubated with commercially available purified cathepsin C (100 μM, 20 μL^[26]) for 2 hours at 23 °C, passed through a size exclusion column (BioRad, Bio-Sil, SEC 125-5) to remove material below 10 kDa, and hybridized to a GenFlex tag array. As shown in Figure 3, panel C, hybridization afforded the expected signal for the probe corresponding to cathepsin C, whereas a control experiment lacking cathepsin C gave no signal (panel B). The same experiment was performed with cathepsin L (10 μM, 20 μL^[26]) using ten-fold less protein. Direct detection of the fluorescein gave a weak signal, but this signal could be amplified using an anti-fluorescein goat antibody followed by a biotinylated anti-goat antibody and phycoerythrin-labeled streptavidin (Figure 3, panel D). These results show that the proposed size exclusion separation is effective to separate the bound PNA–ligand conjugates from the unbound ones, that PNA is efficient for positional encoding, and that small molecule–PNA conjugates can be used to probe protein function in a microarray format.

Cathepsins have been postulated to play important roles in many physiological and pathological functions including antigen presentation,^[27] tumor progression,^[28] osteoporosis,^[29] cataract formation,^[30] and parasitic virulence.^[31] A library based on the described tools enables the identification of specific cathepsin inhibitors, the rapid diagnostic identification of specific cathepsin activity in biological samples, and the identification of novel proteases. The ability to array small molecule libraries prepared by split-pool combinatorial synthesis in a spatially addressable format allows for multiplexed screening in a highly miniaturized format to generate profiles of cellular activity. This approach should also be applicable to other classes of proteases, kinases, and phosphatases, as well as receptors. Screening large libraries as a single mixture in solution prior to hybridization may



Scheme 2. Chemical structure of designed PNA-tagged cysteine protease inhibitors **3–8**. FITC = fluorescein.

prove useful as it minimizes nonspecific interactions of surfaces with the targets. Finally, this technology may prove to be a general and practical solution to prepare and screen microarrays of other biomolecules including antibodies and other proteins.

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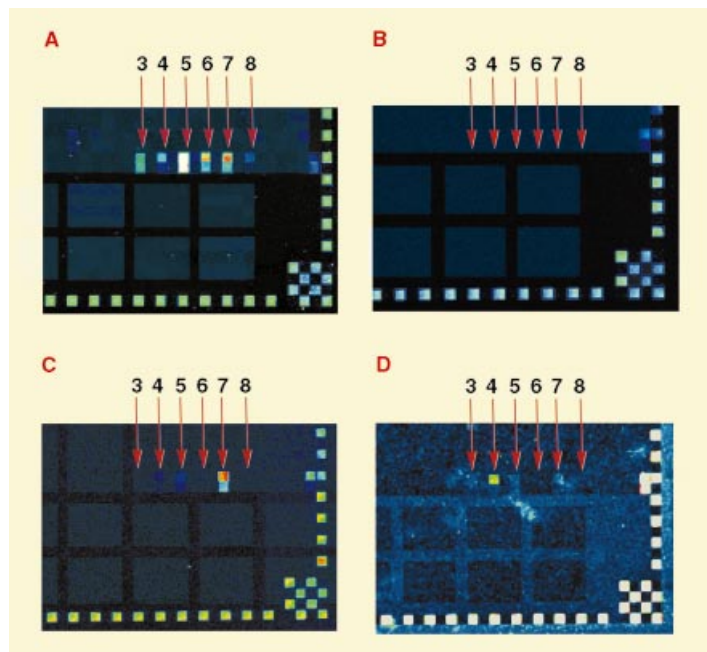


Figure 3. Hybridization of probes **3–8**. A) Hybridization of 0.45 pmol (3.0 nM) of each probe; B) control: incubation of **3–8** (1.4 μ M, 20 μ L) for 2 h at pH 5.5,^[26] size-exclusion chromatography, hybridization; C) incubation of **3–8** (1.4 μ M) with cathepsin C (100 μ M, 20 μ L) for 2 h at pH 5.5,^[26] size-exclusion chromatography, hybridization; D) incubation of **3–8** (1.4 μ M) with cathepsin L (10 μ M) for 2 h at pH 5.5,^[26] size-exclusion chromatography, hybridization.

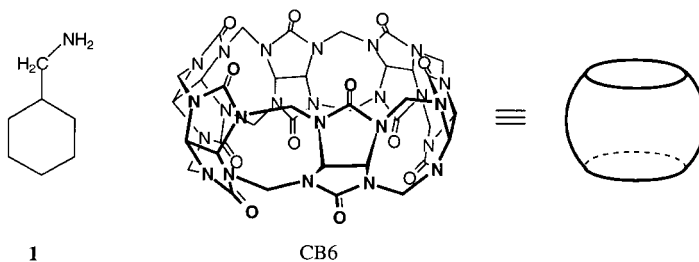
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Two Mechanisms of Slow Host–Guest Complexation between Cucurbit[6]uril and Cyclohexylmethylamine: pH-Responsive Supramolecular Kinetics**

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Supramolecular materials capable of performing specific functions are of great current interest.^[1–3] Ultimately, their suitability will greatly depend, in particular for the case of prospective supramolecular machines, on the speed by which their operations and functions are performed. A prediction and optimization of this speed requires knowledge of the sequence and the rates by which the elementary supramolecular processes occur, which defines the emerging field of supramolecular kinetics.^[4] We have recently introduced a fluorescent probe for measurement of very fast host–guest association processes with cyclodextrins^[5] and now report a study of the factors which govern the other extreme of very slow host–guest complexation kinetics between cyclohexylmethylamine (**1**) and the container compound cucurbit[6]uril (CB6). The pH proved crucial for the kinetics and the rate



constants turned out to be unrelated to the thermodynamics of complexation; rather, they are related to the degree of protonation of the guest. The distinct pH dependence of the kinetics of this host–guest complexation process points to two different mechanisms, in which the ingress of the protonated guest is retarded by the formation of an association complex, while the unprotonated guest can enter the cavity directly with a rate constant 20-fold larger. Such a regulation of the supramolecular kinetics between an organic guest and a container-type host molecule through pH is unprecedented.

CB6 is a glycoluril macrocycle with two “crowns” of six ureido-carbonyl groups on both rims suitable of complexing organic ammonium salts through ion–dipole and hydrogen bonding interactions.^[6, 7] The small cavity openings of CB6 have been considered as portals, the size of which (about 4 Å)^[8] determines whether and how fast complexation occurs. The inner, wider cavity (5.5 Å in width and 6 Å in height)^[8] is suitable to accommodate nonpolar organic residues through

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stabilizing van der Waals interactions. CB6 is a water-soluble molecular container compound^[9] with molecular recognition properties^[3] and forms complexes which closely resemble those of molecule-within-molecule structures,^[10, 11] hemicarceplexes,^[12] and caviplexes.^[13] Besides this function, CB6 has been successfully employed as a supramolecular component in the construction of supramolecular switches,^[6] polyrotaxanes,^[14, 15] and catenanes.^[16] Further, applications for removal of contaminants^[17] from air and water have been reported, as well as the use in fluorescent solids.^[18] Note also that cucurbit[*n*]urils of different sizes ($n = 5-8$),^[19] as well as a permethylated derivative^[20] have been characterized.

The complexation kinetics of CB6 with most organic guests is slow on the NMR time scale, which results in a separate set of resonances attributable to the complexed and the uncomplexed components.^[6, 9, 21] This has allowed Mock et al. to measure the complexation kinetics of cycloalkylmethylamines by a displacement–competition kinetics or the NMR signal-coalescence method (Table 1).^[21] The observed maximum in the binding constants (K) for the medium-sized cyclobutyl-

Table 1. Ingression (k_{ingress}) and egression rate constants (k_{egress}) and binding constants ($K = k_{\text{ingress}}/k_{\text{egress}}$) for the inclusion complexes between CB6 as a host and cycloalkylmethylamines as guests.^[a]

Guest	k_{ingress} [M ⁻¹ s ⁻¹]	k_{egress} [s ⁻¹]	K [M ⁻¹]
cyclopropylmethylamine ^[b]	$\geq 10^6$	$> 10^7$	1.5×10^4
cyclobutylmethylamine ^[b]	5.9×10^3	1.6×10^{-2}	3.7×10^5
cyclopentylmethylamine ^[b]	5.5×10^0	1.6×10^{-5}	3.3×10^5
cyclohexylmethylamine 1 ^[c]	8.8×10^{-4}	1.1×10^{-5}	8.0×10^1

[a] Determined by ¹H NMR spectroscopy in a 1/1 (v/v) D₂O/formic acid mixture at 40 °C. [b] Data from refs. [6, 21]. [c] This work with 3 mM CB6 and 6 mM **1**. The error in the values is 5 %. Data in a D₂O solution with 0.2 M Na₂SO₄ at 40 °C and pH 7.0 are $k_{\text{ingress}} = 8.2 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, $k_{\text{egress}} = 7.3 \times 10^{-5} \text{ s}^{-1}$, and $K = 1.1 \times 10^2 \text{ M}^{-1}$.

methylamine reveals an interplay between stabilizing and repulsive van der Waals interactions between the guest and the walls of the cavity. Interestingly, we have now also observed binding of cyclohexylmethylamine **1**, which has been previously considered as being too bulky to be included.^[22] For this large amine the exchange occurs on the time scale of hours to days, which bypasses an indirect assessment through coalescence or competitive reactions^[21] and allows a direct monitoring of the kinetics in the classical way, namely by taking spectra at different times.

The fact that the kinetics of complexation by CB6 (Table 1), which possesses two tight openings, falls far below the values observed for host systems with unobstructive openings like cyclodextrins^[5] or calixarenes^[23] suggests a physical (steric) barrier towards complexation and decomplexation. The rate constants for ingress (k_{ingress}) and egress (k_{egress}) resemble those observed for some hemicarcerands for which the entrance of the guest through the portals is rate-determining.^[24–29] Such a *constrictive* binding^[30] may also apply for CB6, which resembles hemicarcerands in the size of its portals (about 4 Å).^[6, 21, 22] This is supported by the observed decrease of the k_{ingress} values with increasing steric demand of the guest (Table 1) and the slight decrease of the value of k_{egress} upon

going from cyclopentylmethylamine to the larger cyclohexylmethylamine (Table 1), which is opposite to the expectations arising from the binding constants of these two amines.

The study of rate constants under varying reaction conditions provides an important mechanistic tool. We have analyzed in detail the pH dependence of k_{ingress} and k_{egress} for **1** and compared it to the binding constants K , which can be obtained from the equilibrium concentrations on sufficiently long time scales (Table 2). The experiment could not be performed at very high pH values due to low binding

Table 2. Ingression (k_{ingress}) and egression rate constants (k_{egress}) and binding constants ($K = k_{\text{ingress}}/k_{\text{egress}}$) for the inclusion complex between CB6 and **1** in dependence on pH.^[a]

pH	$10^4 k_{\text{ingress}}$ [M ⁻¹ s ⁻¹]	$10^6 k_{\text{egress}}$ [s ⁻¹]	K [M ⁻¹]
0.6	0.18	0.49	37
1.6	4.0	3.1	130
2.4	6.5	4.0	165
3.6	7.6	4.6	165
4.2	7.6	4.5	170
4.8	8.1	4.9	165
6.0	8.1	4.8	170
7.5	8.1	4.8	170
8.2	8.7	5.2	170
8.6	9.7	5.7	170
10.0	52	30	170
11.0	110	92	120
12.2	140	1100	13
14.0	145 ^[b]	1450 ^[b]	10 ^[b]

[a] Determined by ¹H NMR spectroscopy in D₂O at ambient temperature with 3 mM CB6 and 6 mM **1**. The pH was adjusted through addition of D₂SO₄ or NaOH solutions. The total sodium concentration was adjusted to 0.2 M through addition of appropriate amounts of Na₂SO₄ to achieve a sufficient water solubility of CB6. The error in the values is 5 %. [b] Extrapolated by fitting Equations (1) and (2) to the experimental data, see text.

constants, very fast kinetics, and competitive binding of sodium at the very high NaOH concentrations.^[13] The variation in the binding constant with pH (Figure 1 b) can be accounted for in a straightforward manner. The complex is destabilized at low pH values (<2), at which protonation of CB6 occurs,^[13] and thereby reduces the driving force for complexation of organic ammonium salts; it is also destabilized at high pH values (>11), at which the ammonium complex is converted to the amine complex that lacks the stabilizing ion–dipole interactions with the CB6 ureido-carbonyl groups. In between these two extreme pH values, the binding constant reaches a plateau value of 170 M⁻¹.

The k_{ingress} and k_{egress} values cover a range of more than two orders of magnitude. However, k_{ingress} proved to be unrelated to the driving force for complexation. The data at high pH values demonstrate that although the amine form has a lower binding constant, its k_{ingress} value is higher. Strikingly, a dramatic increase was observed beyond pH 10. Hence, while the equilibrium for inclusion under the experimental conditions was reached only after 1 week at pH 7.5, the same process occurred within hours at pH 10.0 (inset of Figure 1 a). On the other hand, guest release from the complex in dilute solution takes place with a half-life of around 40 h at neutral

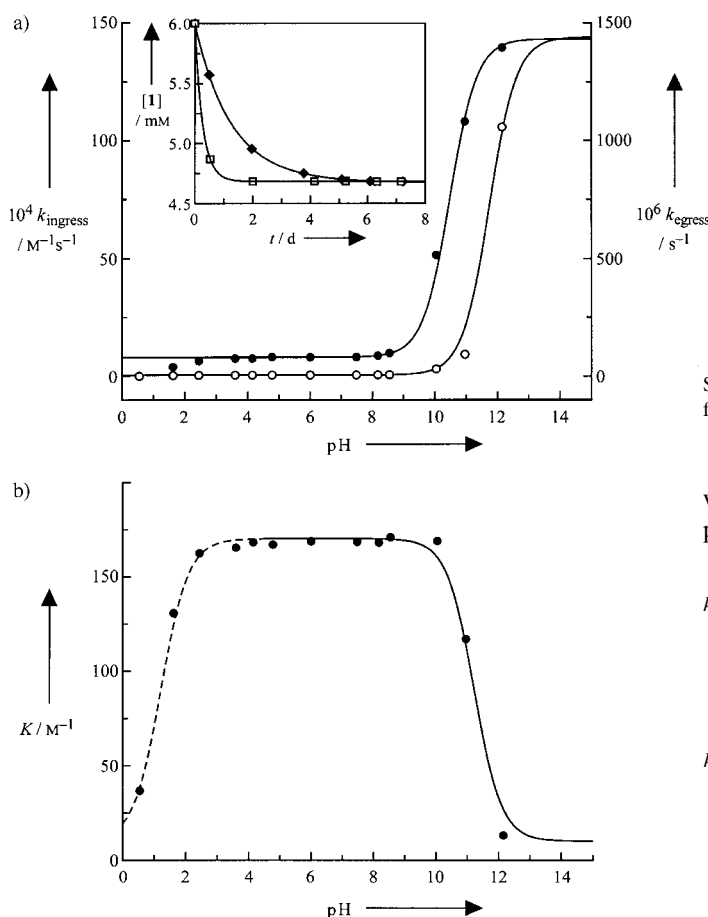
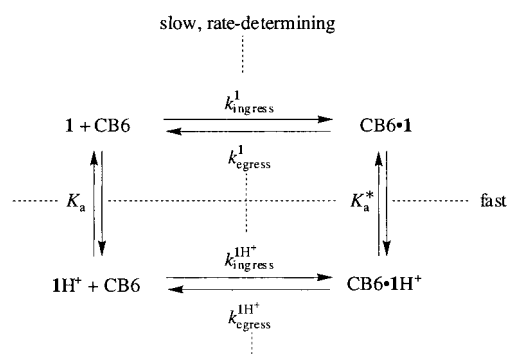


Figure 1. Effect of pH on the kinetics and thermodynamics for complexation between CB6 and **1** according to the data in Table 2: a) Plot of the ingress rate constants (k_{ingress} , ●, left scale) and the egress rate constants (k_{egress} , ○, right scale) versus pH. The deviations for the three data points at lowest pH are due to competitive protonation of CB6, see text; these data points were not included in the regression analysis according to Equations (1) and (2) (interpolated lines). The inset shows representative kinetic decays at pH 7.5 (◆) and pH 10.0 (□). b) Plot of the binding constant ($K = k_{\text{ingress}}/k_{\text{egress}}$) versus pH.

pH but requires merely two hours at pH 11, although the thermodynamics of binding is hardly affected in this pH range.

Since the large change in the kinetics occurred near the $\text{p}K_{\text{a}}$ value of **1**, which was determined as 10.50 in this work by ^1H NMR, in agreement with a previous report,^[31] a competition between ingress and egress processes of the unprotonated and protonated amine and its complex according to Scheme 1 was presumed. Here, the protonation equilibria of the free amine (K_{a}) and the amine complex (K_{a}^*) are fast compared to the slow, rate-determining ingress and egress rates. This is confirmed by the fact that the protonated and unprotonated species are in a dynamic equilibrium on the NMR time scale. The larger k_{ingress} and k_{egress} values at high pH values indicate that the amine form (**1**) undergoes faster ingress and egress than the ammonium form (1H^+). According to Scheme 1, the measured k_{ingress} and k_{egress} values can be expressed as a composite of the rate constants for ingress and egress of the protonated ($k_{\text{ingress}}^{\text{1H}^+}$, $k_{\text{egress}}^{\text{1H}^+}$) and unprotonated forms ($k_{\text{ingress}}^{\text{1}}$, $k_{\text{egress}}^{\text{1}}$), each



Scheme 1. Kinetics for ingress and egress and acid–base equilibria for complexation of **1** and its ammonium form (1H^+) with CB6.

weighted with the fraction of the relevant concentration at a particular pH [Eq. (1), (2)].

$$k_{\text{ingress}} = \frac{[\text{1H}^+]}{[\text{1H}^+] + [\text{1}]} k_{\text{ingress}}^{\text{1H}^+} + \frac{[\text{1}]}{[\text{1H}^+] + [\text{1}]} k_{\text{ingress}}^{\text{1}} \quad (1)$$

$$= \left(\frac{[\text{H}^+]}{K_{\text{a}} + [\text{H}^+]} \right) k_{\text{ingress}}^{\text{1H}^+} + \frac{K_{\text{a}}}{K_{\text{a}} + [\text{H}^+]} k_{\text{ingress}}^{\text{1}}$$

$$k_{\text{egress}} = \frac{[\text{CB6} \cdot \text{1H}^+]}{[\text{CB6} \cdot \text{1H}^+] + [\text{CB6} \cdot \text{1}]} k_{\text{egress}}^{\text{1H}^+} + \frac{[\text{CB6} \cdot \text{1}]}{[\text{CB6} \cdot \text{1H}^+] + [\text{CB6} \cdot \text{1}]} k_{\text{egress}}^{\text{1}} \quad (2)$$

$$= \left(\frac{[\text{H}^+]}{K_{\text{a}}^* + [\text{H}^+]} \right) k_{\text{egress}}^{\text{1H}^+} + \frac{K_{\text{a}}^*}{K_{\text{a}}^* + [\text{H}^+]} k_{\text{egress}}^{\text{1}}$$

Equation (1) can be directly fitted to the experimental k_{ingress} values by using the known $\text{p}K_{\text{a}}$ of **1**. The fit in Figure 1a provided a value of $0.0145 \text{ M}^{-1}\text{s}^{-1}$ for the ingress rate constant of the free amine ($k_{\text{ingress}}^{\text{1}}$), nearly 20-fold larger than for the ammonium form ($k_{\text{ingress}}^{\text{1H}^+} = 8.0 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}$). Equation (2) can be fitted to the experimental k_{egress} values according to [Eq. (3)] if the unknown K_{a}^* value of the complex is expressed through the thermodynamic cycle of Scheme 1.

$$K_{\text{a}}^* = \frac{k_{\text{ingress}}^{\text{1}} k_{\text{egress}}^{\text{1H}^+}}{k_{\text{egress}}^{\text{1}} k_{\text{ingress}}^{\text{1H}^+}} K_{\text{a}} \quad (3)$$

Substitution of Equation (3) into Equation (2) and use of the known values for K_{a} , $k_{\text{ingress}}^{\text{1}}$, and $k_{\text{ingress}}^{\text{1H}^+}$ allows for an excellent fit (Figure 1a) and provides the egress rate constants of the amine and ammonium complexes, $k_{\text{egress}}^{\text{1}}$ and $k_{\text{egress}}^{\text{1H}^+}$, as $1.45 \times 10^{-3} \text{ s}^{-1}$ and $4.7 \times 10^{-6} \text{ s}^{-1}$. The ratio of the rate constants for the free amine ($k_{\text{ingress}}^{\text{1}}/k_{\text{egress}}^{\text{1}}$) provides a weak binding constant of 10 M^{-1} for the unprotonated complex. The kinetic and thermodynamic constants for the free amine are entered in Table 2 as extrapolated to pH 14 values. These can then be used in Equation (3) to derive a $\text{p}K_{\text{a}}^*$ value of 11.75 for the CB6 complex, more than one unit above the free amine. It should be noted that the fitted pH rate profiles in Figure 1a present essentially titration curves, from which the $\text{p}K_{\text{a}}$ values and rate constants (plateau values) can be directly read. The increase in $\text{p}K_{\text{a}}$ upon complexation (from 10.50 to 11.75) is expected in view of the additional stabilization of the ammonium form due to ion–dipole interactions in the

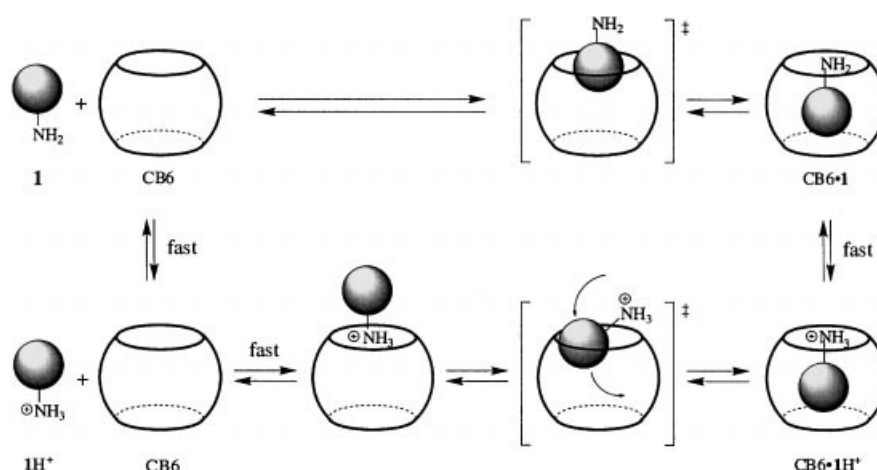
complex. Such complexation-induced pK_a shifts of ammonium ions play an important role not only in supramolecular but also biomolecular systems.^[32]

The overall phenomenon can be qualitatively understood in terms of a change of the preferred pathway for complexation and decomplexation (Scheme 1). At low pH, complexation of the free ammonium form and decomplexation of the ammonium complex predominate. Above pH 10 the complexation proceeds mainly through the free amine, but it is only above pH 11 that the decomplexation of the unprotonated complex becomes important and dominates the egression rate. Consequently, and as can be seen from the comparison of the evolution of the thermodynamics and kinetics with pH (Figure 1a versus 1b), there exists a very narrow region between pH 10 and 11 where the binding constant is similar to the value at lower pH but where the kinetics increases dramatically. From a kinetic point of view, it is thus preferable to assemble such complexes at $pH \approx pK_a$. Knowledge of such protonation effects is important, since amino and ammonium groups are frequently used in supramolecular chemistry.^[11, 33–35]

The fact that the binding constant remains large in a region where most of the free amine is already deprotonated is a consequence of the higher pK_a^* value of the complex. Accordingly, complex formation between pH 10 and 11 does not occur through a direct complexation of the remaining ammonium form but through a faster complexation of the amine form, followed by an immediate protonation of the more basic complex (Scheme 1). Below pH 11 the complex is mainly protonated, such that k_{egress} remains rather constant. Only at larger pH values does deprotonation of the complex become significant, which causes a large increase of k_{egress} and a concomitant decrease in the binding constant. For the present system the binding constant remains similar as the ingress rate constant increases steeply. However, the general reaction mechanism according to Scheme 1 could, in principal, also account for an increase in the binding constant in the $pH \approx pK_a$ region, whenever $pK_a^* \gg pK_a$.

The lower egression rate constant k_{egress} of the ammonium form is consistent with its higher binding affinity. In contrast, the lower $k_{ingress}$ value of the ammonium form is opposite to expectations based on thermodynamic data. A mechanistic explanation must take into account that both the degree of protonation of the amino group (Table 2) as well as the size of the organic residue affect the kinetics (Table 1). Based on the results from molecular dynamics calculations,^[36] we suggest the reaction mechanisms in Scheme 2.

Accordingly, the amine enters by a direct penetration of the organic residue into the nonpolar cavity. This pathway is unfavorable for the ammonium ion, which forms rapidly an intermediary *association* complex, in which the ammonium site coordinates with the ureido-carbonyl rim while the organic residue is still exposed to the aqueous phase.^[37] The subsequent rate-determining ingress of the organic residue



Scheme 2. Proposed mechanisms for complexation of **1** and its ammonium form (**1H⁺**) with CB6.

occurs in a flip–flop manner, namely through a *different* transition state. The transition state for conversion of the association to the inclusion complex requires a significant distortion of the portal, which renders this pathway slower than for the amine, but avoids a complete loss of the stabilizing ion–dipole interactions since it allows the ammonium ion to remain at least partly coordinated with the portal. Typical calculated structures for ingress of the amine and the ammonium form are shown in Figure 2. The involvement

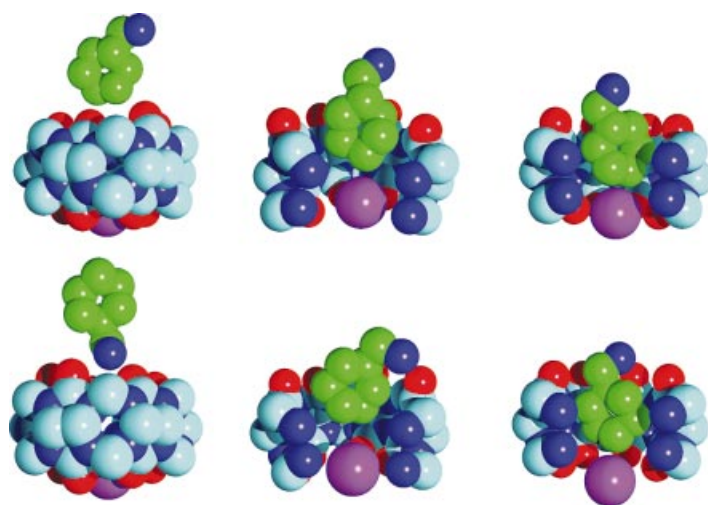


Figure 2. Representative structures obtained in molecular dynamics runs for ingress of unprotonated (top) and protonated (bottom) **1** into CB6. The middle and right-hand structures are shown as intersections. The calculations were run with a sodium cation complexed to the lower portal, which is vital experimentally to promote solubility of the host. Note that the ammonium form (bottom) retains ion–dipole and hydrogen bonding interactions with the carbonyl rim during ingress, while the amine form (top) does not.

of different transition states receives strong support from the rate constants for egression, which differ by more than a factor of 300 for the protonated and unprotonated complex. This difference cannot be entirely accounted for by the ground-state energy difference ($K_a/pK_a^* \approx 18$) and, hence, requires a discrimination of the transition state energies.

Alternative explanations for the observed kinetic effects were also considered. First, differential desolvation of the amine and ammonium forms cannot be decisive for the observed rates since ion and solvent replacement occur on the much faster nano- to microsecond time scale akin to the fast complexation equilibria of inorganic cations, including ammonium, with crown ethers.^[38] Accordingly, the association complexes should be part of a rapid pre-equilibrium, although their spectroscopic properties are not sufficiently distinct to allow direct detection. Further, the possibility that the formation of the association complex reduces the concentration of free ammonium form and uncomplexed CB6, and thereby slows down the kinetics, was also tested. In this case, the association complex does not convert to the inclusion complex, that is, it acts as a dead end and serves merely as a reservoir. However, for such a reservoir effect to cause the observed effect on the ingress rate constant (a factor of 20), a much higher binding constant for the association than for the inclusion complex is required, which contrasts the presumed weak binding in such association complexes.^[37] Moreover, a reservoir effect cannot account for the large decrease of the unimolecular egression rate constant which is higher than expected from the ground state energy difference (see above). A different transition state as suggested in Scheme 2 is therefore required. Note that the refined mechanism in Scheme 2 does not require a modification of the kinetic analysis according to Equations (1) and (2) as long as the association complex is in a rapid pre-equilibrium.

The interesting implication from this mechanistic peculiarity is that the ion–dipole and hydrogen bonding interactions, which stabilize the complex thermodynamically, retard the kinetics of complexation through the formation of an intermediary association complex, which prevents direct inclusion. A closely related example in which such an association complex actually retards the kinetics has been documented by Rebek et al. for the slow complexation of organomercury species by crown ethers.^[39] Clearly, mechanistic details of the elementary reaction steps, which can be assessed through measurement of rate constants under varying reaction conditions, are crucial for the understanding and optimization of the kinetics and thermodynamics of supramolecular processes. Many supramolecular assemblies rely on a combination of different noncovalent interactions like van der Waals forces, hydrogen bonding, π , π interactions, charge-transfer stabilization, or ion–dipole interactions.^[1–3] As demonstrated herein for a prototypal host–guest complex, which results from a combination of ion–dipole and van der Waals interactions as well as steric hindrance, the relative rate constants of the processes governed by a particular interaction and the sequence by which they occur may be an important supramolecular design criterion.

Returning finally to the original context of supramolecular functionality, we have obtained experimental evidence that the presently examined operation (“inclusion of an organic ammonium ion into CB6”) cannot only be “switched on and off” (Figure 1b) but that the operational speed can be “adjusted up and down” by more than an order of magnitude through fine tuning of the pH (Figure 1a). This host–guest system can be considered as a model for a pH-responsive

kinetic regulator as opposed to a thermodynamic switch. Kinetic regulation of supramolecular functions may be crucial, for example, in the cases of supramolecular drug delivery,^[40] to induce an accelerated or retarded release on the time scale of hours to weeks through natural or external stimuli. The pH-responsive guest exchange of CB6 in this attractive yet rarely encountered time range^[28, 34, 41] as well as its water solubility in the presence of physiological electrolyte concentrations are promising features along this train of thought.

Experimental Section

The cyclohexylmethylammonium salt was prepared from the commercial amine (Fluka) through precipitation from diethyl ether upon addition of D₂SO₄. CB6 was from Merck; formic acid, NaOH, and Na₂SO₄ were from Fluka (> 98%). D₂O (Glaser AG, Switzerland, > 99.8%) was used as solvent for all measurements. NMR spectra were obtained at different times after sample preparation on a Bruker DPX 400 MHz Avance spectrometer and the data analysis was performed with the SwaN-MR program.^[42]

Kinetic analysis. The kinetics were in line with a reversible 1:1 host–guest complexation. The restricted solubility of CB6 in nonacidic solutions and the relatively low sensitivity of the NMR integration method precluded the adjustment of pseudo first-order kinetic conditions. We have employed the method described by Mauser^[43] to extract the rate constants for ingress and egression under second-order kinetic conditions. The decrease of the guest concentration, which could be most accurately followed, is given by Equations (4) and (5), which applies for the present conditions where

$$[\text{guest}] = [\text{guest}]_{\infty} - \frac{([\text{guest}]_0 - [\text{guest}]_{\infty})re^{-rt}}{r + k_{\text{ingress}}([\text{guest}]_0 - [\text{guest}]_{\infty})(re^{-rt} - 1)} \quad (4)$$

$$r = \sqrt{(k_{\text{egress}} + k_{\text{ingress}}([\text{host}]_0 - [\text{guest}]_0))^2 + 4k_{\text{ingress}}k_{\text{egress}}[\text{guest}]_0} \quad (5)$$

$[\text{guest}]_0 \neq [\text{host}]_0$ and $[\text{complex}]_0 = 0$. Here, the concentrations of host and guest refer to the sum of the protonated and unprotonated forms, which are in a dynamic equilibrium on the NMR time scale. The kinetics were followed until no further change in concentration was detected. Nonlinear least-squares fitting to the experimental data provided the pertinent rate constants and binding constants in Table 2.

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Combinatorial Discovery of New Photocatalysts for Water Purification with Visible Light**

Christian Lettmann, Heike Hinrichs, and Wilhelm F. Maier*

Photocatalytic conversion of air and water pollutants belongs to the most promising methods of environmental protection. Irradiation of a semiconducting oxide (SCO) with light of energy equal to or larger than the bandgap energy leads to the generation of electron–hole pairs, which can subsequently induce redox reactions at the SCO surface. Heterogeneous photocatalysis is an environmentally friendly method for the detoxification of water and air by sunlight, by which organic pollutants are converted to CO₂, H₂O, and mineral acids. Recent reviews were published discussing the underlying mechanisms and summarizing the state of the art.^[1, 2] Research has been concentrated on anatase (TiO₂), which is photostable, nontoxic, cheap, and active. Due to its large bandgap of 3.2 eV, UV light ($\lambda < 400$ nm) is necessary to generate the electron–hole pairs. This is a major drawback for an efficient water detoxification with TiO₂, since only 3 % of the solar spectrum has wavelengths shorter than 400 nm.

We have discovered recently, that a variety of dopants in sol–gel derived TiO₂, such as Pt, Ir, and even coke, provide photocatalytic activity with visible light at wavelengths > 400 nm.^[3, 6, 7, 12] These discoveries have convinced us, that there may be more photocatalytically active materials than presently known, and we have developed a combinatorial high-throughput technique in order to search more effectively for promising novel compositions. In recent years the potential power of combinatorial approaches and high-throughput (HT) screening for materials and catalysts has already been amply demonstrated,^[4] while reports on the discovery of superior novel substances are still limited.

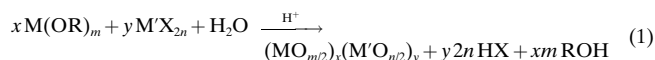
The aim of this work was to develop a reliable HT technique for the efficient discovery of new photocatalysts, capable of photodegrading water pollutants with visible light ($\lambda > 400$ nm). Due to their well-known photocatalytic properties, doped titanium oxides were selected as reference materials during the development of the method. Since nothing is known about the photocatalytic properties of the doped SCOs WO₃ or SnO₂ with visible light, these oxides have been selected as base materials for the discovery libraries.

The “library design” chosen consists of 45 transparent HPLC glass flasks (2 mL) arranged in an addressable rack of nine rows and five columns. The potential catalysts were

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synthesized directly in the HPLC flasks by means of a synthesis robot (Tecan Miniprep 50). For synthesis, a simple sol–gel procedure [Eq. (1)] was applied, which has already been shown to provide excellent photocatalysts.^[3]



Here, M stands for the base oxide (Ti, W, Sn), M' for the dopant, and X for monovalent anions. The recipes were optimized for robotic synthesis. During the whole preparation procedure the reaction mixtures were agitated by a microtiterplate–orbital shaker (Heidolph Titramax 100) to ensure rapid and complete mixing of the reagents. The sol compositions are given in Table 1. For TiO₂ and SnO₂, isopropanol

Table 1. Calcination temperature *T* of the gels, pipetted volumes *V*, and concentrations *c* of the precursor solutions.

SCO	<i>V</i> (iPrOH) [μL]	<i>V</i> (SCO) [μL]	<i>c</i> (SCO) [mol L ^{−1}]	<i>V</i> (dopant) [μL] (<i>c</i> = 0.05 N)	<i>V</i> (HCl) [μL] (<i>c</i> = 12 N)	<i>T</i> [°C]
TiO ₂	251.6	396.9	1.0	79.4	7.8	400
SnO ₂	159.3	396.9	0.282	67.6	5.7	400
WO ₃	600	396.9	0.5	40.4	–	400

solutions of the corresponding tetraisopropoxides were employed as precursors; for WO₃, a synthesis procedure reported by Reisfeld and co-workers^[5] was slightly modified, namely, a solution of H₂WO₄ in water was employed, prepared directly before the synthesis by passing a Na₂WO₄ solution through an acidic ion exchanger (Dowex 50WX8-400). Doping by addition of 1 or 3 mol % metal salts (0.05 N solutions in isopropanol) to the sol was used to modify the light-absorbing properties of the SCOs. For TiO₂ and SnO₂, hydrochloric acid was added to catalyse the gelation. For Pb and Ag as dopants, nitric acid was added instead of hydrochloric acid to avoid precipitation of the corresponding metal chlorides. In the case of WO₃, there was no addition of acid. This approach to doping does not require additional preparation steps and provides a mixed oxide, in which the dopant is homogeneously distributed throughout the solid matrix of the material.

After gelation, the libraries were aged at room temperature for two weeks and subsequently calcined at 400 °C for 3 h to yield the mixed oxides, which remained in the HPLC flasks and were used as obtained. To determine the activity of the catalysts for photodegradation of water pollutants upon irradiation with visible light, 4-chlorophenol (4-CP) was chosen as model pollutant. 1 mL of a 4-CP solution (*c* = 2.5 × 10^{−4} mol L^{−1}), previously saturated with air, was added to each HPLC flask containing a catalyst, and the whole library was then subjected to irradiation with visible light. The light was provided by eight conventional fluorescence lamps (Osram Dulux SG23, 11 W; Figure 1 A), which emit light of wavelengths >400 nm. The lamps were mounted symmetrically side by side in order to assure homogeneous light intensity. A potassium chromate solution (*c* = 1 mol L^{−1}) was placed in a frosted-glass bath (solution depth ~1 cm) to

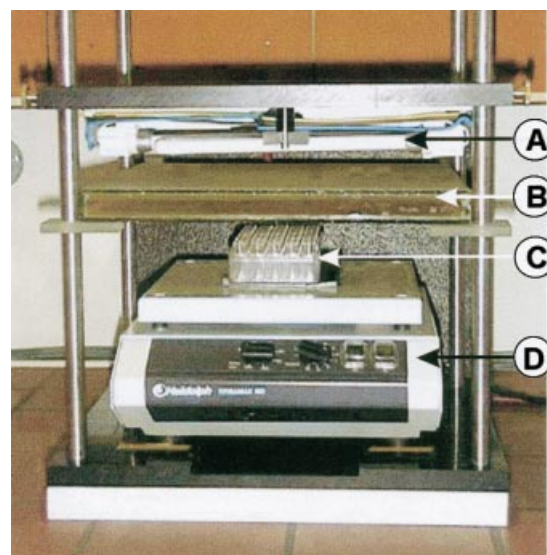
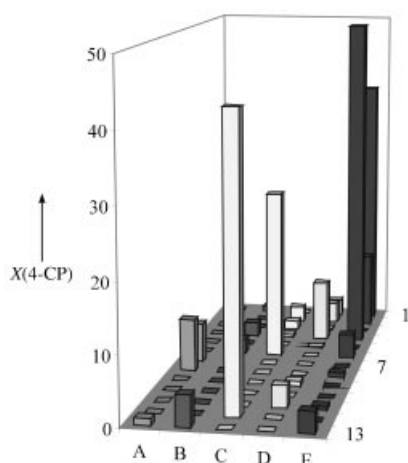


Figure 1. Front view of the experimental setup for visible-light irradiation of the libraries. A) Array of lamps (Osram Dulux S G 23, 11 W); B) bath of frosted glass filled with 1M K₂CrO₄ solution; C) library of 45 HPLC flasks arranged in five columns and nine rows; D) orbital shaker (Heidolph Titramax 100).

remove remaining traces of UV light and to improve the homogeneity of the light-intensity distribution due to multiple scattering (Figure 1 B). The filter was positioned under the lamps, directly above the array of HPLC flasks containing the potential catalysts with aqueous pollutant solutions. The center of the flask array was aligned with the center of the lamp arrangement. The 5 × 9-library of HPLC flasks was illuminated for 2.5 h in the setup shown in Figure 1. During the whole experiment the library was agitated at the maximum shaker speed. After irradiation, the whole library was centrifuged at 4000 min^{−1} to ensure particle-free solutions. The solutions were analysed semi-automatically by HPLC using a standard HPLC sampling robot to quantify the 4-CP conversion.

The adsorption of 4-CP on the porous mixed oxides is a potential source of error. Adsorption experiments with the most active samples on the libraries confirmed that the decrease in solution concentration of 4-CP caused by adsorption at the catalyst surface remained below 5 %. Therefore, only conversions >5 % were associated with photocatalytic degradation. Clearly, the method does not yield identical solid catalysts with respect to particle size and mass-transport conditions. The gel catalysts on the flask bottom were used “as is” after the calcination; some remained clear gels, some showed severe cracks, others were powdery. Therefore the relative activity can be strongly affected by this state of the nature of the solid. This shortcoming was accepted, after we found that no false activities occurred and that the clear as well as cracked gels exhibit photocatalytic activity. However, the relative activities detected in the high-throughput experiments should not be taken quantitatively.

TiO₂ library: In Figure 2 the results obtained from two TiO₂ libraries are summarized. As expected, no conversion was found with the undoped TiO₂ (position D5 and E10) confirming the absence of UV irradiation. For a few catalysts, high 4-CP conversions have been observed. Neglecting



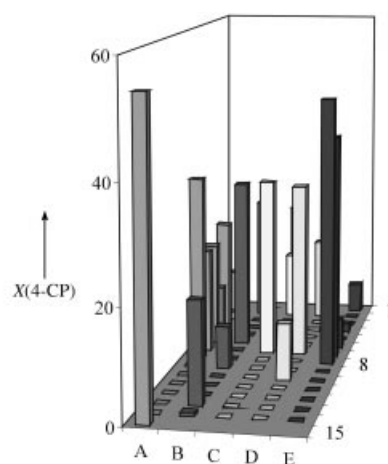
	A	B	C	D	E
1	Sc(NO ₃) ₃ · 5 H ₂ O	V(acac) ₃	VO ₂	OV(OiPr) ₃	CrCl ₃ · 6 H ₂ O
2	MnCl ₂	Mn(OAc) ₃ · 2 H ₂ O	FeBr ₂	FeCl ₃ · 6 H ₂ O	Co(NO ₃) ₂ · 6 H ₂ O
3	Ni(OAc) ₂ · 4 H ₂ O	CuBr	CuCl ₂ · 2 H ₂ O	ZnCl ₂	Y(NO ₃) ₃
4	Zr(OnPr) ₄	NbBr ₅	MoO ₂ Cl ₂	RuCl ₃	RhCl ₃
5	PdCl ₂	La(NO ₃) ₃ · H ₂ O	HfCl ₄	undoped	WCl ₄
6	WCl ₆	ReCl ₅	IrCl ₃	AuBr ₃	Ce(NO ₃) ₃
7	PrCl ₃ · 6 H ₂ O	NdCl ₃ · 6 H ₂ O	SmCl ₃	Eu(NO ₃) ₃ · 6 H ₂ O	Gd(NO ₃) ₃
8	TbCl ₃ · 6 H ₂ O	DyCl ₃ · 6 H ₂ O	HoCl ₃ · 6 H ₂ O	Er(NO ₃) ₃ · 5 H ₂ O	TmCl ₃ · 6 H ₂ O
9	Yb(NO ₃) ₃ · 6 H ₂ O	LuCl ₃ · 6 H ₂ O	Ge(OEt) ₄	In(NO ₃) ₃ · H ₂ O	SnCl ₂
10	SnCl ₄	BiI ₃	Ga(NO ₃) ₃ · 9 H ₂ O	Th(NO ₃) ₄ · 5 H ₂ O	undoped
11	BeCl ₂	MgCl ₂	CaCl ₂	SrBr ₂ · 6 H ₂ O	B(OH) ₃
12	Al(NO ₃) ₃ · 9 H ₂ O	Si(OEt) ₄	Na ₂ [PtCl ₆] · 6 H ₂ O	H ₂ SeO ₃	TeCl ₄
13	LiNO ₃	NaClO ₄	KOAc	Rb(acac)	(NH ₄) ₂ -Ce(NO ₃) ₆

Figure 2. 4-CP conversions X for TiO₂-based mixed oxides after 2.5 h of irradiation. Each library member is identified by its position in the library, given by a column (A–E) and a row (1–13). The table contains the doping salts (1 mol % with respect to TiO₂) added to the corresponding positions during the sol–gel preparation. The library members showing 4-CP conversions > 5% are shaded grey in the table; acac = acetylacetonate.

conversions less than 5%, the following dopands resulted in activation of the TiO₂ matrix: RhCl₃, Na₂[PtCl₆] · 6H₂O, CrCl₃ · 6H₂O, IrCl₃, Co(NO₃)₂ · 6H₂O, RuCl₃, TbCl₃ · 6H₂O, MnCl₂, PrCl₃. The photosensitisation of TiO₂ by RhCl₃, Na₂[PtCl₆] · 6H₂O, and IrCl₃ has already been reported.^[6] The activity of these materials with visible light was discovered by conventional experiments and especially the Na₂[PtCl₆] · 6H₂O-doped TiO₂ was characterized in detail.^[7] The same applies for the Cr- and Co-containing materials.^[8,9] The excellent agreement between our combinatorial with the conventional findings confirms the reliability and reproducibility of the high-throughput method developed. The positive effects of Tb, Mn, and Pr has not been reported in the literature and can be considered new discoveries. These

materials are currently under investigation. Especially the activity of the Mn-doped material is of interest, since manganese oxide is cheap and nontoxic.

SnO₂ library: The ~3.88 eV bandgap of SnO₂^[10] corresponds to an excitation wavelength of 326 nm. This readily explains why the undoped SnO₂ (position E14) is not active under the experimental conditions chosen. Doping of SnO₂ drastically changes the situation (Figure 3): 22 out of the 71 library materials (two arrays) exhibit 4-CP conversions higher than 5% upon visible-light irradiation. As with TiO₂, the Cr-, Mn-, Ru-, Ir-, and Pr-containing samples are active. In contrast to TiO₂, some other dopands also show beneficial effects: transition metals (Hf, V, Ta, Re, Au), lanthanoids (Ce, Tb, Ho), and even main-group metals (Bi, Ca). The origin of

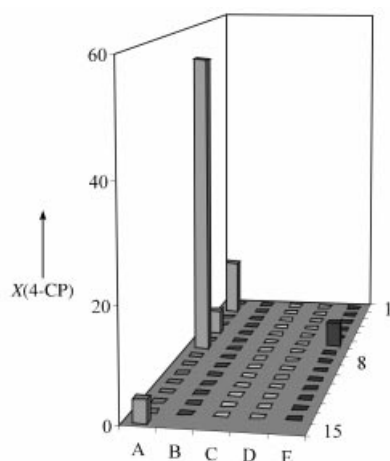


	A	B	C	D	E
1	Sc(NO ₃) ₃ · 5 H ₂ O	Ti(OiPr) ₄	V(acac) ₃	OV(acac) ₂	OV(O'Pr) ₃
2	CrCl ₃ · 6 H ₂ O	Cr(NO ₃) ₃ · 9 H ₂ O	MnCl ₂	Mn(OAc) ₃ · 2 H ₂ O	FeBr ₂
3	FeCl ₃ · 6 H ₂ O	Co(NO ₃) ₂ · 6 H ₂ O	Ni(OAc) ₂ · 4 H ₂ O	CuBr	CuCl ₂ · 2 H ₂ O
4	ZnCl ₂	Y(NO ₃) ₃	Zr(OnPr) ₄	NbBr ₅	MoO ₂ Cl ₂
5	RuCl ₃	RhCl ₃	PdCl ₂	AgNO ₃	La(NO ₃) ₃ · H ₂ O
6	HfCl ₄	Ta(OEt) ₄	WCl ₄	WCl ₆	ReCl ₅
7	IrCl ₃	Na ₂ [PtCl ₆] · 6 H ₂ O	AuBr ₃	Ce(NO ₃) ₃	PrCl ₃ · 6 H ₂ O
8	NdCl ₃ · 6 H ₂ O	SmCl ₃	Eu(NO ₃) ₃ · 6 H ₂ O	Gd(NO ₃) ₃	TbCl ₃ · 6 H ₂ O
9	DyCl ₃ · 6 H ₂ O	HoCl ₃ · 6 H ₂ O	Er(NO ₃) ₃ · 5 H ₂ O	TmCl ₃ · 6 H ₂ O	Yb(NO ₃) ₃ · 6 H ₂ O
10	LuCl ₃ · 6 H ₂ O	BeCl ₂	MgCl ₂	CaCl ₂	SrBr ₂ · 6 H ₂ O
11	B(OH) ₃	Al(NO ₃) ₃ · 9 H ₂ O	Ga(NO ₃) ₃ · 9 H ₂ O	In(NO ₃) ₃ · H ₂ O	Si(OEt) ₄
12	Ge(OEt) ₄	SnCl ₂	SnCl ₄	PbClO ₄ · 3 H ₂ O	SbCl ₃
13	SbCl ₅	BiI ₃	H ₂ SeO ₃	TeCl ₄	Th(NO ₃) ₄ · 5 H ₂ O
14	LiNO ₃	NaClO ₄	KOAc	Rb(acac)	undoped
15	(NH ₄) ₂ -Ce(NO ₃) ₆				

Figure 3. 4-CP conversions X for SnO₂-based mixed oxides after 2.5 h of irradiation. Each library member is identified by its position in the library, given by a column (A–E) and a row (1–15). The table contains the doping salts (1 mol % with respect to SnO₂) added to the corresponding positions during the sol–gel preparation. The library members showing 4-CP conversions > 5% are shaded grey in the table.

these activities is not understood yet. It is assumed that excitation of the dopant ions by visible light results in an excited state high enough in energy to allow transfer of the electron to the conduction band of SnO_2 . None of these materials have been known for their photocatalytic properties.

WO_3 library: At 2.8 eV, the bandgap energy of WO_3 is much smaller than that of TiO_2 and SnO_2 . As a consequence, only visible light of wavelengths $\lambda < 443$ nm is required to generate electron–hole pairs in undoped WO_3 and some activity could have been expected. However, as shown in Figure 4, the undoped WO_3 (position E14) and most other materials on the library are not active. This may be attributed to a high recombination rate of electron–hole pairs and/or the difficulty to photoreduce O_2 .^[11] Only very few dopants cause significant 4-CP conversion, among which Ir is by far the most



	A	B	C	D	E
1	$\text{Sc}(\text{NO}_3)_3 \cdot 5 \text{H}_2\text{O}$	$\text{Ti}(\text{O}i\text{Pr})_4$	$\text{V}(\text{acac})_3$	$\text{OV}(\text{acac})_2$	$\text{OV}(\text{O}i\text{Pr})_3$
2	$\text{CrCl}_3 \cdot 6 \text{H}_2\text{O}$	$\text{Cr}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$	MnCl_2	$\text{Mn}(\text{OAc})_3 \cdot 2 \text{H}_2\text{O}$	FeBr_2
3	$\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$	$\text{Co}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$	$\text{Ni}(\text{OAc})_2 \cdot 4 \text{H}_2\text{O}$	CuBr	$\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$
4	ZnCl_2	$\text{Y}(\text{NO}_3)_3$	$\text{Zr}(\text{O}n\text{Pr})_4$	NbBr_5	MoO_2Cl_2
5	RuCl_3	RhCl_3	PdCl_2	AgNO_3	$\text{La}(\text{NO}_3)_3 \cdot \text{H}_2\text{O}$
6	HfCl_4	$\text{Ta}(\text{OEt})_4$	WCl_4	WCl_6	ReCl_5
7	IrCl_3	$\text{Na}_2[\text{PtCl}_6] \cdot 6 \text{H}_2\text{O}$	AuBr_3	$\text{Ce}(\text{NO}_3)_3$	$\text{PrCl}_3 \cdot 6 \text{H}_2\text{O}$
8	$\text{NdCl}_3 \cdot 6 \text{H}_2\text{O}$	SmCl_3	$\text{Eu}(\text{NO}_3)_3 \cdot 6 \text{H}_2\text{O}$	$\text{Gd}(\text{NO}_3)_3$	$\text{TbCl}_3 \cdot 6 \text{H}_2\text{O}$
9	$\text{DyCl}_3 \cdot 6 \text{H}_2\text{O}$	$\text{HoCl}_3 \cdot 6 \text{H}_2\text{O}$	$\text{Er}(\text{NO}_3)_3 \cdot 5 \text{H}_2\text{O}$	$\text{TmCl}_3 \cdot 6 \text{H}_2\text{O}$	$\text{Yb}(\text{NO}_3)_3 \cdot 6 \text{H}_2\text{O}$
10	$\text{LuCl}_3 \cdot 6 \text{H}_2\text{O}$	BeCl_2	MgCl_2	CaCl_2	$\text{SrBr}_2 \cdot 6 \text{H}_2\text{O}$
11	$\text{B}(\text{OH})_3$	$\text{Al}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$	$\text{Ga}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$	$\text{In}(\text{NO}_3)_3 \cdot \text{H}_2\text{O}$	$\text{Si}(\text{OEt})_4$
12	$\text{Ge}(\text{OEt})_4$	SnCl_2	SnCl_4	$\text{PbClO}_4 \cdot 3 \text{H}_2\text{O}$	SbCl_3
13	SbCl_5	BiI_3	H_2SeO_3	TeCl_4	$\text{Th}(\text{NO}_3)_4 \cdot 5 \text{H}_2\text{O}$
14	LiNO_3	NaClO_4	KOAc	$\text{Rb}(\text{acac})$	undoped
15	$(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$				

Figure 4. 4-CP conversions X for WO_3 -based mixed oxides after 2.5 h of irradiation. Each library member is identified by its position in the library, given by a column (A–E) and a row (1–15). The table contains the doping salts (1 mol % with respect to WO_3) added to the corresponding positions during the sol–gel preparation. The library members showing 4-CP conversions $> 5\%$ are shaded grey in the table.

active one, followed by Cr. Both materials have not been mentioned in the literature and are thus true discoveries.

To confirm the photocatalytic properties of the newly discovered materials not based on TiO_2 , a conventional study was added. The mixed oxides are abbreviated as with the amorphous mixed oxide (AM) notation $\text{AM-M}'_x\text{M}$, where M' is the dopant and M is base oxide ($\text{AM-Ir}_1\text{W}$ than stands for a mixed oxide composed of 1 mol % iridium oxide and 99 mol % tungsten oxide). The $\text{AM-Ir}_1\text{W}$ and $\text{AM-Ce}_3\text{Sn}$ mixed oxides were prepared on a 0.7 g scale by the sol–gel procedure, dried, calcined, and milled. The fine powders were used as photocatalysts in conventional experiments as described elsewhere.^[12] 30 mg of the Sn- and 180 mg of the W-catalyst were dispensed in a 2.5×10^{-4} M aqueous solution of 4-CP in a stirred glass reactor, which was surrounded by a cooled, aqueous solution of K_2CrO_4 to absorb traces of extraneous UV radiation. The solution was irradiated for 100 min by seven fluorescence lamps (Osram Dulux SG23) and maintained at 25 °C. The decrease of 4-CP concentration was followed by HPLC analysis. In control experiments without UV radiation, a lack of photocatalytic activity was confirmed with commercial TiO_2 (Degussa P25). With $\text{AM-Pt}_2\text{Ti}$, a 30 % conversion of 4-CP was obtained after 100 min, with $\text{AM-Ir}_1\text{W}$ 8 %, and with $\text{AM-Ce}_3\text{Sn}$ 12 %. Although the activities of the newly discovered SnO_2 - and WO_3 -based photocatalysts are still lower compared to the well-studied $\text{AM-Pt}_2\text{Ti}$, the photocatalytic activity of the new materials with visible light was confirmed under conventional reaction conditions. Both catalysts were characterized by various methods and compared to the undoped SnO_2 and WO_3 , prepared by the same sol–gel procedure. Argon-adsorption isotherms showed these materials to be mesoporous (Brunauer–Emmett–Teller (BET) surface areas [m^2g^{-1}] were 112/120 and 12/10 for the doped/undoped SnO_2 and WO_3 , respectively). While the tin oxides are both X-ray amorphous, the tungsten oxides both show the diffraction pattern of small-particle WO_3 . High-resolution transmission electron microscopy imaging revealed the presence of small (5 nm) SnO_2 crystallites embedded in the amorphous bulk matrix of the tin oxides, while the size (~ 10 nm) and number of the embedded crystallites in tungsten oxides are significantly larger. However, the microstructure of both photocatalysts is identical to that of the undoped, photocatalytically inactive reference oxides, which concludes that the photocatalytic activity of both materials can be attributed to dopant effects on the electronic structure of the bulk oxides.

The high-throughput method introduced here provides a low-cost, easy to handle, and powerful tool to screen for better photocatalysts. The reliability of the method was confirmed by the excellent agreement between the conventional and the high-throughput results found for Ti-based mixed oxides. Several new photocatalytically active materials based on doped TiO_2 , SnO_2 , and WO_3 were discovered. In few selected cases studied under conventional conditions their unusual photocatalytic activities were confirmed. This study shows that catalysts capable of utilising visible light can be discovered readily by the pragmatic use of a high-throughput technique. The overall activity of a photocatalyst is not only related to intrinsic electronic properties. Physicochemical

characteristics are also of great importance, for example surface area, adsorption rate of pollutants and oxygen, concentration of surface hydroxyl groups, recombination rate of electron–hole pairs, crystallinity, and so forth. The complex nature of the origin of good photocatalytic properties in combination with our lack of knowledge related to the effects of oxide doping underlines the difficulty to predict the composition of the most active catalyst. The success of this simple study suggests that there may be many more photocatalytically active materials. It also indicates how little we know about the structure–activity relationship for visible-light photocatalysts.

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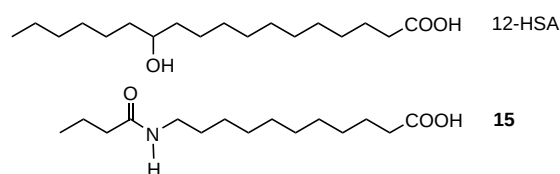
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Rational Design of Low Molecular Mass Organogelators: Toward a Library of Functional *N*-Acyl-1, ω -Amino Acid Derivatives**

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The gelation of organic fluids by low molecular mass organic gelators is a fascinating phenomenon in that it represents a spectacular macroscopic expression of molecular self-assembly.^[1, 2] Organogels have especially attracted much interest as a unique class of nanostructured organic materials with far-reaching applications.^[1–3] A major challenge that arises thus concerns the elaboration of novel design strategies enabling the synthesis of new series of gelator molecules. In that connection, structurally simple compounds,^[4] easy to synthesize and available in large amount from cheap starting materials, are desirable from both fundamental and practical standpoints. Several design approaches have been already proven successful, that are based on the use of various self-recognition units.^[5] Hydrogel formation with amphiphilic molecules was also reported recently.^[6, 7]

Fatty acid compounds are known to form gelatinous and curd-fiber phases in organic solvents, but typically at high concentrations.^[8] Within this series of amphiphile compounds, the behavior of 12-hydroxystearic acid (12-HSA, Scheme 1) attracted special attention.^[9] Indeed, this compound displays a remarkably improved gelation ability in organic solvents as compared to analogous molecules lacking a hydrogen bonding group in the hydrophobic chains.^[10] It occurred to us that *N*-acyl-1, ω -amino acid compounds presenting similar structural features to 12-HSA could also provide gelation ability,



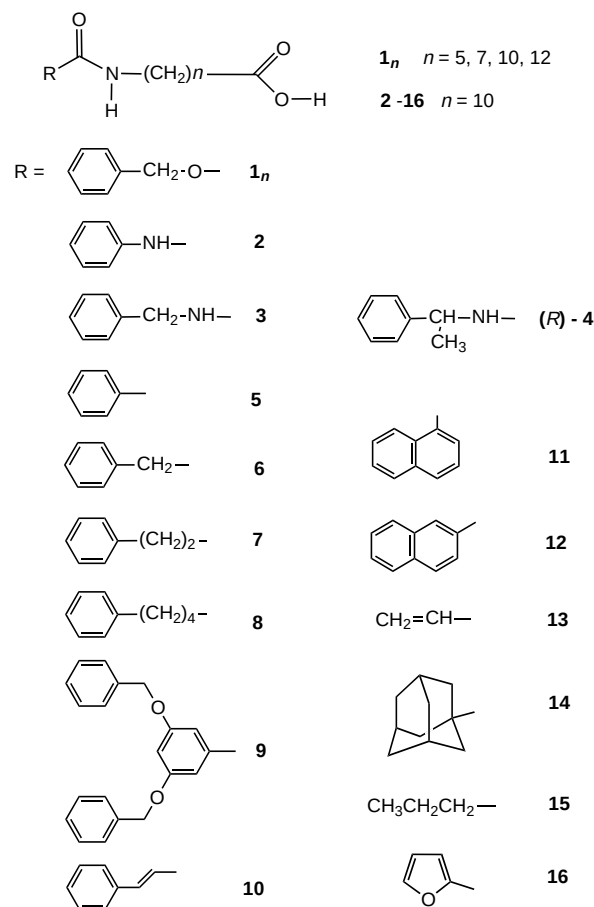
Scheme 1. Structure of 12-HSA in comparison with the new gelator **15**.

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particularly those derivatives containing a long aliphatic chain as spacer between the terminal carboxylic functionality and an amide, urethane, or urea group serving as strong, directional hydrogen bonding unit. Our question was if unbranched achiral molecules like *N*-butyryl-11-aminoundecanoic acid would act as gelators. It should be mentioned that a few studies by different research groups illustrated the formation of fibrous molecular assemblies and gels in the particular case of *N*-acyl derivatives of short amino acids, such as α - or β -alanine, and glutamic acid.^[11]

We report herein on the pronounced gelation ability in different organic solvents of a representative series of selected *N*-acyl-1, ω -amino acid compounds, **1_n** and **2–16**, in which the nature of the pendant *N*-acyl group, the amino acid chain length, and the ionization state of the carboxylic acid functionality differ (Scheme 2). The *N*-benzyloxycarbonyl amino acids, **1_n**, were investigated as prototypes of urethane derivatives with different chain lengths ($n = 5, 7, 10, 12$). The ureas and amide derivatives **2–16** were obtained from amino 11-undecanoic acid ($n = 10$).



Scheme 2. Structures of the compounds **1_n** and **2–16** investigated in this study.

The neutral carboxylic acid derivatives were found to be insoluble in hot CCl_4 and very soluble in DMF at room temperature ($> 20 \text{ mg mL}^{-1}$). Only compounds **13** and **15** were observed to gelate toluene at a concentration of $2 \times 10^{-2} \text{ M}$.

The gelation ability of the sodium salts was investigated with DMF as a solvent, and the results are collected in Table 1.

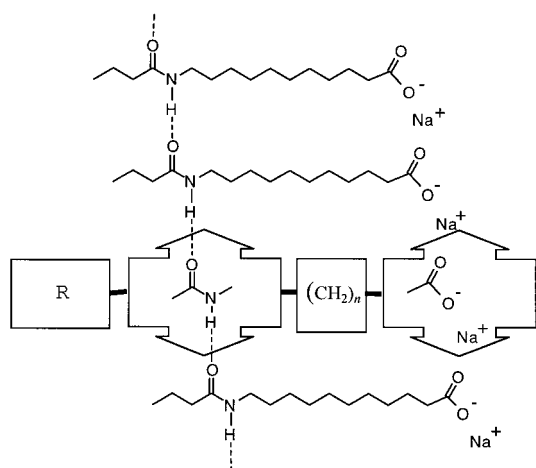
Table 1. Gelation properties of the sodium salts of *N*-acyl amino acids **1_n**, **2–16** in DMF.^[a]

Compound	<i>c</i> [mg mL ⁻¹]	Compound	<i>c</i> [mg mL ⁻¹]
1₁₀ -Na	< 1	9 -Na	< 6
2 -Na	p	10 -Na	< 1
3 -Na	p	11 -Na	< 1.5
4 -Na ^[b]	< 1.3	12 -Na	< 0.6
5 -Na	< 1	13 -Na	< 0.4
6 -Na	p	14 -Na	< 1
7 -Na	p	15 -Na	< 6
8 -Na	< 3	16 -Na	< 2

[a] *c* = minimum gelation concentration (20 °C); p = precipitate.

Efficient gelators of DMF are found in the three classes of compounds. Other aprotic polar solvents, such as DMSO, propylene carbonate, or *N,N*-dimethylacrylamide, were also found to be gelled similarly. The minimum gelation concentration in DMF was in the millimolar range; the acrylamide derivative **13**-Na is the most efficient as only 0.04 wt % is sufficient to immobilize DMF at room temperature. Gelation was observed to be fully thermoreversible, the gel–sol transition for a DMF gel with compound **12**-Na, for example, being observed at about 72 °C at $4 \times 10^{-3} \text{ M}$. Typically, a DMF gel of **1₁₀**-Na was observed to remain stable and transparent for more than one year in a stoppered tube. The data in Table 1 also show that the nature of the covalent link between the amide carbonyl functionality and the aromatic endgroup is critical for the observed gelation. Indeed, amide compounds **6**-Na and **7**-Na with one and two methylene groups, respectively, do not form a gel whereas **5**-Na and **8**-Na, incorporating zero and four methylene units respectively, do gelate DMF. In this regard, it is remarkable that gelation is observed for the chiral urea (*R*)-**4**-Na but not for the achiral analogue **3**-Na, which lacks the methyl substituent. Urethane compounds with a short chain ($n = 5, 7$) gelate DMF but at a higher concentration (about 10 mM, 4 mg mL⁻¹) as compared to the longer analogues ($n = 10, 12$). Noticeably, the gelation propensity is also retained for those compounds that bear a bulky substituent, such as dendron and adamantane moieties in **9**-Na and **14**-Na, respectively. The sodium salts were observed to be insoluble in apolar solvents (cyclohexane, toluene, CCl_4). However, when one drop of methanol was deposited onto the solid gelator compound in a test tube, subsequent addition of these solvents caused instant gelation at room temperature (methanol content 5–7 vol %). The compounds described here are shown to gelate preferably as their sodium carboxylate form. It is likely that both sodium cation coordination and hydrogen bond formation play a major role in the establishment of directional aggregates, as sketched in Scheme 3 on the basis of crystallographic data obtained for long-chain carboxylates.^[6]

This study shows the versatile construction of new gelator systems based on the *N*-acyl amino acid scaffold by incorporating appropriate molecular segments including chiral ones. A combinatorial approach could be helpful to generate a great diversity of *N*-acyl amino acid gelators that can be obtained simply in one step from commercially available amino acids and activated acids libraries. They are thus easy to



Scheme 3. Illustration of one-dimensional self-aggregation in the case of sodium *N*-acyl aminocarboxylate compound **15**. R represents a pendant aliphatic chain or aromatic group.

prepare in large amounts and at low cost. These features open exciting perspectives toward the development of functional material based on this family of organogelators.

Experimental Section

The synthesis of compounds **1_n** and **2–16** was performed according to classical procedures and will be published elsewhere. Chemicals and solvents were used as received.

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A New Method toward Microengineered Surfaces Based on Reactive Coating**

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The control of engineered microenvironments on device surfaces has been addressed by several approaches including soft lithographic methods, such as microcontact printing (μ CP) and micromolding (MIMIC).^[1] These procedures have been used for the formation of a wide range of surface patterns, for example protein and cell arrays,^[2] and for micro- and nanofabrication of devices. Potential applications include the regulation of cell shapes,^[3] the development of micro-electronic elements, such as optical displays,^[4] circuits, or lasers,^[5] and the fabrication of complex three-dimensional microstructures^[6] or microfluidic devices.^[7] A key step is generally the spatially controlled self-assembly of monolayers on a substrate.^[8] Although several systems have been investigated, only assemblies of siloxanes on silicon oxide^[9] and of alkanethiolates on gold^[10] are widely exploited. Biomedical devices are however mostly manufactured from polymers and metals other than gold. For these materials, the microengineering of patterns is very challenging and only addressed in a few cases.^[11] The main limitation is the lack of sufficient and homogeneously distributed functional groups on the substrate surface being necessary for the build-up of further structural elements. Treatment with high-energy sources, such as plasma,^[12] laser,^[13] or ion beams,^[14] has been used to create functionalized surfaces for biomedical systems. Recently, poly(ethylene terephthalate) was surface modified via a multistep synthesis to generate a surface for the μ CP of biological ligands.^[15] Alternatively, CVD-based polymer coatings were used in order to provide amino- or hydroxyl-functionalized surfaces for the conjugation of biomolecules.^[16] Although CVD polymerization has been known for more than 30 years,^[17] the exploitation of functionalized [2.2]paracyclophanes for CVD polymerization was realized only recently.^[18]

Amino- or hydroxyl-functionalized poly(*para*-xylylene) coatings require an additional activation step for linkage of proteins or ligands. Typically, bivalent spacers, such as hexamethylene diisocyanate, are used for amino- or hydroxyl-functionalized polymers.^[16] The additional activation step not only limits the feasibility of microengineering but also causes the contamination of the substrate with organic solvents and volatile chemicals. These contaminations reduce crucial advantages of CVD coatings, such as their low intrinsic cytotoxicity due to the absence of harmful solvents, initiators, or accelerators during polymerization. Therefore, an one-step

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coating procedure that provides linkable reactive groups is highly desirable.

Herein, we report an approach toward engineered micro-environments based on coating with poly(*para*-xylylene carboxylic acid pentafluorophenolester-*co*-*para*-xylylene) (**5**). The technique is applicable to all substrates that are not affected by reduced pressure. For simplicity, we suggest "reactive coating" for polymers like compound **5**. Reactive coating **5** provides active ester groups on the surface being suitable for straightforward linkage of biomolecules presenting amino groups. As a proof-of-principle, we used μ CP of biotin-based ligands. The evaluation was done by means of fluorescein-conjugated streptavidin.

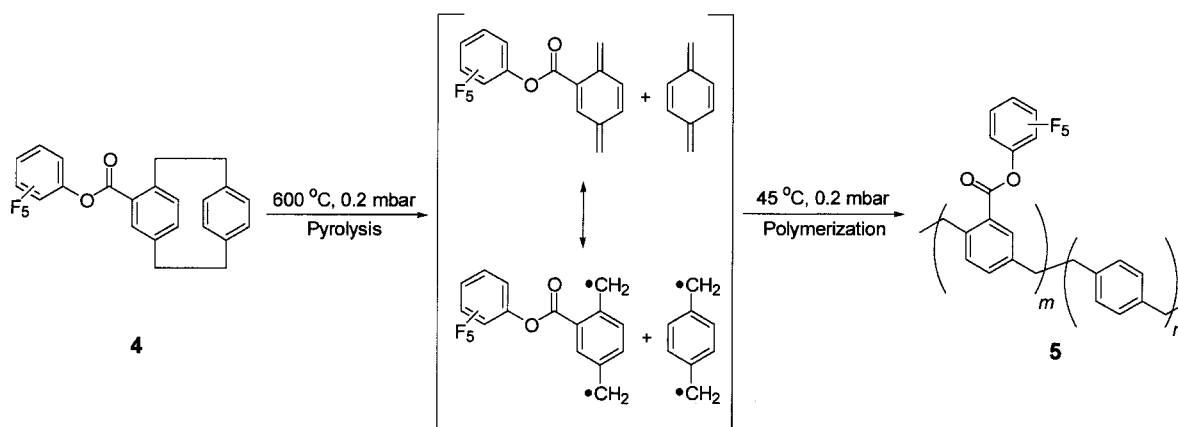
[2.2]Paracyclophane-4-carboxylic acid pentafluorophenolester (**4**) was synthesized from [2.2]paracyclophane (**1**) via a three-step synthesis. Friedel–Crafts acylation of **1** with trifluoroacetic acid anhydride using an excess of AlCl_3 resulted in 4-trifluoroacetyl [2.2]paracyclophane (**2**) in 92% yield. Hydrolysis of **2** to 4-carboxy [2.2]paracyclophane (**3**) (93% yield) and subsequent conversion with pentafluorophenol trifluoroacetate led to the final product **4**.

CVD polymerization of **4** resulted in a vacuum-deposited film of polymer **5** on the substrate (Scheme 1). Carefully purified **4** (dimer) was sublimated under a reduced pressure of 0.2 mbar at temperatures between 120 and 130 °C. Sublimated **4** was transferred to the pyrolysis zone, which was heated to 600 °C to ensure cleavage of the C–C bonds resulting in the corresponding quinodimethanes (monomers). In the last step, monomers were adsorbed on the substrate at temperatures around 45 °C and spontaneously polymerized. The CVD polymerization of **4** resulted in transparent and topologically uniform polymer films of thicknesses between 90 and 600 nm. The film thickness is mainly determined by the amount of **4** used for polymerization. The thickness of a film produced by the deposition of 30 mg of **4** was determined by means of spectroscopic ellipsometry (SE) to be 190.0($\pm$ 5.8) nm. Atomic force microscopy was used to characterize the surface topology: The root-mean square roughness was determined to be 0.4 nm (1 μm^2 spot). The reactive coating showed excellent chemical stability in a dry-air environment. No significant change in composition or chemical behavior was found for

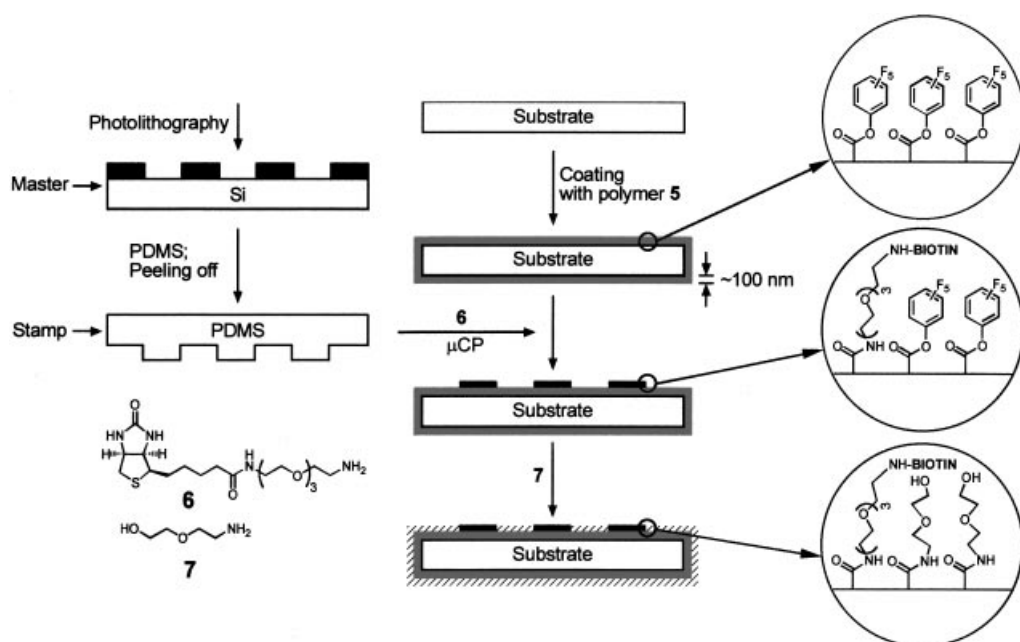
samples stored under dry air for several weeks as compared to freshly prepared samples.

The elemental composition of **5** was determined by X-ray photoelectron spectroscopy (XPS) to be in good accordance with the theoretical composition. Decomposition of the pentafluorophenol ester group was negligible when pyrolysis temperatures under 600 °C and a working pressure between 0.1 and 0.2 mbar were chosen. At higher pyrolysis temperatures, however, significant loss of fluorine due to decomposition of the pentafluorophenol ester group was detected by means of XPS. The IR spectrum of polymer **5** confirmed the presence of the intact ester bond, as indicated by a characteristic signal at 1762 cm^{-1} . Further evidence is provided by characteristic bands of the C–F stretching vibrations between 997 and 1036 cm^{-1} and of symmetric and asymmetric C–O–C stretching vibrations at 1176 and 1246 cm^{-1} . The reactive coating showed excellent adhesion on various substrates, such as poly(dimethylsiloxane) (PDMS), poly(tetrafluoroethylene), poly(carbonate), chrome nitride, gold, and silicon. Polymer **5** is insoluble in common solvents, such as dimethylformamide, chloroform, acetone, ethanol, or aqueous solutions. Incubation of a gold substrate coated with polymer **5** in an aqueous PBS buffer (pH 7.4) for seven days at room temperature did not affect its mechanical stability. Similarly, the reactive coating showed excellent adhesion after Soxhlet extraction for six hours in acetone. Adhesion of the reactive coating to the gold substrate was examined by gently pressing a 1 cm^2 area of a Scotch tape onto the polymer coating. After subsequently peeling off the tape, the sample was examined by optical microscopy and infrared spectroscopy and was mechanically and chemically intact.

(+)-Biotinyl-3,6,9-trioxaundecanediamine (**6**) was used for μ CP of different patterns on gold substrates coated with polymer **5** (Scheme 2). Biotin-based ligands were chosen since biotin is a prototype of a small ligand. Its interaction with streptavidin is characterized by strong noncovalent interaction and allows the facile patterning of streptavidin on the surface. Streptavidin is a widely used immobilization protein that has two pairs of binding sites on opposite faces and therefore represents a universal platform for further patterning of biotin-labeled biomolecules. In this study, fluorescein-labeled streptavidin was used to examine the micropatterns. A



Scheme 1. Reactive coating with polymer **5** by means of CVD polymerization of paracyclophane **4**; polymerization involves quinodimethanes intermediates being in equilibrium with the corresponding diradicals.



Scheme 2. Spatially controlled surface modification of the reactive coating covering a substrate using μ CP. A PDMS stamp, manufactured as a replica from a silicon master, is used for printing ligand **6** onto polymer **5**. The remaining surface area is passivated by reaction with compound **7**.

PDMS stamp was casted from a photolithographically produced silicon master.^[19] Prior to use, the PDMS stamp was oxidized by means of an oxygen plasma.^[20] Ligand **6** was printed via a PDMS stamp on the substrate surface coated with polymer **5**; the contact time was 60 s. Subsequently, the remaining pentafluorophenol ester groups were treated with 2-(aminoethoxy)ethanol (**7**) to passivate nonprinted areas of the surface. The covalent nature of the linkage of the amino ligands to the reactive coating was examined by IR spectroscopy. After μ CP of ligand **6** for 60 s, the IR spectrum of polymer **5** showed characteristic bands at 1653 and 1578 cm^{-1} indicating primary amide bonds. By patterning the substrate

into regions that alternately promote or prevent the binding of streptavidin, spatially controlled self-assembly was expected to occur. A patterned substrate was then incubated with fluorescein-conjugated streptavidin in an aqueous phosphate buffer (pH 7.4) containing 0.1 % (w/v) bovine serum albumin and 0.02 % (v/v) Tween 20. Fluorescence microscopy was used to visualize the microengineered patterns. As shown in Figure 1, the fluorescein-labeled streptavidin was spatially restricted to the biotin-coated areas of the substrate surface. Sharp contrasts were observed. Both micrographs demonstrate homogeneity and reproducibility of the reactive coating on the substrate.

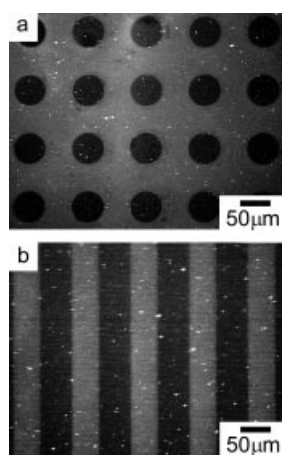


Figure 1. Fluorescence micrographs of fluorescein-labeled streptavidin bound to the ligand **6**, which was patterned onto the reactive coating by μ CP. a) Dot profile with a dot radius of 25 μm ; b) line profile with a line width of 50 μm .

An unique and basically substrate-independent technique toward reactive coatings has been developed and used for spatially designed microenvironments. The resulting surfaces may have value for several biomedical applications, such as tissue engineering, drug development, or molecular diagnostics.

Experimental Section

2: Trifluoroacetic acid anhydride (6.75 mL, 47.75 mmol) dissolved in dichloromethane (30 mL) was added to a suspension of AlCl_3 (5.7 g, 43.13 mmol) in dichloromethane (120 mL) at 0 °C. After 15 min, **1** (5 g, 24.03 mmol) was added while the temperature was held under 5 °C. After stirring for 30 min at room temperature, the reaction mixture was quenched at 0 °C by adding concentrated aqueous HCl (4.4 mL). Extraction with dichloromethane and subsequent column chromatography delivered **2** in 92 % yield. ^1H NMR (300 MHz, CDCl_3 , TMS): δ = 2.90–3.25 (7 H; CH_2), 3.95 (1 H; CH_2), 6.47 (4 H; CH), 6.63 (1 H; CH), 6.78 (1 H; CH), 7.11 (1 H; CH); ^{13}C NMR (75 MHz, CDCl_3 , TMS): δ = 34.54, 35.07, 35.09, 36.31, 118.47, 130.10, 131.36, 132.42, 132.98, 134.67, 136.96, 138.87, 139.39, 139.92, 140.26, 145.41, 145.21, 182.00; IR (KBr): $\tilde{\nu}$ = 844, 986, 1136, 1204, 1708, 2857, 2928, 3047 cm^{-1} ; MS (70 eV): m/z : 304 [M^+], 235 [$\text{C}_{16}\text{H}_{15}\text{CO}^+$], 200 [$\text{C}_8\text{H}_7\text{COCF}_3^+$], 131 [$\text{C}_8\text{H}_7\text{CO}^+$], 105 (100) [C_8H_9^+], 78 [C_6H_6^+], 77 [C_6H_5^+].

3: Compound **2** (5 g, 16.45 mmol) was incubated in aqueous KOH (10 % (w/v), 125 mL) and heated under reflux for 4.5 h. The solid residue was separated by filtration and subsequently washed with chloroform. The organic phase was then extracted with water. Titration of the aqueous phase with concentrated aqueous HCl delivered a precipitate **3** that was purified by recrystallization from a mixture of acetic acid and water (9/1, v/v) (yield 86 %). ^1H NMR (300 MHz, CDCl_3 , TMS): δ = 2.86–3.25 (7 H; CH_2), 4.22 (1 H; CH_2), 6.52 (2 H; CH), 6.59 (3 H; CH), 6.71 (1 H; CH), 7.28 (1 H; CH), 14.03 (1 H; COOH); ^{13}C NMR (100 MHz, DMSO, TMS): δ = 34.79, 34.81, 34.99, 35.79, 131.45, 131.92, 132.36, 132.99, 133.45, 135.56, 136.23, 136.34, 139.53, 139.72, 139.84, 142.27, 168.47; IR (KBr): $\tilde{\nu}$ = 867, 928, 1304, 1686, 2854, 2938, 3010, 3425 cm^{-1} ; MS (70 eV): m/z : 252 [M], 148 [$\text{C}_8\text{H}_7\text{COOH}^+$], 105 (100) [C_8H_9^+], 104 [C_8H_8^+], 91 [C_7H_7^+], 78 [C_6H_6^+].

4: A solution containing **3** (1 g, 3.94 mmol), pentafluorophenol trifluoroacetate (0.7 mL, 4.07 mmol), and pyridine (0.4 mL, 5.0 mmol) in tetrahydrofuran (10 mL) was stirred at room temperature for 12 h. The solution was concentrated under reduced pressure and the remaining crude product

was dissolved in ethyl acetate (35 mL). Extraction with ethyl acetate and subsequent column chromatography delivered 1.15 g of product **4** (88%). ¹H NMR (300 MHz, CDCl₃, TMS): δ = 2.92–2.99 (1H; CH₂), 3.07–3.23 (6H; CH₂), 4.10 (1H; CH₂), 6.51 (1H; CH), 6.54 (1H; CH), 6.60 (1H; CH), 6.65 (1H; CH), 6.68 (1H; CH), 6.81 (1H; CH), 7.39 (1H; CH); ¹³C NMR (75 MHz, CDCl₃, TMS): δ = 34.80, 35.32, 35.45, 36.38, 125.79, 127.50, 131.74, 132.51, 133.28, 133.53, 136.59, 136.81, 138.01, 138.52, 139.71, 139.94, 140.10, 140.12, 140.80, 141.24, 143.50, 145.10, 159.51; IR (KBr): $\tilde{\nu}$ = 513, 630, 693, 790, 852, 902, 994, 1040, 1122, 1163, 1255, 1516, 1757, 2853, 2898, 2926, 2955, 3015 cm⁻¹; MS (70 eV): *m/z*: 418 [M⁺], 314 [C₈H₇CO₂C₆F₅⁺], 251 [C₁₆H₁₅CO₂⁺], 235 [C₁₆H₁₅CO⁺], 131 [C₈H₇CO⁺], 104 (100) [C₈H₈⁺], 77 [C₆H₅⁺].

5: Compound **4** was polymerized using a self-designed CVD installation consisting of a sublimation zone, a pyrolysis zone, and a deposition chamber.^[21] Compound **4** (30 mg, 0.07 mmol) was placed in the sublimation zone and a sample, such as a gold-coated silicon substrate, was fixed in the deposition chamber at 45 °C. The pressure was adjusted to 0.2 mbar and the pyrolysis zone was heated to 600 °C. Subsequently, **4** was sublimated slowly by increasing the temperature of the sublimation zone from 120 to 130 °C. Under these conditions, the deposition rate was 0.4 Å s⁻¹.

The spectroscopic ellipsometry was done on a variable-angle spectroscopic ellipsometer (J. A. Woollam Inc., USA) using a Cauchy model for curve fitting. AFM studies were conducted in tapping mode on a NanoScope III (Digital Instruments Inc., USA).

XPS (atomic ratios): F_{1s}/C_{1s}: 31.7% (calcd: 21.7%), O_{1s}/C_{1s}: 7.4% (calcd: 8.7%), C–F/C–C: 28.3% (calcd: 31.3%), C–O/C–C: 5.7% (calcd: 6.3%), C=O/C–C: 5.4% (calcd: 6.3%); IR (grazing angle 85°): $\tilde{\nu}$ = 658, 829, 997, 1036, 1176, 1246, 1450, 1471, 1497, 1523, 1762, 2862, 2931, 3025, 3054 cm⁻¹.

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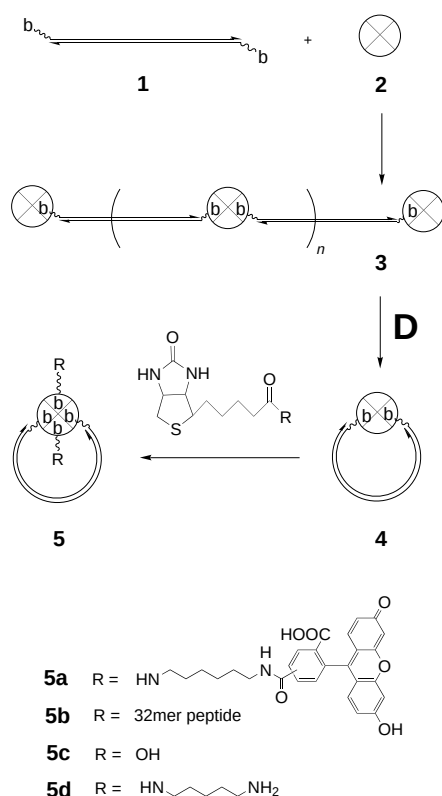
Hapten-Functionalized DNA-Streptavidin Nanocircles as Supramolecular Reagents in a Competitive Immuno-PCR Assay**

Christof M. Niemeyer,* Ron Wacker, and Michael Adler

The self-assembly of small building blocks to form structural and functional elements is an important goal of molecular nanotechnology.^[1] In this respect, DNA is a promising construction material which has already been employed in the fabrication of nanometer-scale scaffolds and surface architectures,^[2] to selectively position proteins^[3] and nanoclusters,^[4] and to generate mechanical molecular devices.^[2] We recently reported the nanostructured oligomeric conjugates **3**, formed by self-assembly of bisbiotinylated DNA **1** and the biotin-binding protein streptavidin (STV) **2** (Scheme 1).^[5] Despite its tetravalent binding capacity for biotin, the STV molecules are predominantly present as bi- or trivalent linkers between double-stranded DNA (dsDNA) fragments within **3**. Because of this remaining biotin-binding capacity, the DNA–STV oligomers **3** are powerful reagents for immuno-PCR (IPCR; PCR = polymerase chain reac-

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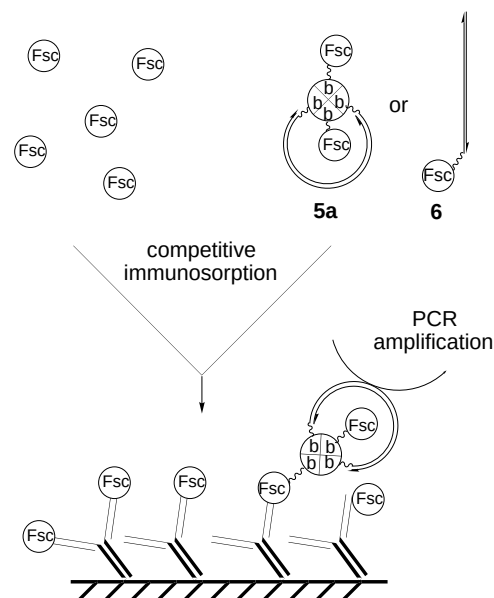


Scheme 1. Synthesis of hapten-functionalized DNA-STV nanocircles **5** from 5',5'-bisbiotinylated 169 bp dsDNA fragments (b = biotinyl) and STV **2**. The oligomeric conjugates **3** are transformed into the nanocircles **4** by thermal treatment, and the crude products are coupled with an excess of biotinylated fluorescein (Calbiochem; **5a**), biotinylated 32mer peptide β -endorphine (EGKKYANKIIANKFLWLPWQSKESWMFGGYK-biotin, Sigma; **5b**), D-Biotin (**5c**), or aminobiotin^[15] (**5d**), and purified by electrophoresis. The complementary DNA strands are drawn as parallel lines. The 3'-ends are indicated by the arrow heads.

tion),^[5] a highly sensitive method for the trace analysis of proteins and other antigens.^[6] Moreover, the oligomers **3** can be transformed by thermal treatment into well-defined supramolecular nanocircles **4** which have two unoccupied biotin-binding sites.^[7] Herein, we report the functionalization of **4** with biotinylated hapten groups, as well as the use of the conjugates **5** as reagents in a novel competitive immuno-PCR (cIPCR) assay for the ultrasensitive detection of low molecular weight substances.

The specific detection of hormones, toxins, pesticides, and other low molecular weight analytes is commonly achieved by methods based on chromatography, mass spectrometry, and biosensors. In addition, antibody-based techniques, such as radioimmunoassay (RIA) or competitive enzyme-linked immunosorbent assays (cELISA) are often applied.^[8] The competitive assay format is necessary since for steric reasons the low molecular weight analyte can only bind to a single antibody. Typically, in cELISA, a conjugate of the traceable hapten and a signal-generating enzyme is employed. The signal-generating conjugate competes with the free analyte for binding sites on a surface.^[8d] As a result of our experience with the ultrasensitive detection of proteins by means of IPCR,^[5] we attempted to investigate whether the PCR amplification of a DNA marker might be applicable to the

competitive detection of low molecular weight analytes (Scheme 2). In such a cIPCR, the hapten-functionalized dsDNA fragment **6** might be used as the signal-generating conjugate. Conjugates such as **6** can be obtained by chemical oligonucleotide synthesis and preparative PCR. However, despite the power of today's solid-phase DNA synthesis, this



Scheme 2. Detection of low molecular weight analytes by cIPCR, illustrated with the model analyte fluorescein (Fsc). The free analyte Fsc competes with the immunosorptive binding of the signal-generating hapten-DNA conjugate to an antibody-modified surface. The covalent Fsc functionalized dsDNA **6** or the hapten functionalized nanocircle **5a** are employed as the hapten-DNA conjugate. After competitive binding and washing, the surface-immobilized hapten-DNA conjugate can be detected by means of PCR amplification.

approach would be laborious since, for instance, appropriate hapten-phosphoramidite precursors would need to be prepared for each particular application. In comparison, the coupling of a hapten with a biotin group is often easier to achieve.^[9] Thus, the modular generation of hapten-DNA conjugates by means of the highly specific biotin-STV interaction appeared very attractive to us. Because of their simple and efficient preparation, as well as their well-defined stoichiometry and structure, the DNA-STV nanocircles **4** were chosen as basic building blocks of a supramolecular construction kit for generating hapten-DNA conjugates.

To experimentally verify our concept, the nanocircle **5a** was prepared containing the model hapten fluorescein. According to the established procedure,^[7] oligomers **3** were generated from 5',5'-bisbiotinylated 169 base pair (bp) dsDNA fragments and STV, the oligomers were thermally denatured, and the crude products were coupled with an excess of biotinylated fluorescein. Subsequently, **5a** was purified by native electrophoresis. The intact circular structure of hapten-functionalized **5** was verified by its characteristic electrophoretic mobility, which is identical to that of **4**. The presence of the fluorescein moiety in **5a** was verified by antibody-induced gel-shift. Since the hapten-containing nanocircles

were supposed to be applied as storable stock solutions for the cIPCR, we investigated the thermal stability of **5a** by incubating aliquots of **5a** for 90 seconds at various temperatures and subsequent analysis by gel electrophoresis (Figure 1). As determined by densitometry, the purity of **5a** was about 93 % (position c in Figure 1). The brief heating of the nanocircle led to the temperature-dependent formation of degradation products which were assigned by their electrophoretic mobility as free DNA (position a), a STV conjugate containing single-stranded DNA (position b), the linearized, opened dsDNA–STV nanocircle (position d), as well as the (dsDNA)₂–STV conjugate (position e). To differentiate between the closed and linearized (opened) nanocircles, which show an almost identical electrophoretic mobility (position c and d, respectively, in Figure 1), an excess of STV was added to an aliquot of the heat-denatured products. This procedure retarded the movement of the opened circle and allowed the quantification of the band intensity of the remaining intact nanocircles. An increase in temperature led to an increase in the amount of degradation products, and a distinctive change in degradation-product distribution was observed between 75 and 80 °C. At temperatures greater than 80 °C an approximately constant amount of a single-stranded DNA–STV conjugate was formed.^[10] The further increase of the temperature over 90 °C led to almost complete degradation of nanocircle **5a**.

Similar experiments were carried out with the nanocircles **5b–d** (Scheme 1). Based on product distributions determined by densitometry, temperature dependent degradation curves were prepared in which the amount of intact nanocircle **5** remaining was plotted against the temperature (Figure 2). To compare the relative thermal stability of **5a–d**, the temperature was extrapolated to give the temperature at which 50 % of nanocircles remained intact (TD₅₀ value). As indicated from the TD₅₀ values listed in the legend to Figure 2, the thermal stability of **5a** through **5d** continuously decreases. It can be estimated that the stability of fluorescein-modified nanocircle **5a** is reduced by about 100 J mol⁻¹, while the stability of aminobiotin-modified circle **5d** is enhanced by

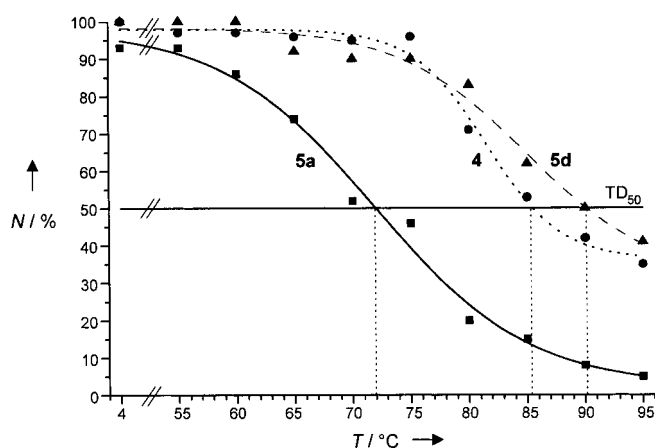


Figure 2. Thermal stability of nanocircles **4** and **5**. The relative product distributions from the experiments in Figure 1 were determined by densitometric analysis. The percentage amounts (*N*) of the remaining intact nanocircles **4** or **5** are plotted against the temperature. The temperature point at which 50 % of the nanocircles remained intact (TD₅₀ value) was extrapolated to be 85 ± 3 °C (**4**), 73 ± 3 °C (**5a**), 77 ± 3 °C (**5b**), 80 ± 3 °C (**5c**), and 90 ± 3 °C (**5d**).

about 40 J mol⁻¹ compared to that of unmodified circle **4**. Functionalization with the biotinylated 32mer neuropeptide β-endorphine^[11] in **5b** and the coupling of D-Biotin in **5c** induces a small destabilization of about 40–50 J mol⁻¹ compared to unmodified **4**. The destabilization of **5a** by the coupling of biotinylated fluorescein, and the slight stabilization of **5d** with aminobiotin might be a result of Coulomb interactions between the negatively charged phosphate backbone of the dsDNA and the negatively charged fluorescein groups in **5a**, or the positively charged amino groups in **5d**.^[12]

To demonstrate the functionality of the hapten-nanocircles **5** in a model cIPCR assay, samples containing a constant amount of **5a** were mixed with increasing amounts of free fluorescein. For competitive immunosorption, these mixtures were added to wells of a microplate previously coated with an antibody directed against fluorescein (Scheme 2). After incubation and washing of the microplate, the DNA immunosorbed was amplified by PCR, and the PCR products were

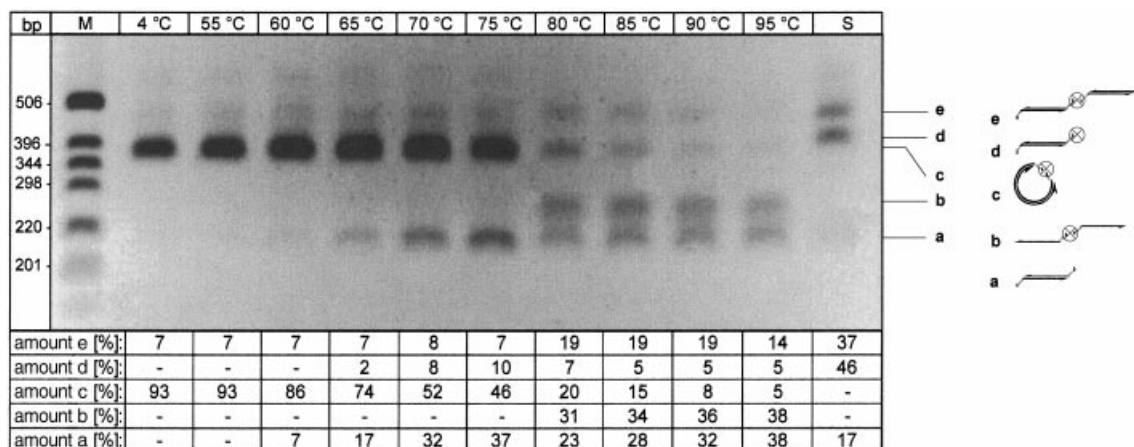


Figure 1. Thermal stability of fluorescein-modified nanocircle **5a**. A nondenaturing 1.5 % agarose gel stained by ethidiumbromide is shown. Samples of purified **5a** were briefly heated to the temperatures listed at the top of the lanes. The percentage yields of the various products formed, listed at the bottom of the lanes, were determined by densitometric analysis. The products a–e were assigned by comparison of the mobility of standards, such as, conjugates of STV and monobiotinylated dsDNA applied to lane S. Lane M contains a DNA molecular-weight marker; the lengths are given in base pairs.

quantified.^[13] For comparison, similar experiments were carried out using the DNA conjugate **6**, which contains a covalently bound fluorescein moiety. As shown in Figure 3, the signals obtained in the cIPCR assays clearly indicate that this novel immunoassay allows trace amounts of the model analyte to be detected. Although direct comparison of **5a** and

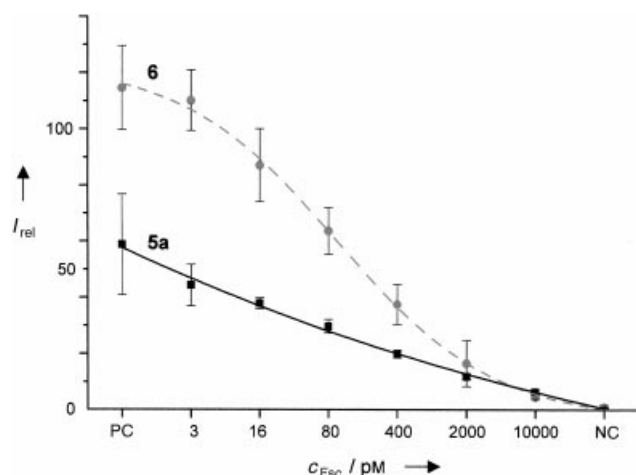


Figure 3. Functionality of the fluorescein–DNA conjugates **5a** and **6** in the cIPCR assay (see Scheme 2). A constant amount of **5a** or **6** was mixed with increasing amounts of free fluorescein (c_{Fsc}) and the mixtures were incubated in microplate wells, previously coated with an antibody directed against fluorescein. After competitive immunosorption, the DNA conjugate immobilized was amplified by PCR. Quantification of the PCR products led to signals which were normalized to relative intensities (I_{rel}) using a PCR negative control (NC) in which no DNA-conjugate was applied to the wells. PC = positive control.

6 reveals greater signal intensities for **6**, the use of **5a** led to significantly smaller errors as well as an improved limit of detection of about 10 pM of the analyte. Further experiments indicated that, as expected, the intensity and the detection limit of cIPCR depends on the amount of both the hapten–DNA conjugate and the antibody immobilized. No reference data on the ELISA detection of fluorescein were available for comparison. However, typical limits of detection of low molecular weight haptens are about ≥ 10 nM by using mass spectrometry, or about ≥ 100 pM by using ELISA and RIA.^[14] Thus, the detection limit of 10 pM for cIPCR observed here with the nonoptimized model-system fluorescein, is similar to the 10- to 1000-fold enhancement of the detection limit regularly obtained by the transition from ELISA to the analogous IP-CR assay.^[5]

We reported here the simple and efficient formation of well-defined functional DNA–protein conjugates by non-covalent assembly of biotinylated dsDNA, biotinylated hapten groups, and streptavidin. The supramolecular complexes can be used as reagents in a novel PCR-based immunoassay, which we term competitive immuno-PCR (cIPCR). Our model studies suggest that cIPCR allows for around a 10- to 1000-fold improvement over the detection limit of conventional antibody-based assays. Further optimization of the cIPCR and applications regarding the detection of biomedical-relevant analytes are in progress. For instance, these

studies indicate that the model system **5a** can be applied to the β -endorphine–peptide nanocircle **5b**, as well as to similar conjugates prepared from biotinylated testosterone.^[9]

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$S_2N_3^+$: An Aromatic SN Cation with an N_3 Unit**

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During a study of the known^[1] thiazyl dichloride ion, $[NSCl_2]^-$, we investigated the Cl^- ion donor–acceptor properties of thiazyl chloride (NSCl). Since the NSX_2 group ($X = F, Cl$) can also act as a ligand and is stabilized by a covalent interaction of the N atom with metals (e.g. $Hg(NSF_2)_2$),^[2] it was of interest to study the reaction of $NSCl/(NSCl)_3$ with $HgCl_2$ in CH_2Cl_2 as a potential route to $Hg(NSCl_2)_2$. By ^{14}N NMR spectroscopy small amounts of the NS^+ ion ($\delta = +201$), as well as the starting material ($NSCl/(NSCl)_3$), were detected, which indicates that in this reaction NSCl can function as a Cl^- ion donor. Surprisingly, crystals

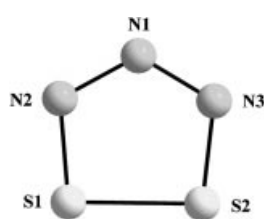


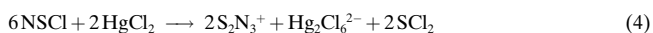
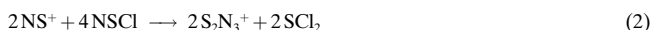
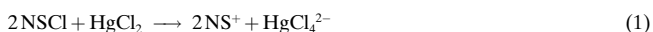
Figure 1. Molecular structure of the $S_2N_3^+$ ion.

were obtained from the yellow CH_2Cl_2 solution. Raman, infrared (IR), and single-crystal X-ray studies of these crystals revealed a ring-shaped planar $S_2N_3^+$ ion (Figure 1) and an $Hg_2Cl_6^{2-}$ ion.^[3]

The $S_2N_3^+$ ion represents the first binary SN ring with an N_3 unit. The other ring isomer of the $S_2N_3^+$ ion with one N atom between the two S atoms is not known. Although, the isovalence-electronic $S_3N_2^{2+}$ ion has

been observed in the solid state by vibrational spectroscopy, this ring readily dissociates in liquid SO_2 into SN^+ and SNS^+ ions.^[4] In contrast, the $\{[S_2N_3]^+\}_2[Hg_2Cl_6]^{2-}$ salt is stable at room temperature, is neither heat nor shock sensitive, and decomposes only above $80^\circ C$. The formation of the $S_2N_3^+$ ion in the reaction of $NSCl/(NSCl)_3$ with $HgCl_2$ indicates a complex reaction. The $S_2N_3^+$ ion might be regarded as the product of a $[3+2]$ cycloaddition of N_3^- and S_2^{2+} ions but this is rather unlikely in view of the starting materials. No evidence was found for the intermediate formation of N_3^- and S_2^{2+} ions. We assume that in the first step a Cl^- ion is abstracted from NSCl by dissolved $HgCl_2$ resulting in the formation of NS^+ and $HgCl_4^{2-}$ ions [Eq. (1)]. In the second step the NS^+ ion could formally react with two NSCl molecules yielding $S_2N_3^+$

and SCl_2 [Eq. (2)]. The formation of the anion [Eq. (3)] gives the overall reaction in Equation (4).



Interestingly, upon layering the CH_2Cl_2 solution with hexane, the $S_2N_3^+$ ion crystallizes with the previously unknown $Hg_3Cl_{10}^{4-}$ counterion (Figure 2). Because of the low solubility of $HgCl_2$ in CH_2Cl_2 , the yield strongly depends on

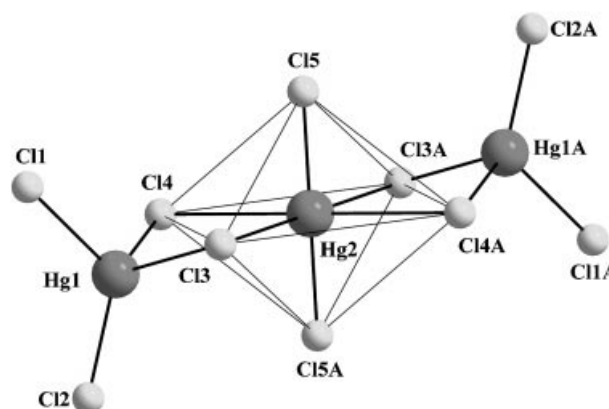


Figure 2. Molecular structure of the $Hg_3Cl_{10}^{4-}$ ion.

the reaction time and only small yields of the $S_2N_3^+$ salts were obtained (5–12 %). In addition, the formation of $S_4N_3^+$ salts was observed. However, the formation of both salts, $\{[S_2N_3]^+\}_4[Hg_3Cl_{10}]^{4-}$ and $\{[S_2N_3]^+\}_2[Hg_2Cl_6]^{2-}$, can be reproduced.

Ab initio (CCSD(T)) and density functional theory (DFT) calculations (B3LYP) indicate that the isolated $S_2N_3^+$ ion in the gas phase possesses a planar C_{2v} structure (Table 1). This prediction turns out to be correct for the salts as well: $\{[S_2N_3]^+\}_2[Hg_2Cl_6]^{2-}$ crystallizes in the triclinic space group $P\bar{1}$ with one independent $S_2N_3^+$ ion; $\{[S_2N_3]^+\}_4[Hg_3Cl_{10}]^{4-}$ crystallizes in the monoclinic space group $P2_1/n$ with two symmetry independent $S_2N_3^+$ ions. The C_{2v} structure of the $S_2N_3^+$ ion in $\{[S_2N_3]^+\}_2[Hg_2Cl_6]^{2-}$ is slightly distorted; the same holds for one $S_2N_3^+$ ion in $\{[S_2N_3]^+\}_4[Hg_3Cl_{10}]^{4-}$, whereas the second has almost exact C_{2v} symmetry (Table 1). The N–N bond lengths of 1.329–1.363 Å are substantially shorter than the sum of the covalent radii ($d_{cov}(N-N) = 1.4$ Å, $d_{cov}(N=N) = 1.2$ Å;^[5] cf. 1.318–1.364 Å in tetrazoles),^[6] which indicates partial double bond character for the N_3 unit. A similar situation is found for the N–S bonds (1.574–1.596 Å) which also lie in the range between a single and a double bond ($d_{cov}(S-N) = 1.74$, $d_{cov}(S=N) = 1.54$ Å;^[6] cf. 1.572–1.593 Å in $S_4N_4Cl^+$, 1.58 Å in $S_3N_2^{2+}$, and 1.55 Å in $S_4N_3^+$).^[4c, 7] The S–S bond length of 2.016–2.031 Å corresponds to a single bond ($d_{cov}(S-S) = 2.08$, $d_{cov}(S=S) = 1.88$ Å).^[5] The N–N–N angle (ca. 120°) is rather

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

Table 1. Experimental and theoretical structural data for the $S_2N_3^+$ ion.

	$[S_2N_3]^+ [Hg_2Cl_6]^{2-}$	$[S_2N_3]^+ [Hg_3Cl_{10}]^{4-}$		B3LYP ^[a]	
		cation 1	cation 2	6-311 + G(3df)	6-31G(d)
$d(N1-N2)$ [Å]	1.329(9)	1.329(8)	1.336(8)	1.297	1.299
$d(N1-N3)$ [Å]	1.348(10)	1.363(10)	1.337(9)	1.297	1.299
$d(N2-S1)$ [Å]	1.574(6)	1.583(5)	1.582(5)	1.622	1.633
$d(N3-S2)$ [Å]	1.583(5)	1.576(5)	1.596(6)	1.622	1.633
$d(S1-S2)$ [Å]	2.016(2)	2.031(2)	2.023(3)	2.026	2.050
$\angle(N2-N1-N3)$ [°]	119.9(6)	119.6(5)	120.3(6)	118.3	118.7
$\angle(N1-N2-S1)$ [°]	114.9(5)	115.4(4)	114.6(4)	117.3	117.3
$\angle(N1-N3-S2)$ [°]	114.3(5)	114.3(4)	114.5(5)	117.3	117.3
$\angle(N2-S1-S2)$ [°]	95.7(2)	95.0(2)	95.6(2)	93.6	93.3
$\angle(N3-S2-S1)$ [°]	95.3(2)	95.7(2)	95.0(2)	93.6	93.3

[a] CCSD(T)/6-31G(d): $d(N1-N2) = 1.318$, $d(N1-S1) = 1.652$, $d(S1-S2) = 2.038$ Å; $\angle(N2-N1-N3) = 118.3$, $\angle(N1-N2-S1) = 117.0$, $\angle(N2-S1-S2) = 93.9^\circ$.

large compared to the N-N-S ($114-115^\circ$) and N-S-S angles ($95-96^\circ$; cf. N-N-N angles in tetrazoles ca. 113°).^[6]

Over the past 30 years a large number of planar, binary molecules and ions containing only the elements sulfur and nitrogen have been isolated and characterized.^[8,9] This class of planar ring systems (e.g. $S_2N_2(6\pi)$, $S_3N_2^+(7\pi)$, $S_3N_3^-(10\pi)$, $S_4N_3^+(10\pi)$, $S_4N_4^{2+}(10\pi)$, $S_5N_5^+(14\pi)$), to which the novel $S_2N_3^+$ ion (6π) belongs, has electronic structures that are related to those of aromatic hydrocarbons.^[10] With the exception of the radical cation $S_3N_2^+$, which is stabilized by a $\pi^*-\pi^*$ interaction upon dimerization,^[4,9,11] these planar SN rings have $(4n+2)\pi$ electrons and therefore formally obey the Hückel rule for aromatic hydrocarbons.^[12]

The π molecular orbitals (MOs) of the $S_2N_3^+$ ion are related to those of $C_5H_5^-$ and P_5^- . Both sulfur and nitrogen are more electronegative than carbon and phosphorous and therefore the π MOs of the $S_2N_3^+$ ion are of lower energy than those of the $C_5H_5^-$ and P_5^- ions.^[4b] The degeneracy of the e_1'' and e_2'' π MOs is removed because of the lower symmetry of the C_{2v} $S_2N_3^+$ ion (Figure 3). Inspection of the occupied π MOs and the calculated electron-density distribution indicate that the π -electron density is mainly localized between the N-N and N-S centers. The calculated sulfur and nitrogen atoms-in-molecules (AIM) net charges are $Q_{S1/S2} = +0.89 e$, $Q_{N1} = +0.12 e$, and $Q_{N2/N3} = -0.45 e$, respectively. These partial charges and the calculated bond orders ($BO(N-S) = 1.72$, $BO(N-N) = 1.60$, and $BO(S-S) = 1.23$) are in agreement with the experimentally observed short S-N and N-N bonds and the S-S single bond. The rather large positive partial charges (resulting in a large nuclear-nuclear repulsion) on both S atoms also contribute to the longer S-S bond, whereas, because of the small amount of S-S π overlap a shorter S-S bond would be expected.

Structural variety within the chloro-mercurate(II) anions is surprisingly large.^[13,14] The formal stoichiometry rarely reflects the nature of the chloro-mercurate(II) anions in the crystal lattice. This diversity stems from the ability of mercury to form $Hg_xCl_y^{n-}$ ions with coordination numbers from three to six with chloro-mercury fragments

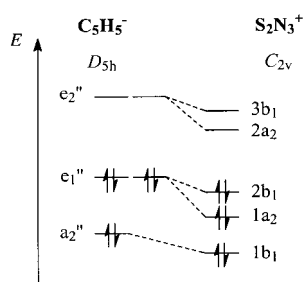


Figure 3. Schematic MO diagram of the aromatic $C_5H_5^-$ and $S_2N_3^+$ ions.

connected through chloro bridges. Hence, mono- ($HgCl_4^{2-}$), di- ($Hg_2Cl_6^{2-}$, $Hg_2Cl_7^{3-}$), tri- ($Hg_3Cl_9^{3-}$, $Hg_3Cl_{12}^{6-}$), tetra- ($Hg_4Cl_{14}^{6-}$) oligo-, or polynuclear chloro-mercurate(II) anions have been observed. To our best knowledge, the $Hg_3Cl_{10}^{4-}$ ion is a new species. It can be assumed that in the reaction mixture of NSCl/ $HgCl_2$ different chloro-mercurate(II) anions coexist in equilibrium.^[13a]

The salts of the known $Hg_2Cl_6^{2-}$ ion (edge-sharing tetrahedra) crystallize from the polar solvent CH_2Cl_2 and have been identified in many compounds.^[13,14] Upon layering the CH_2Cl_2 solution with hexane the novel $Hg_3Cl_{10}^{4-}$ ion crystallizes (Figure 2). The decrease of the solvent polarity causes the insertion of a neutral $HgCl_2$ molecule between two $HgCl_4^{2-}$ ions. The interaction between these three fragments can be considered as a closed-shell interaction.^[15,16] Selected structural data of the $Hg_2Cl_6^{2-}$ and $Hg_3Cl_{10}^{4-}$ ions are summarized in Tables 2 and 3. The $Hg_3Cl_{10}^{4-}$ ion consists of two strongly distorted tetrahedral $HgCl_4^{2-}$ units each sharing one edge unsymmetrically ($d(Cl3-Hg2) = 2.958$ Å and $d(Cl4-Hg2) =$

Table 2. Experimental structural data for the $Hg_2Cl_6^{2-}$ ion.

Bond lengths [Å]		Angles [°]	
Hg1-Hg1A	3.948(2)	Hg1-Cl3-Hg1A	92.11(4)
Hg1-Cl1	2.3662(13)	Cl1-Hg1-Cl2	144.40(7)
Hg1-Cl2	2.369(2)	Cl3-Hg1-Cl3A	87.89(4)
Hg1-Cl3	2.629(2)	Cl1-Hg1-Cl3	111.51(5)
Hg1-Cl3A	2.851(1)	Cl1-Hg1-Cl3A	97.77(5)

Table 3. Experimental structural data for the $Hg_3Cl_{10}^{4-}$ ion.

Bond lengths [Å]		Angles [°]	
Hg1-Hg2	3.865(0)	Cl1-Hg1-Cl2	113.86(5)
Hg1-Cl1	2.578(1)	Cl1-Hg1-Cl3	93.28(5)
Hg1-Cl2	2.406(1)	Cl1-Hg1-Cl4	106.71(5)
Hg1-Cl3	2.635(1)	Cl2-Hg1-Cl3	105.06(4)
Hg1-Cl4	2.433(2)	Cl2-Hg1-Cl4	130.43(5)
Hg2-Cl3	2.958(1)	Cl3-Hg1-Cl4	99.67(5)
Hg2-Cl4	3.079(1)	Cl3-Hg2-Cl4	79.84(4)
Hg2-Cl5	2.335(1)	Cl3-Hg2-Cl4A	100.16(4)

3.079 Å; Table 3) with the $HgCl_2$ unit resulting in a formal highly distorted octahedral environment for the central mercury atom (Hg2). The $Hg_3Cl_{10}^{4-}$ ion has inversion symmetry and shows a great variety of different Hg-Cl bond lengths (2.335–3.079 Å; cf. 2.25–2.34 Å in $HgCl_{2(gas)}$, 2.25 Å

in $\text{HgCl}_{2(\text{solid})}$ ^[17] and Cl–Hg–Cl bond angles ($\angle \text{Cl–Hg–Cl}$ ca. 80–180°). Both bridging Hg1–Cl bonds (2.635 and 2.433 Å) and the terminal Hg1–Cl bonds (2.578 und 2.406 Å) are considerably longer than the axial Hg2–Cl5 bond (2.335 Å).

Experimental Section

General remarks: all experiments and manipulations were carried out under argon (Schlenk techniques). Prior to use all solvents were freshly distilled, dried, and stored under nitrogen. The starting material (NSCl)₃ was prepared according to the literature method.^[18] HgCl_2 was purchased from Merck and dried under vacuum before use. NMR: Jeol Eclipse 400 (¹⁴N NMR chemical shifts refer to $\delta(\text{CH}_3\text{NO}_2) = 0.00$); IR: Nicolet 520 FT-IR; Raman: Perkin Elmer Spectrum 2000R NIR FT.

$[\text{S}_2\text{N}_3]^+[\text{Hg}_3\text{Cl}_{10}]^{4-}$ (**1**): A solution of (NSCl)₃ (0.25 g, 1.02 mmol) in CH_2Cl_2 (10 mL) was added to HgCl_2 (0.42 g, 1.55 mmol). The light yellow mixture was stirred at ambient temperature for 16 h. The color changed to bright orange. The mixture was filtered through a glass sinter (G4). The clear yellow filtrate was allowed to stand for crystallization after layering with hexane (5 mL). (Besides the $[\text{S}_2\text{N}_3]^+$ salt we were able to isolate the $[\text{S}_4\text{N}_3]^+$ salt which was identified by its Raman spectrum.) Yield: 0.04 g (0.03 mmol, 11 %, based on (NSCl)₃); Raman (400 mW, RT): $\tilde{\nu} = 1180 \text{ cm}^{-1}$ (0.1, ν_a (NNN)), 859 (9, $\delta_{\text{in-plane}}$ (NNN)), 815/812 (2, ν_s (SN)), 516 (10, $\delta_{\text{in-plane}}$ (S_2N_3^+)), 443 (1, ν (SS)); $\text{Hg}_3\text{Cl}_{10}^{4-}$: 387 (3, br), 287(1), 157(2), 132(2), 130(2) cm^{-1} ; IR (KBr): $\tilde{\nu} = 1238$ (s, br, ν_s (NNN)), 846 (s, $\delta_{\text{in-plane}}$ (NNN)), 812 (s, br, ν_s (SN)), 801 (vs, br, ν_a (SN)), 570 cm^{-1} (m, br, $\delta_{\text{in-plane}}$ (S_2N_3^+)).

Crystal structure analysis of **1**: $\text{Hg}_3\text{Cl}_{10}\text{N}_{12}\text{S}_8$, $M = 1380.91$, crystal size: $0.46 \times 0.05 \times 0.04 \text{ mm}$, yellow rod, monoclinic, space group $P2_1/n$, $a = 8.8890(1)$, $b = 9.5688(1)$, $c = 17.1702(2) \text{ Å}$, $\beta = 95.3469(4)^\circ$, $V = 1454.09(3) \text{ Å}^3$, $Z = 2$, $\rho_{\text{calcd}} = 3.15397(7) \text{ Mg m}^{-3}$, $\lambda = 0.448 \text{ mm}^{-1}$, $F(000) = 1244$. Nonius Kappa CCD, $\text{MoK}\alpha$, $\lambda = 0.71073 \text{ Å}$, $T = 200(3) \text{ K}$, θ range = 2.38 to 27.49° in $-11 \leq h \leq 11$, $-12 \leq k \leq 12$, $-22 \leq l \leq 22$, reflections collected: 29665, independent reflections: 3337 ($R_{\text{int}} = 0.0575$), observed reflections: 3014 ($F > 4\sigma(F)$). Structure solution program: SIR97,^[19] direct methods, final R indices ($F > 4\sigma(F)$): $R1 = 0.0298$, $wR2 = 0.0937$ (all data), GOF on $F^2 = 1.269$, largest difference peak/hole: 3.583 , -1.508 e Å^{-3} , program used: SHELXL-97.^[20, 21]

$[\text{S}_2\text{N}_3]^+[\text{Hg}_2\text{Cl}_6]^{2-}$ (**2**): A solution of (NSCl)₃ (0.25 g, 1.02 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a suspension of HgCl_2 (0.42 g, 1.55 mmol). The light yellow mixture was stirred at ambient temperature for 16 h. The color changed via light green to orange. The mixture was filtered through a glass sinter (G4). The clear yellow CH_2Cl_2 phase was allowed to stand for crystallization. (Besides the $[\text{S}_2\text{N}_3]^+$ salt we were able to isolate the $[\text{S}_4\text{N}_3]^+$ salt which was identified by its Raman spectrum.) Yield: 0.05 g (0.06 mmol, 12 %, based on (NSCl)₃); Raman (400 mW, RT): $\tilde{\nu} = 1180 \text{ cm}^{-1}$ (0.1, ν_a (NNN)), 859 (9, $\delta_{\text{in-plane}}$ (NNN)), 812 (2, ν_s (SN)), 514 (10, $\delta_{\text{in-plane}}$ (S_2N_3^+)), 448 (1, ν (SS)); $\text{Hg}_2\text{Cl}_6^{2-}$: 381 (0.5, br); 286 (2, br), 142 (3, br), 120 (2) cm^{-1} ; IR (KBr): $\tilde{\nu} = 1238$ (s, br, ν_s (NNN)), 846 (s, $\delta_{\text{in-plane}}$ (NNN)), 812 (s, br, ν_s (SN)), 801 (vs, br, ν_a (SN)), 570 cm^{-1} (m, br, $\delta_{\text{in-plane}}$ (S_2N_3^+)).

Crystal structure analysis of **2**: $\text{Hg}_2\text{Cl}_6\text{N}_6\text{S}_4$, $M = 826.2$, crystal size: $0.20 \times 0.04 \times 0.04 \text{ mm}$, yellow rod, triclinic, space group $P\bar{1}$, $a = 6.0086(1)$, $b = 8.2445(2)$, $c = 9.2830(2) \text{ Å}$, $\alpha = 105.0320(11)$, $\beta = 99.2003(11)$, $\gamma = 106.4211(14)^\circ$, $V = 412.289(15) \text{ Å}^3$, $Z = 1$, $\rho_{\text{calcd}} = 3.32766(12) \text{ Mg m}^{-3}$, $\mu = 20.062 \text{ mm}^{-1}$, $F(000) = 368$. Nonius Kappa CCD, $\text{MoK}\alpha$, $\lambda = 0.71073 \text{ Å}$, $T = 200(3) \text{ K}$, θ range = 2.72 to 27.48° in $-7 \leq h \leq 7$, $-10 \leq k \leq 10$, $-12 \leq l \leq 12$, reflections collected: 7569, independent reflections: 1881 ($R_{\text{int}} = 0.0581$), observed reflections: 1722 ($F > 4\sigma(F)$). Structure solution program: SIR97,^[19] direct methods, final R indices ($F > 4\sigma(F)$): $R1 = 0.0330$, $wR2 = 0.0836$ (all data), GOF on $F^2 = 1.073$, largest difference peak/hole: 1.957 , -1.979 e Å^{-3} , program used: SHELXL-97.^[20, 21]

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[NdI₂(thf)₅], the First Crystallographically Authenticated Neodymium(II) Complex**

Mikhail N. Bochkarev,* Igor L. Fedushkin, Sebastian Dechert, Anatolii A. Fagin, and Herbert Schumann*

Dedicated to Professor Glen Deacon on the occasion of his 65th birthday

Over the last three years the series of molecular complexes of divalent lanthanides has been extended for three lanthanide metals. The well known complexes of divalent samarium, europium, and ytterbium were supplemented by the solvated diiodide complexes of divalent neodymium, dysprosium, and thulium, [LnI₂(dme)₃] (Ln = Nd,^[1] Dy,^[1] Tm;^[2] dme = 1,2-dimethoxyethane) and [LnI₂(thf)₅] (Ln = Nd,^[1] Dy^[1]). The thulium and dysprosium compounds proved to be useful agents in inorganic^[3] and organic syntheses.^[4,5] Apart from complexes of divalent samarium, europium, and ytterbium, [DyI₂(dme)₃]^[5] and [TmI₂(dme)₃]^[2] are the only two molecular lanthanide(II) complexes which have been studied by single-crystal X-ray diffraction methods. Crystals of [DyI₂(dme)₃] contain two independent molecules with linear and bent I-Dy-I units; the dysprosium atoms are η²-chelated by all three DME molecules. The molecules of [TmI₂(dme)₃] consist of linear I-Tm-I fragments with the metal atoms being further coordinated by one terminal η¹-bonding and two chelating η²-bonding DME molecules. Here we report on the first reliable structure determination of a molecular neodymium(II) complex, [NdI₂(thf)₅] (**1**), as well as on the structure of a new thulium(II) complex, [TmI₂(dme)₂(thf)] (**2**).

NdI₂ was prepared from neodymium powder and iodine according to the procedure recently reported by Bochkarev and Fagin.^[1] Crystallization of NdI₂ from THF at –28 °C affords the molecular complex [NdI₂(thf)₅] (**1**) as black crystals. All manipulations have to be carried out at low temperatures otherwise **1** reacts with THF. Cooling of solutions of [TmI₂(dme)_x] in 1:1 mixtures of DME and THF to 0 °C causes precipitation of [TmI₂(dme)₂(thf)] (**2**) as loush emerald green crystals.

[NdI₂(thf)₅] (**1**) (Figure 1) crystallizes in the monoclinic space group P2₁/n. The unit cell contains 16 molecules of **1** and

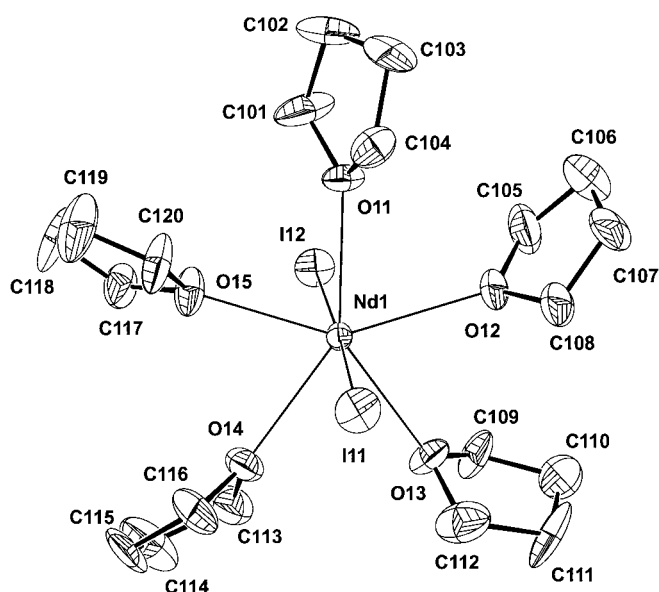


Figure 1. Structure of one of the four crystallographically independent molecules of **1** (ORTEP drawing; thermal ellipsoids with 30 % probability. The hydrogen atoms are omitted for clarity). Relevant bond lengths [Å] and angles [°]: Nd1–I11 3.2566(10), Nd1–I12 3.2861(10), Nd1–O11 2.617(7), Nd1–O12 2.629(8), Nd1–O13 2.624(7), Nd1–O14 2.593(7), Nd1–O15 2.584(8); O11–Nd1–O12 71.8(3), O13–Nd1–O12 72.5(3), O14–Nd1–O13 71.8(3), O15–Nd1–O14 72.0(3), O15–Nd1–O11 72.8(3), I11–Nd1–I12 177.60(3), O11–Nd1–I11 94.44(17), O12–Nd1–I11 87.3(2), O13–Nd1–I11 93.60(18), O14–Nd1–I11 86.79(19), O15–Nd1–I11 89.91(19), O11–Nd1–I12 87.35(17), O12–Nd1–I12 94.8(2), O13–Nd1–I12 85.90(18), O14–Nd1–I12 90.82(19), O15–Nd1–I12 89.10(19).

noncoordinating THF solvent molecules.^[6] The four crystallographically independent molecules of **1** show similar molecular parameters. [TmI₂(dme)₂(thf)] (**2**) (Figure 2) crystallizes in the monoclinic space group C2/c with four molecules in the unit cell.^[6] The comparison of the crystallographic data of **1**

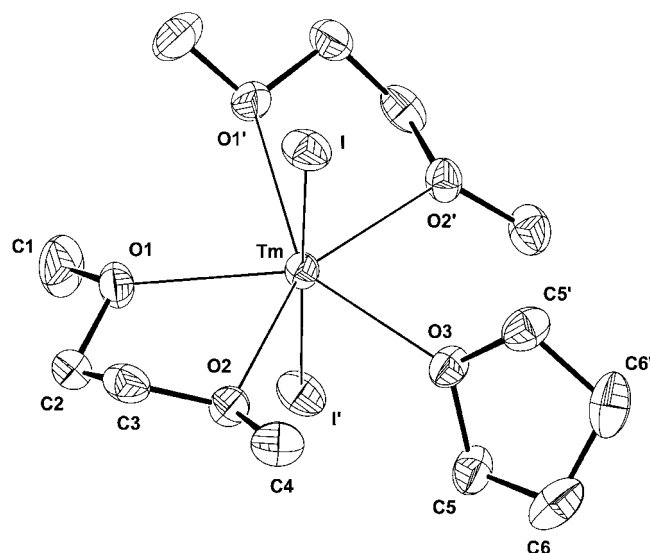


Figure 2. Structure of **2** (ORTEP drawing; thermal ellipsoids with 50 % probability. The hydrogen atoms are omitted for clarity; symmetry transformations used to generate equivalent atoms: (') –x, y, 0.5–z). Relevant bond lengths [Å] and angles [°]: Tm–I 3.1511(5), Tm–O1 2.501(6), Tm–O2 2.551(6), Tm–O3 2.438(8); O1–Tm–O3 141.66(13), O1–Tm–O1' 76.7(3), O2–Tm–O3 77.06(13), O1–Tm–O2 66.95(19), O1–Tm–O2' 137.98(19), O2–Tm–O2' 154.1(3), O1–Tm–I 96.65(15), O1–Tm–I' 84.15(15), O2–Tm–I 80.00(13), O3–Tm–I 89.490(18), O2–Tm–I' 99.77(13).

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with those of the analogous samarium complex $[\text{SmI}_2(\text{thf})_5]^{[7]}$ (same space group, similar unit cell parameters, equal number of molecules in the unit cell), suggests that they are isotopic compounds. However, due to the limited quality of the crystallographical data of $[\text{SmI}_2(\text{thf})_5]$, only the arrangement of the atoms, but no precise values for the bond lengths and bond angles could be established. Complex **2** is isostructural with $[\text{SmI}_2(\text{dme})_2(\text{thf})]^{[7]}$. The molecules of **1** and **2** exhibit a pentagonal-bipyramidal structure with the two iodine atoms in the axial positions. Similar seven-coordinate structures were reported for the solvent adducts of samarium and ytterbium diiodide $[\text{SmI}_2(\text{dme})(\text{thf})_3]^{[7]}$ and $[\text{YbI}_2(\eta^2\text{-dme})_2(\eta^1\text{-dme})]^{[8]}$. Furthermore, the structures of the cationic lanthanide(III) complexes $[\text{SmI}_2(\text{thf})_5]^+$,^[9a] $[\text{LaI}_2(\text{thf})_5]^+$,^[9b] $[\text{CeCl}_2(\text{thf})_5]^+$,^[9c] $[\text{TbCl}_2(\text{thf})_5]^+$,^[9d] $[\text{YbCl}_2(\text{thf})_5]^+$,^[9e,f] $[\text{GdCl}_2(\text{thf})_5]^+$,^[9e,g] $[\text{DyCl}_2(\text{thf})_5]^+$,^[9h,i] $[\text{ErCl}_2(\text{thf})_5]^+$,^[9b,g] and $[\text{LuCl}_2(\text{thf})_5]^+$,^[9j] show an analogous arrangement, whereas for $[\text{YbI}_2(\text{thf})_4]^{[10]}$ a six-coordinate and for $[\text{SmI}_2(\text{dme})_3]^{[11]}$ an eight-coordinate structure was ascertained. Depending on the crystallization conditions, $[\text{SmI}_2(\text{dme})_3]$ crystallizes either as an enantiomer (Δ or Λ) or as a racemic mixture. The only crystallographically completely characterized penta(tetrahydrofuranato)metal(II) complex is the strontium compound $[\text{SrI}_2(\text{thf})_5]^{[12]}$.

The Nd–I distances of the four crystallographically independent molecules of **1** range from 3.2566(10) to 3.2910(10) Å. The average Nd–I distance of 3.276 Å is about 0.035 Å longer than the average metal–iodine distance in $[\text{SmI}_2(\text{dme})_2(\text{thf})]$ or $[\text{SmI}_2(\text{dme})(\text{thf})_3]^{[7]}$ and exceeds significantly the Tm–I distances in **2** (3.1511(5) Å) and in $[\text{TmI}_2(\eta^2\text{-dme})_2(\eta^1\text{-dme})]^{[2]}$ (3.141(2) and 3.186(2) Å). These data are consistent with the differences in the radii^[17] of the respective metal(II) ions. The I–Nd–I angle in **1** (av 178.1°) and the I–Tm–I angle in **2** (178.98(4)°) are in accordance with a rather regular pentagonal-bipyramidal structure. The O–Nd–O angles in **1** are close together and range from 70.8(3) to 73.5(3)°. Also the Nd–O bond lengths vary only slightly and show an average value of 2.612 Å for the four crystallographically independent molecules. Because of the two different ligands the O–Tm–O angles in **2** deviate from the ideal value of 72°. The O(dme)–Tm–O(dme) angles formed by the two chelating DME molecules (66.95(19)°) are smaller than the O(thf)–Tm–O(dme) angle (77.06(13)°). A similar value (76.7(3)°) was determined between the two DME molecules. As in $[\text{SmI}_2(\text{dme})_2(\text{thf})]^{[7]}$ the Tm–O(thf) distance (2.438(3) Å) is shorter than the [Tm–O(dme)] distances (2.501(6) and 2.551(6) Å). From the fact that the difference of 0.095 Å between the M–I distances in **2** and in $[\text{SmI}_2(\text{dme})_2(\text{thf})]$ agrees well with the difference of 0.092 Å between the M–O(thf) and M–O(dme) bond lengths of these two compounds, it may be concluded that for the seven-coordinate compounds the difference in ionic radii of Sm^{II} and Tm^{II} will range between 0.092 and 0.095 Å.

Experimental Section

All manipulations were performed in vacuum and in a nitrogen atmosphere. The extreme sensitivity of **1** and **2** towards air and moisture and the low thermal stability of **1** prevented satisfactory elemental analysis.

1: After combustion of a mixture of neodymium (0.5 g) and iodine (0.5 g) in an evacuated glass tube (8 × 100 mm), the formed ingot of NdI_2 was broken down using an ultrasonic bath, and the powder formed was transferred under vacuum to a 50-mL Schlenk flask. While cooling the flask with liquid nitrogen, THF (ca. 25 mL) was condensed on to the NdI_2 powder. Subsequently, the flask was cooled with dry ice for 12 h and was then allowed to warm slowly to temperatures between –10 and –5 °C. Gentle stirring of the reaction mixture caused the formation of a deep violet solution which was then placed into a refrigerator (–28 °C). In the course of 36 h, black crystals of $[\text{NdI}_2(\text{thf})_5]$ precipitated from the solution.

2: A mixture of thulium powder (3.0 g, 17.79 mmol) and iodine (0.5 g, 1.97 mmol) in DME (20 mL) was stirred at room temperature until the iodine color disappeared completely and colorless thulium triiodide precipitated. The mixture was sonicated at 70 °C for 30 min. The emerald green solution of TmI_2 formed was decanted from unreacted metal and concentrated in vacuum to a volume of 10 mL. Subsequently THF (10 mL) was added to the DME solution and the mixture was heated to 60 °C until the crystalline product was completely dissolved. Cooling of the solution to 0 °C gave **2** (0.97 g, 73 %) as large longish emerald green crystals. M.p. > 90 °C (decomp); elemental analysis calcd (%) for $\text{C}_{12}\text{H}_{28}\text{O}_5\text{I}_2\text{Tm}$ (675.09): Tm 25.02; found: Tm 25.13.

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- [6] X-ray structure data: Siemens SMART CCD diffractometer, ω scans, MoK_α radiation ($\lambda = 0.71093$ Å), graphite monochromator, $T = 173$ K, SADABS^[13] for absorption correction, structure solution with direct methods (SHELXS-97^[14]), refinement against F^2 (SHELXL-97^[15]) with anisotropic thermal parameters for all non-hydrogen atoms, hydrogen positions with fixed isotropic thermal parameters ($U_{\text{iso}} = 0.08$ Å²) on calculated positions. Data collection for **1**: crystal dimensions $0.63 \times 0.38 \times 0.18$ mm, monoclinic, space group C_{2h}/n , $a = 23.1057(3)$, $b = 26.1191(4)$, $c = 23.9426(1)$ Å, $\beta = 110.896(1)^\circ$, $V = 13499.0(3)$ Å³, $Z = 16$, $\rho_{\text{calcd}} = 1.688 \times 10^3$ kg m^{–3}, $\mu = 3.401$ mm^{–1}, $F(000) = 6736$, $2.1 \leq 2\theta \leq 50.0^\circ$, $-27 \leq h \leq 27$, $-27 \leq k \leq 31$, $-27 \leq l \leq 28$, 76040 data collected, 23563 unique data ($R_{\text{int}} = 0.0867$), 11496 data with $I > 2\sigma(I)$, 1099 refined parameters, $\text{GOF}(F^2) = 1.001$, final R indices ($R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$): $R_1 = 0.0643$, $wR_2 = 0.1277$, max/min residual electron density 1.186/–1.188 e Å^{–3}. The THF molecules coordinated to the metal center in **1** are disordered about multiple positions in a small range which results in large anisotropic displacement parameters of the atoms and shortening of the C–C and C–O bond lengths. It was not possible to find a satisfactory model for the disorder. The unit cell of **1** contains 22 noncoordinating THF solvent molecules of which only eight could be refined. The remaining 14 molecules occupy an area of 2354 Å³ (17 %) in the unit cell. For further refinement the contribution of the missing solvent molecules was subtracted from the reflection data by the SQUEEZE routine of the PLATON^[16] program. Data collection for **2**: crystal dimensions $0.40 \times 0.24 \times 0.16$ mm, monoclinic, space group C_{2h}/c , $a = 12.9457(4)$, $b = 11.3172(2)$, $c = 15.3120(5)$ Å, $\beta = 114.900(1)^\circ$, $V = 2034.81(10)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 2.204 \times 10^3$ kg m^{–3}, $\mu = 7.407$ mm^{–1}, $F(000) = 1260$, $5.0^\circ \leq 2\theta \leq 55.0^\circ$, $-16 \leq h \leq 16$, $-14 \leq k \leq 12$, $-18 \leq l \leq 19$, 7412 data collected, 2328 unique data ($R_{\text{int}} = 0.0538$), 1389 data with $I > 2\sigma(I)$, 94 refined parameters, $\text{GOF}(F^2) = 1.050$, final R indices ($R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$): $R_1 =$

- 0.0473, $wR_2 = 0.0917$, max/min residual electron density 2.057/–1.062 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-163342 (**1**) and CCDC-163343 (**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).
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Mass-Spectrometric Monitoring of a PNA-Based Ligation Reaction for the Multiplex Detection of DNA Single-Nucleotide Polymorphisms**

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The genetic variation that occurs most frequently is the exchange of single nucleobases (single-nucleotide polymorphisms, SNP).^[1, 2] These single-base mutations are associated with diseases, such as cystic fibrosis,^[3] familial hypercholes-

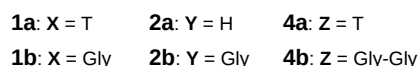
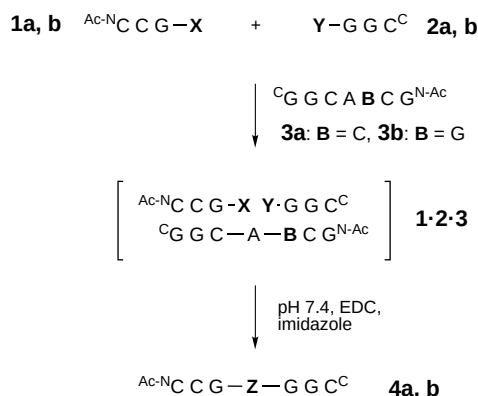
terolemia,^[4] cancer,^[5] and many others. DNA diagnostic assays depend on a binding event between the target DNA and a complementary oligonucleotide probe. The hybridization alone, however, barely exhibits sufficient selectivity in distinguishing matched from single-base mismatched DNA targets. An enhancement of the discriminatory power is possible by accommodating a probe-modifying event such as oligonucleotide ligation^[6] or primer extension.^[7] There is an increasing demand for the development of assay systems in which a single analysis is able to report on more than one diagnostic event (multiplexing). Most commonly fluorescence-based techniques, such as fluorescent dye-terminator-extension,^[7] TaqMan assays,^[8] molecular beacons,^[9, 10] or scorpion probes^[11] are used. A drawback is the need for labor- and cost-intensive fluorescence labeling. More importantly, the degree of multiplexing that can be achieved is limited by the number of spectrally resolved fluorophores. In contrast, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass-spectrometry (MS) offers unparalleled resolution and has been demonstrated to enable a genotyping of single-base mutations.^[12, 13] However, the fragmentation and the known tendency to form DNA metal-salt adducts can impede the resolving power of MALDI-based detection when oligonucleotides are used as probes.^[14, 15] Herein, a novel approach for single-base-mutation detection is proposed that capitalizes upon the use of peptide nucleic acids (PNAs)^[16]—a chemically stable and non-ionic DNA analogue that combines superior base-pairing properties with ease and accuracy of detection.^[17, 18] Contrary to previous reports on PNA-based MALDI-TOF genotyping^[19, 20] it is a DNA-controlled chemical ligation of two short probes that is monitored rather than the hybridization of one long probe.^[21]

PNA exhibits an unparalleled suitability for MALDI-TOF MS analysis in terms of molecular-weight resolution and accuracy.^[19, 20] The discriminative power of PNA-hybridization itself, however, depends on base content, sequence, and particularly on the length of the oligomer. Oligomers that are longer than 16 base pairs are required to provide a unique sequence. However, the longer PNA segments are relatively unselective as DNA binders.^[22] Conversely, short-length PNA oligomers exhibit a sufficiently high DNA affinity combined with a very good discrimination for single-base mismatches. We thought that a highly specific sequence analysis of a unique gene segment should be possible by employing a PNA-ligation strategy. Since PNA is not a substrate of any known ligase, any attempt to employ PNA as a ligation probe has to rely solely on chemical methods.

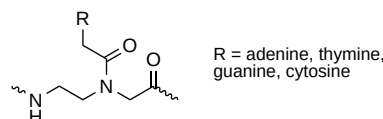
PNA is a pseudopeptide and ligation chemistry can easily be implemented by using amide-bond forming reactions.^[21] For the development of a sequence-specific PNA-based ligation reaction we examined the all-PNA model system shown in Scheme 1 and the carbodiimide-mediated condensation of PNA-fragments **1a** and **2a** was investigated. It became apparent that the addition of the complementary template **3a** increased the rate and the yield of the ligation reaction (Figure 1A). Surprisingly, the presence of template **3b**, which contained a single-base mismatch, gave an even higher increase in the ligation yield.

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structure of a PNA-monomer:



Scheme 1. PNA-fragment condensation of the tetramer **1a** and the trimer **2a** as well as the abasic-site ligation of PNA–glycine conjugates **1b** and **2b** on fully complementary PNA template **3a** and the single-mismatched template **3b** (the mutation site (B) is marked in bold). Concentration of ligation probes **1** and **2** and the PNA templates: 160 μM . (EDC = 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide-hydrochloride).

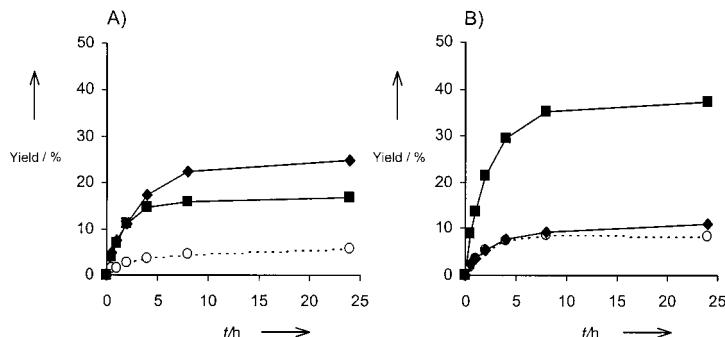
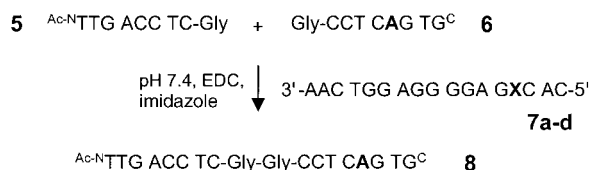


Figure 1. Kinetics of product formation in the presence of match template **3a** (■), mismatch template **3b** (◆), and in the absence of template (○). A) PNA-fragment ligation of **1a** and **2a**; B) abasic-site ligation of PNA–glycine conjugates **1b** and **2b**. Yields of ligation products were determined by subjecting aliquots to high-pressure liquid chromatography (HPLC) analysis.

A solution to the problem of poor template selectivity was found by performing the ligation reaction so that an abasic site was introduced. We speculated that the incorporation of an abasic site would disrupt the cooperative base stacking, thereby rendering the ternary complex **1**·**2**·**3** less tolerant to further disturbances.^[23] The abasic site was integrated by replacing a central PNA monomer such that an isosteric dipeptide would be formed upon ligation of two PNA–glycine conjugates, such as **1b** and **2b**. The model experiments indicated the validity of this approach (Figure 1B). The

addition of the complementary template **3a** greatly enhanced ligation efficiency and led to the formation of **4b**. More importantly, the ligation displayed a remarkable sequence selectivity: the single-mismatch template **3b** was ineffective in accelerating the formation of PNA–dipeptide hybrid **4b**.

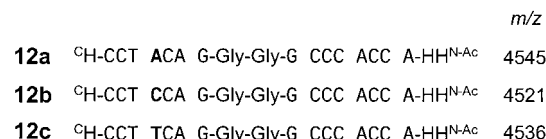
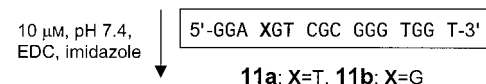
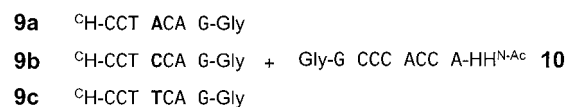
The conditions that enabled a highly sequence-selective ligation in the model system were employed for the MALDI-TOF-based detection of a single-base mutation in a 17mer DNA target.^[24] We studied the ligation of the 8mer PNA–glycine conjugates **5** and **6** in the presence of the DNA targets **7a–d**. The oligonucleotides **7a–d** differ in one central position, each one contains a different nucleobase (Scheme 2). The reactions were performed at a 10 μM



Scheme 2. Abasic-site ligation of PNA–glycine conjugates **5** and **6**. The DNA targets **7** differ in one central position (highlighted in bold, X = T (**7a**), G (**7b**), C (**7c**); A (**7d**)). The PNA base opposite to the mutation site is also in bold. Concentration of ligation probes **5** and **6** and target DNA **7a–d**: 10 μM .

concentration of DNA and ligation probes, which completely suppressed the blank reaction. After 6 h the DNA templates were degraded by a treatment with trifluoroacetic acid (TFA). MALDI-TOF MS analysis indicated that only with the fully complementary DNA (X = T) **7a** was a significant peak for a ligation product **8** m/z 4437 observed (Figure 2). All other DNA templates (X = A, C, G) proved unproductive and only trace amounts of **8** were detected.

To assess the discriminative power of the abasic-site ligation strategy we examined a competition ligation reaction with three PNA–glycine conjugates that would compete with the template. The aim was to detect the G→T mutation in the synthetic DNA segment **11** (Scheme 3). Three nucleophilic



Scheme 3. Detection of a G→T mutation (marked in bold) in the DNA **11**. The three variable PNA–glycine probes **9a–c** (probing sites marked in bold) were treated with one common PNA–glycine probe **10** in the presence of either mutant DNA **11a** (X = T) or wild-type DNA **11b** (X = G). Concentration of ligation probes **9a–c** and **10** and target DNA **11a, b**: 10 μM . (H = histidine).

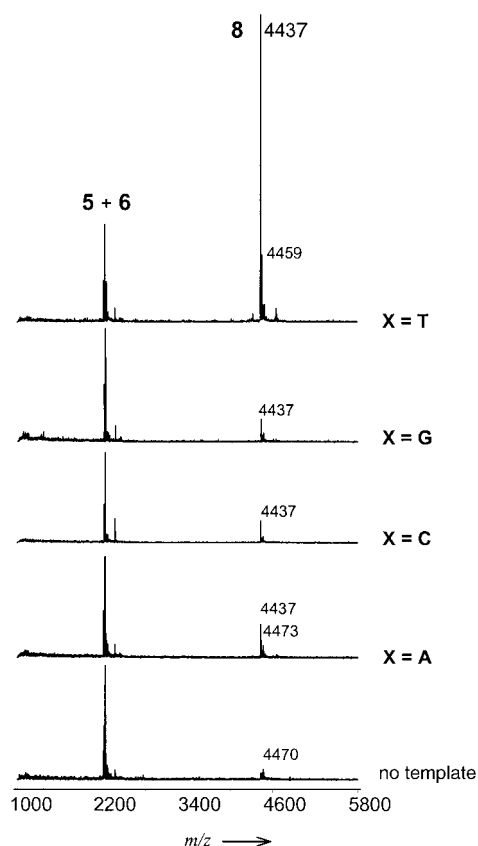


Figure 2. MALDI-TOF MS of the abasic-site ligation of **5** and **6** after 6 h in the presence of DNA templates **7a–d** (m/z [$M+H$] $^+$: 2237 (**5**); 2219 (**6**); 4437 (**8**)). Matrix: sinapinic acid.

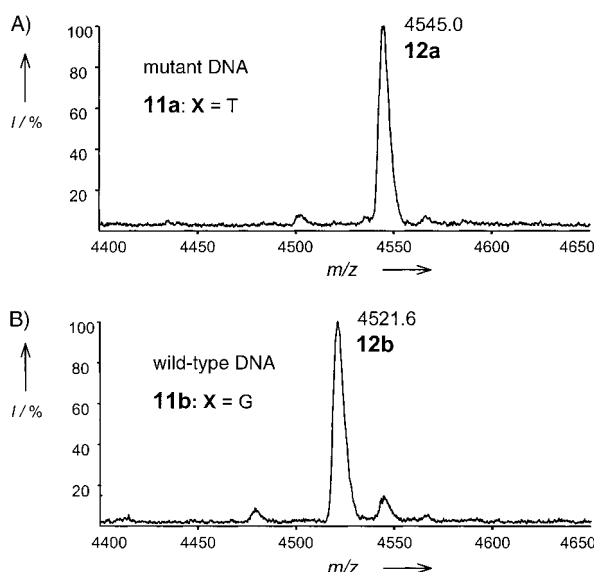


Figure 3. MALDI-TOF MS analysis of the competition ligation for the detection of a single-base mutation in the presence of A) mutant DNA **11a** ($X=T$) and B) wild-type DNA **11b** ($X=G$). Single peaks detected corresponded to the ligation product **12a** (m/z : 4545 [$M+H$] $^+$) and the ligation product **12b** (m/z : 4521 [$M+H$] $^+$). Matrix: sinapinic acid.

PNA–glycine conjugates (**9a–c**) served to probe the mutation site and were allowed to react with the acyl-donor segment **10**. The MALDI-TOF analysis revealed that in the presence of the mutant DNA **11a** ($X=T$) only one of the

three possible ligation products was formed (Figure 3). The m/z ratio of 4545 corresponded to the molecular mass of the PNA–diglycine hybrid **12a**, which indicated that only the A-containing probe **9a** was involved in the ligation. When the reaction was carried out on the wild-type DNA target **11b** ($X=G$), predominantly the C-containing product **12b** was observed. The high selectivity of the ligation process was also demonstrated in that the T-containing PNA probe **9c** showed no sign of ligation.

The positive results obtained in the competition ligation encouraged us to investigate whether the high ligation fidelity and the accuracy of mass-spectrometric detection would enable a multiplexed ligation assay to be performed. The goal was to obtain information about three potential mutations in the pig-*ras* gene segment in a single experiment.^[25] The mutations examined corresponded to the carcinogenic G12V ($G \rightarrow T$), F28L ($T \rightarrow C$), and the E63H ($G \rightarrow C$, $G \rightarrow T$) transformations. Each potential mutation site was probed by a ligation of a common PNA–glycine acyl donor with a wild-type or a mutant PNA–glycine conjugate (Figure 4A). In total, the multiplexed ligation comprised six nucleophilic PNA–glycine probes and three acyl-donor segments such that six specific ligation products and 12 cross-ligation products could form. The multiplex ligation was performed at a 500 nM concentration of DNA and ligation probes. After 4 h the ligation reactions were analyzed by MALDI-TOF MS. Of the 18 ligation products that could form only three were observed when the nine ligation probes were allowed to react with the mutant DNAs (Figure 4B). The ligation events were highly specific and a comparison with the calculated masses of the possible ligation products revealed that significant product formation was observed only for the three mutant ligation products. As a control the multiplex ligation was examined in the presence of the wild-type DNAs. Clearly, the ligation product spectrum showed the occurrence of the wild-type DNAs and the observed molecular masses matched those of the wild-type ligation products (Figure 4C).

To probe the sensitivity of the detection system, the ligation was performed on a mixture of templates corresponding to codon 12 wild-type and mutant *ras* sequences. The mutant DNA was easily detected when present as a mixture of wild-type/mutant-sequences in ratio of 9/1 and at a 500 nM total DNA concentration. The signal from the mutant DNA still exceeded the noise level when the ligation was carried out on a 99/1 mixture of wild-type/mutant-gene segments. On a pure wild-type template, formation of the mutant ligation product was not measurable. These preliminary results indicate that the ligation system may deliver reliable results at 5 nM concentrations of target DNA.

Starting from a simple model system we have devised a DNA-controlled PNA-ligation strategy that has been made single-mismatch specific by “engineering” an abasic site into the ligation site. PNA contrary to DNA is stable against acid hydrolysis. This acid stability was employed to remove DNA-based background signals, thereby eliminating the need to perform solid-phase based hybridization techniques. From the data acquired with six different sequences we deduce that the nucleobase opposite to the glycyglycine abasic site plays no important role since adenine, guanine, and thymine were

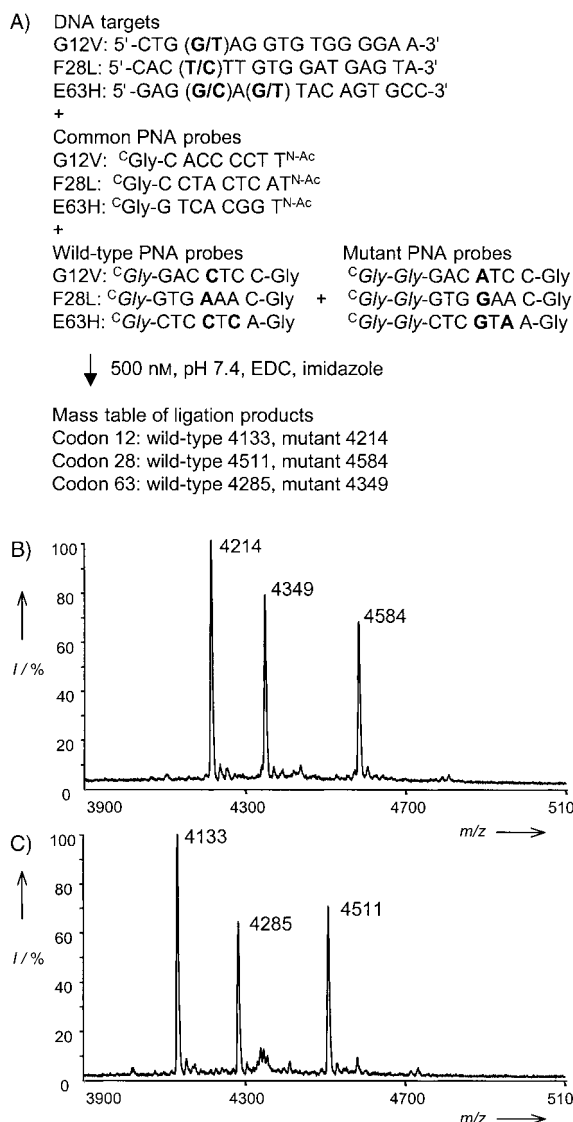


Figure 4. A) For the multiplex detection of three carcinogenic mutations in the *pig-ras* gene segment (G12V (G → T), F28L (T → C), E63H (G → C, G → T)) three common PNA probes were treated with three wild-type- and three mutant-PNA probes (probing sites marked in bold) in a single experiment. The glycine residues in italics were incorporated for mass-tagging. Concentration of ligation probes and target DNA: 500 nM. Positive-mode MALDI-TOF MS in the presence of B) the three mutant-DNA targets; C) the wild-type DNA targets. Matrix: sinapinic acid.

tolerated. It thus seems likely that the abasic site ligation is a general reaction which might allow for the multiplex detection of single-base mutations in homogeneous solution. In future studies we will apply the PNA-ligation assay to PCR-amplified DNA samples.

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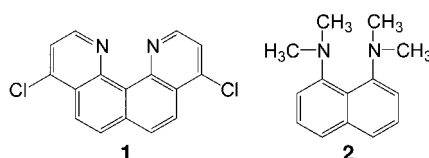
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Transition Metal Complexes with the Proton Sponge 4,9-Dichloroquinolo[7,8-*h*]quinoline: Highly Twisted Aromatic Systems and an Extreme “Out-of-Plane” Position of the Coordinated Transition Metal Atom**

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Ferdinand Schüth,* Galen D. Stucky, Xianhui Bu, and
Bernt Krebs

*Dedicated to Professor Heinz A. Staab
on the occasion of his 75th birthday*

We report the first successful synthesis and complete characterization of transition metal complexes of the “proton sponge” 4,9-dichloroquinolo[7,8-*h*]quinoline **1**. The novel proton sponge **1** and its derivatives were prepared and described fifteen years ago by Zirnstein and Staab.^[1, 2] Even at that time



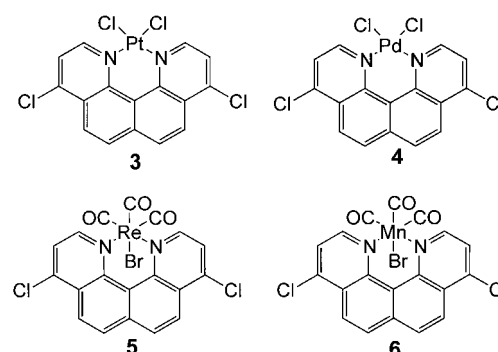
these strong bases already had a complicated and controversial history: Although several authors had reported the synthesis of these compounds, the products were incorrectly formulated.^[3, 4] To our knowledge, all attempts to synthesize stable metal complexes with proton sponges as ligands have been unsuccessful.

The first proton sponge, 1,8-bis(dimethylamino)naphthalene **2**,^[5] is not suitable as a ligand for metal complexes because the methyl groups shield the nitrogen atoms and thereby sufficient thermodynamic stability is not conferred on the complex that could be formed.^[6] Although 1,8-diamino-naphthalene^[7, 8] is not a proton sponge a few transition metal complexes are known.

The proton sponge **1**, in contrast, is a molecule in which the complexing nitrogen atoms are part of the aromatic system. In addition, this compound is planar and not helical and thus

very suitable as a ligand for metal complexes. **1** is in comparison a better ligand than the widely used *o*-phenanthroline for two reasons: The unusually high basicity of **1** ($pK_a = 10.9$ ^[8]) compared to *o*-phenanthroline ($pK_a = 4.81$ ^[9]) and the short nitrogen–nitrogen distance of 2.685 Å forces the complexed metal atom into an “out-of-plane” position. This confers a very high thermal stability on the metal complexes. The extreme “out-of-plane” position of the metal atom leads to a very reactive, accessible metal center and exposes one side for a variety of interactions including substitution and addition reactions. Compounds with such strong “out-of-plane” conformations are now a “hot” research theme.^[10–12]

The small nitrogen–nitrogen distance along with the high basicity of **1** limits the use of protic solvents in the metal complex synthesis. However, dichloromethane or chloroform are suitable for the formation of the platinum and palladium complexes **3** and **4**, and THF for the rhenium and manganese complexes **5** and **6**. The synthesis of the platinum complex **3**



was successfully completed by treating Zeise’s dimer [$Pt_2(H_2C=CH_2)_2Cl_4$] in dichloromethane with compound **1**. The complex precipitated as a yellow solid. The X-ray molecular analysis revealed a very unusual structural feature: The aromatic system is strongly bowed (Figure 1). The extent of the bowing can be determined by measuring the deviation

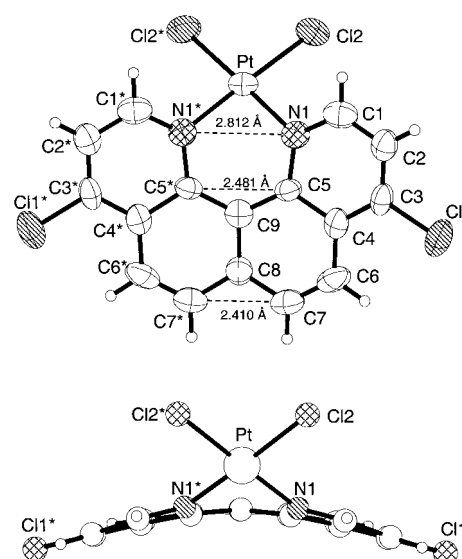


Figure 1. Diamond plot of **3** in front and side view.

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of the lowest and highest atoms of the aromatic system from a "best" plane. This plane results when the sum of the squared deviations of all single carbon and nitrogen atoms from the aromatic system is minimized. For example, the ligand atoms N1 (−0.359 Å) and C3 (0.383 Å), which lie farthest outside this plane, define the bend of the bow: 0.742 Å. The corresponding value for the nearly planar ligand **1** is 0.151 Å. The central platinum atom assumes an extreme "out-of-plane" position relative to the best plane and lies 1.43 Å over this plane. In spite of this, the platinum atom is coordinated in a square planar environment that is relatively undisturbed.

The rhenium complex was prepared by heating the ligand in THF with the prepared rhenium(II)tetracarbonylbromide dimer. The distance from the rhenium atom to the "best" plane is 1.42 Å which is similar to that of the platinum atom in complex **3** (Figure 2). The extent of bending of the aromatic

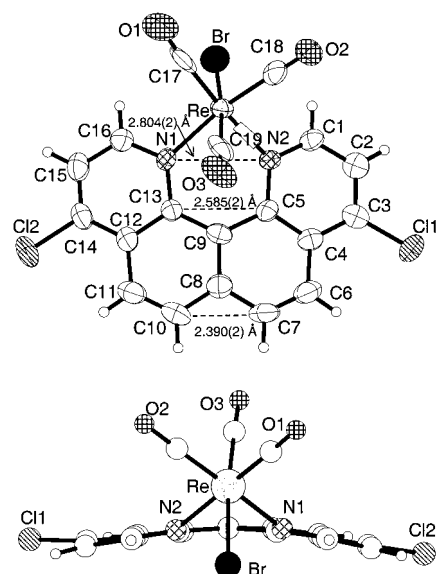


Figure 2. Diamond plot of **5** in front and side view.

system is however 0.498 Å, much less than in the platinum complex **3**. Structurally it is remarkable that the N–N distances in the platinum and rhenium complexes are 2.812 and 2.804 Å, respectively, which is significantly larger than that in the parent compound, 2.685 Å. This is readily explained by the strong nitrogen–metal bond of the large transition metal ions. Although the nitrogen–nitrogen distance is larger, this is balanced by a compression in the naphthalene residue. This is evident at the periphery of the naphthalene residue, where the C5–C13 and C5–C5* separation as well as that of C7–C10 and C7–C7* atoms are less than in 4,9-dichloroquino[7,8-*h*]quinoline **1** (Table 1).

Table 1. Important bond lengths of **1** as well as of the metal complexes **3** and **5**.

Compound	N–N [Å]	C5–C13 or C5–C5* [Å]	C7–C10 or C7–C7* [Å]
1	2.685(6)	2.604(6)	2.455(6)
3	2.812	2.481	2.410
5	2.804(2)	2.585(2)	2.390(2)

The unusual thermal and chemical stability of the metal complexes are a consequence of the unique "out-of-plane" position and conformational stability of the metal atom in the aromatic system. Thus the Pt complex can be heated to 380 °C for several days without decomposition and it is stable in hot concentrated sulfuric acid.

Moreover, this system is excellent for structure-activity studies in catalysis because the basicity of the chelating nitrogen atoms can be adjusted over a wide range with different substituents at the 4,9-positions without the influence of steric effects.

Structure–activity relationships suggest the use of the platinum-complex **3** as a cytostatic agent.

Experimental Section

1: The ligand 4,9-dichloroquino[7,8-*h*]quinoline was synthesized with modifications of Zirnstein's procedure.^[9] The thermal decarboxylation step was performed in small quantities in order to increase the yield. Moreover, the use of active charcoal RO0.8 (Aldrich) was important in purification of the ester in the third step. M.p. 236 °C (decomp.); ¹H NMR (300 MHz, [D₆]DMSO, 25 °C, TMS): δ = 7.99 (d, ³J = 4.8 Hz, 2H; 3,10-H), 8.38 (AB, ³J = 8.9 Hz, 2H; 6,7-H), 8.54 (AB, ³J = 8.9 Hz, 2H; 5,8-H), 9.19 (d, ³J = 4.8 Hz, 2H; 2,11-H); IR (KBr): $\tilde{\nu}$ = 3097 (w), 3074 (w), 3036 (w), 1626 (m), 1606 (s), 1587 (s), 1541 (m), 1522 (m), 1496 (m), 1478 (s), 1409 (s), 1339 (m), 1226 (m), 1013 (s), 840 (s), 769 (s), 691 (s), 681 cm^{−1} (m); MS (EI): *m/z* (%): 298 (100, [M]⁺, isotopic pattern for 2 Cl), 263 (29.95, isotopic pattern for 1 Cl), 236 (4.19, isotopic pattern for 1 Cl), 228 (8.06), 201 (9.33), 174 (4.65), 149 (10.12, [M]²⁺); HR-MS (EI): *m/z*: found: 298.00642 ([M]⁺), calcd.: 298.00645.

3: A solution of **1** (59.8 mg, 0.2 mmol) in 20 mL of dry dichloromethane under argon was added over a 2 h period to a boiling solution of [di-μ-chlorodichlorobis(ethylene)platinum(II)] (58.8 mg, 0.1 mmol). After heating for an additional hour, the solution was filtered to give a clear yellow solution. This solution was reduced in volume and the resultant yellow solid was recrystallized from dichloromethane. Yield: 107.3 mg (91.2 %). M.p. > 315 °C; ¹H NMR (300 MHz, CD₂Cl₂, 25 °C, TMS): δ = 7.84 (d, ³J = 6.0 Hz, 2H; 3,10-H), 8.31 (AB, ³J = 9.1 Hz, 2H; 6,7-H), 8.61 (AB, ³J = 9.1 Hz, 2H; 5,8-H), 9.66 (d, ³J = 6.0 Hz, 2H; 2,11-H); IR (KBr): $\tilde{\nu}$ = 3123 (w), 3091 (m), 3060 (m), 3039 (w), 1611 (s), 1575 (s), 1559 (m), 1520 (w), 1478 (s), 1409 (s), 1348 (s), 1221 (s), 1199 (s), 1028 (s), 857 (s), 845 (s), 766 (m), 704 (s), 677 cm^{−1} (m); MS (EI): *m/z* (%): 563 (4.98, [M]⁺, isotopic pattern for 4 Cl), 528 (10.24, [M⁺ – Cl], isotopic pattern for PtCl₃), 492 (6.18, [M⁺ – H – 2 Cl], isotopic pattern for PtCl₂), 456 (8.56).

5: A suspension of [bromotetracarbonylrhenium(II)] dimer (75.6 mg, 0.1 mmol) and **1** (59.8 mg, 0.2 mmol) in 50 mL of THF under argon was heated at reflux for 5 days. The solution became red-orange after 2 days and a dark red-orange solid formed after 5 days. The product was filtered under argon, washed with cold THF and dichloromethane, and recrystallized from THF. Yield: 83.7 mg (64.5 %). M.p. 288 °C (decomp); ¹H NMR (300 MHz, CD₂Cl₂, 20 °C, TMS): δ = 7.81 (d, ³J = 5.8 Hz, 2H; 3,10-H), 8.24 (AB, ³J = 9.0 Hz, 2H; 6,7-H), 8.60 (AB, ³J = 9.0 Hz, 2H; 5,8-H), 9.73 (d, ³J = 5.8 Hz, 2H; 2,11-H); IR (KBr): $\tilde{\nu}$ = 2962 (s), 2927 (s), 2854 (m), 2020 (s, ν(C–O)), 1920 (s, ν(C–O)), 1890 (s, ν(C–O)), 1730 (w), 1614 (w), 1577 (w), 1553 (w), 1467 (w), 1023 (s), 864 (m), 843 (m), 701 cm^{−1} (m); MS (EI): *m/z* (%): 648 (51.71, [M]⁺, isotopic pattern for ReCl₂Br), 620 (31.18, [M⁺ – CO]), 592 (94.95, [M⁺ – 2 CO]), 564 (28.06, [M⁺ – 3 CO]); HR-MS (EI): *m/z*: found: 647.866, calcd.: 647.8655.

Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-164921 (**3**) and CCDC-164920 (**5**). 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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Regioselective Cross-Coupling Reactions as an Entry into Biologically Relevant Bithiazoles: First Total Synthesis of Cystothiazole E**

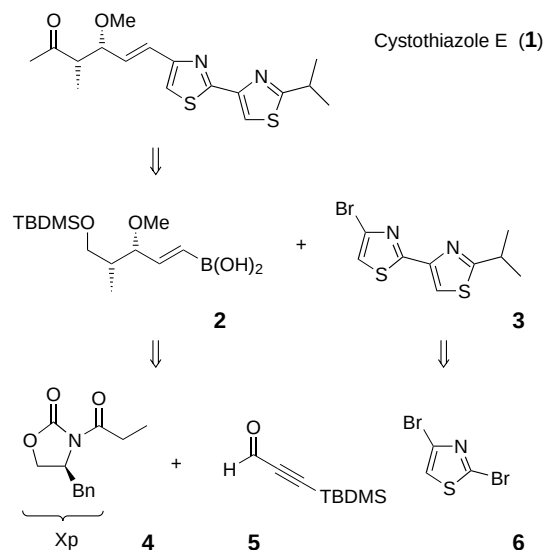
Thorsten Bach* and Stefan Heuser

*Dedicated to Professor Rolf Gleiter
on the occasion of his 65th birthday*

2',4-Disubstituted 2,4'-bithiazoles are incorporated in several biologically relevant natural products which exhibit anti-infective and cytotoxic properties.^[1] In previous syntheses access to the heterocyclic bithiazole core was based exclusively on the classical Hantzsch thiazole methodology.^[2] Because of their regioselectivity^[3] Pd-catalyzed cross-coupling reactions^[4] of 2,4-dibromothiazole^[5] appeared to offer an alternative shorter entry into bithiazoles which would allow a great variability in the choice of substituents at the 2'- and 4-positions. Herein we report the application of this strategy to the total synthesis of cystothiazole E (**1**).

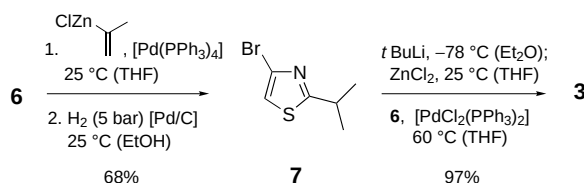
The bithiazole **1** and several structurally related products were isolated by Sakagami et al. from a culture broth of the

myxobacterium *Cystobacter fuscus* AJ-13278.^[1a] The absolute and relative configuration of the stereogenic centers of **1** could not be determined. In analogy to related bithiazoles a syn-arrangement of the methyl and the methoxy group was likely. Following the above-mentioned concept we envisioned a Suzuki cross-coupling^[6] of the building blocks **2** and **3** as a possible key step for the construction of the carbon skeleton of **1** (Scheme 1). While the vinyl boronic acid **2** was to be derived from the fragments **4** and **5** by a stereoselective aldol reaction,^[7] the synthesis of bithiazole **3** was planned starting from 2,4-dibromothiazole (**6**).



Scheme 1. Retrosynthetic disconnection of the target molecule cystothiazole E (**1**); TBDMS = *tert*-butyldimethylsilyl.

Initial attempts aimed at a cross-coupling of isopropyl zinc chloride with 2,4-dibromothiazole (**6**) were not promising and delivered only minor quantities of the desired product **7**. In contrast, isopropenyl zinc chloride reacted smoothly (96% yield, Scheme 2). The subsequent hydrogenation proceeded



Scheme 2. Synthesis of the bithiazole building block **3** by a regioselective cross-coupling.

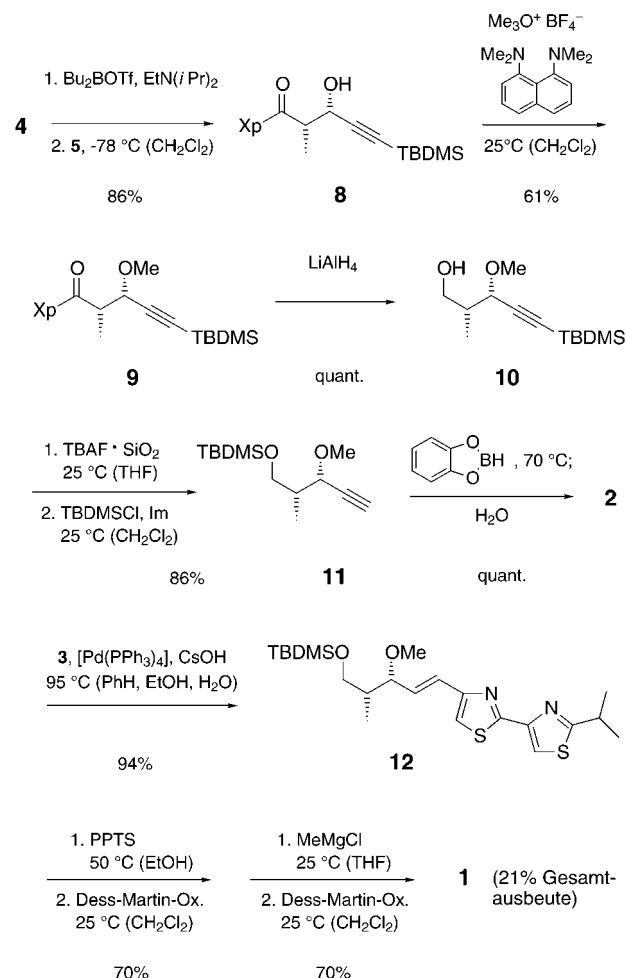
slowly at atmospheric pressure. At elevated pressure (5 bar) the conversion went to completion, however, the product is prone to hydrodebromination under these conditions and a prolonged hydrogenation should be avoided. The 4-bromothiazole **7** was converted into a carbon nucleophile by a halogen–metal exchange reaction.^[8] After transmetalation with zinc chloride the aryl metal compound reacted regioselectively with **6** to afford the desired intermediate **3** (Scheme 2).

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

For the construction of the anticipated *syn*-aldol functional group we employed the L-phenylalanine derived *N*-propionyl oxazolidinone **4** (Scheme 3).^[9] The reaction of **4** and the TBDMS-protected aldehyde **5** afforded **8** with excellent



Scheme 3. Overview of the individual steps in the total synthesis of (–)-cystothiazole E (**1**); Tf = trifluoromethanesulfonate, TBAF = tetrabutylammonium fluoride, im = imidazole, PPTS = pyridinium *p*-toluenesulfonate.

control of the facial and simple diastereoselectivity.^[10] After alkylation to methyl ether **9**^[11] and subsequent reduction the alkyne part of alkynol **10** was deprotected and the hydroxy group was TBDMS-protected (Scheme 3). The hydroboration of the alkyne with catecholborane proceeded quantitatively.^[12] The choice of base in the Suzuki cross-coupling of boronic acid **2** with the bithiazole building block **3** required some optimization; the best result was with CsOH (94% yield). As in other coupling reactions^[13] 5 mol % of the Pd-catalyst was employed. A variation of the catalyst ratio and further mechanistic studies have not yet been performed. In successive steps the TBDMS-protected primary alcohol **12** was converted into a methyl ketone.^[14] The product **1** so obtained proved to be identical to the natural product in all scalar spectroscopic properties which supported the presumed relative configuration.^[15] The direction of the specific rotation, however, was opposite to the one reported for the natural product ($[\alpha]_D^{20} = -17.5$ ($c = 0.12$, CHCl₃); natural

product:^[1a] $[\alpha]_D^{24} = +17.8$ ($c = 0.2$, CHCl₃)). Consequently, we assign the (3*R*,4*S*)-configuration to the naturally occurring (+)-cystothiazole E (*ent*-**1**), whereas (–)-cystothiazole E (**1**) as prepared by us has the (3*S*,4*R*)-configuration.

In summary, we have achieved the synthesis of (–)-cystothiazole E in a convergent route, which includes 10 synthetic operations, with an overall yield of 21%. Further applications of the reported bithiazole synthesis are being studied in our laboratories.

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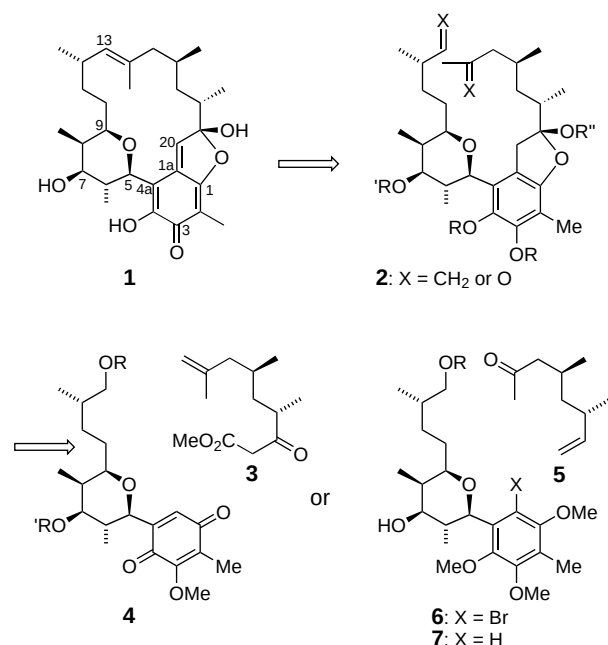
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Synthesis of the C1–C13 Fragment of Kendomycin: Atropisomerism around a C–Aryl Glycosidic Bond**

Harry J. Martin, Martina Drescher, Hanspeter Kählig, Sabine Schneider, and Johann Mulzer*

Kendomycin (**1**), a novel ansamycin compound produced from various *Streptomyces* species, was recently described as a potent endothelin receptor antagonist and anti-osteoporotic compound.^[1] Recently, remarkable antibacterial activity and cytostatic effects were also reported.^[2] The structure of **1** features an aliphatic carbon ansa chain with a highly substituted tetrahydropyran ring attached to a unique quinone methide chromophore. Its diverse pharmacological activity and the challenging molecular architecture have motivated us to embark on a laboratory synthesis of **1**.

Our synthetic plan (Scheme 1) is centered around a ring-closing metathesis or McMurry reaction for macrocyclization of the seco intermediate **2**, which was either to be assembled

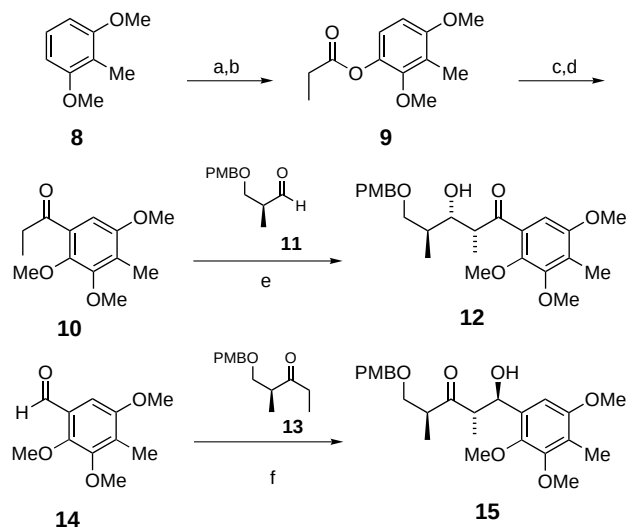


Scheme 1. Retrosynthesis of kendomycin (**1**).

by a Michael addition of keto ester **3** to quinone **4** or by a Heck coupling of alkene **5** and aryl bromide **6**. Herein we report the synthesis of the tetrahydropyran compounds **6** and **7** as well as the observation of a hindered rotation around the C–aryl glycosidic bond. As outlined in Schemes 2 and 3, the C5–C8 stereotetrad (C5–C8) of the tetrahydropyran ring

was constructed by aldol addition^[3] and diastereoselective reduction of β -hydroxyketones.

In the *syn*-selective aldol addition (Scheme 2) our synthesis started with a Friedel Crafts acylation of dimethoxytoluene (**8**) followed by a Baeyer–Villiger oxidation to deliver phenyl



Scheme 2. a) EtCOCl, TiCl₄, benzene, 0 °C, 0.5 h, 96 %; b) mCPBA, CH₂Cl₂, RT, 85 %; c) BF₃OMe₂, 90 °C, 3 h, 76 %; d) (MeO)₂SO₂, K₂CO₃, acetone, RT, 40 h, 99 %; e) **10**, LiHMDS, –78 °C, THF, 0.75 h, Ti(OiPr)₃Cl, –78 °C, addition of **11**, –78 °C, 1 h, –78 °C, addition of **11**, –78 °C, 3 h, then aq. NH₄F, 58 %; f) **13**, NEt₃, (c-C₆H₁₁)₂BCl, Et₂O, –78 °C, –40 °C, 1.5 h, –78 °C, addition of **14**, –78 °C, 16 h, then NaBO₃, 62 %. mCPBA = *m*-chloroperbenzoic acid; LiHMDS = lithium hexamethyldisilazide; PMB = *p*-methoxybenzyl.

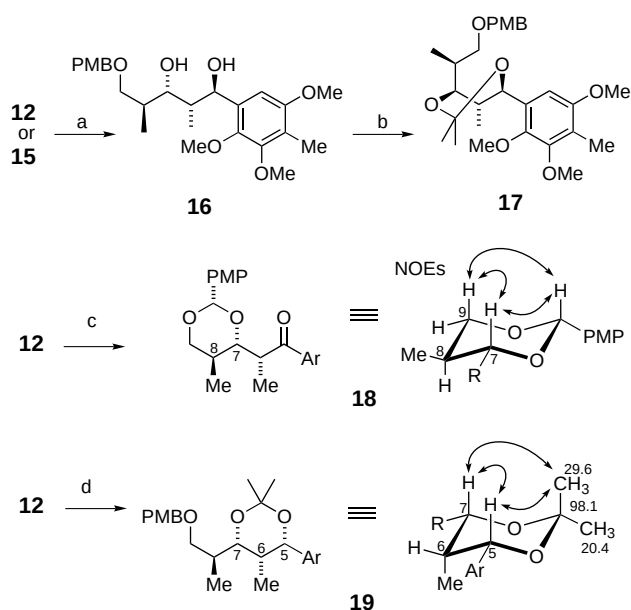
propionate **9**. *ortho*-Fries rearrangement and O-methylation gave ketone **10**,^[4] which after conversion into the titanium enolate was added to the known aldehyde **11**^[5] to furnish *syn* aldol adduct **12** as the main isomer (58 %). In the alternative *anti*-selective version, known ketone **13**^[6] was converted into the enol borinate and then treated with the known aldehyde **14**^[7] to give adduct **15** as the main isomer (62 %). Either version affords the main diastereomer stereochemically pure in gram quantities.

Both aldol adducts **12** and **15** gave the same acetonide (**17**) in high yield and diastereoselectivity on diastereoselective reduction^[8] with Me₄NBH(OAc)₃ (to give *anti*-diol **16**) and subsequent treatment with 2,2-dimethoxypropane (Scheme 3). For configurational assignments keto alcohol **12** was converted into benzylidene acetal **18** and acetonide **19**. The ¹H NMR coupling constants (**18**: ³J_{7,8} = 10.0, ³J_{8,9ax} = 11.2, ³J_{8,9eq} = 4.5 Hz; **19**: ³J_{5,6} = 2.0, ³J_{6,7} = 1.8 Hz) and NOESY experiments corroborated the relative configurations of C7/C8 in **18** and of C5/C6/C7 in **19**, and hence of the relative and absolute configurations of **12**, **15**, and **17**.

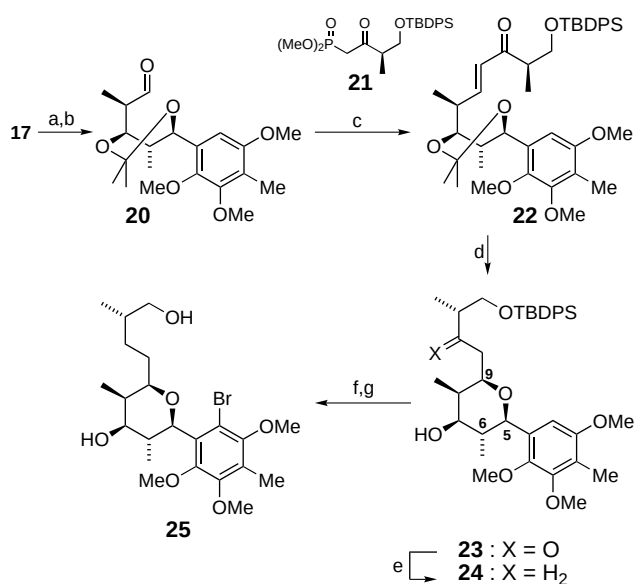
An intramolecular 1,4-addition of the C5–OH group to a C9–C11 enone was used for the construction of the tetrahydropyran ring (Scheme 4).^[9] Thus, aldehyde **20**, prepared from **17** in two steps, was coupled with ketophosphonate **21**^[10] in a Horner–Wadsworth–Emmons reaction to afford pure (*E*)-enone **22**. On removal of the acetonide with dilute HCl in methanol the free diol underwent the desired Michael-type

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Scheme 3. a) $\text{Me}_4\text{NBH}(\text{OAc})_3$, $\text{MeCN}:\text{AcOH}$ (1:1), $-30 \rightarrow 20^\circ\text{C}$, 16 h, 92–95%; b) $(\text{MeO})_2\text{CHMe}_2$, RT, 18 h, 88–93%; c) DDQ, 4-Å molecular sieves, CH_2Cl_2 , 3 h, 65%; d) 1. NaBH_4 , EtOH , 0°C , 0.5 h, 90%, separation of the ca. 1.5:1 mixture of *syn* and *anti* diols; 2. *syn* diol, $(\text{MeO})_2\text{CHMe}_2$, CSA (0.1 equiv), 22°C , 3 h, 92%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; CSA = (+)-camphorsulfonic acid; PMP = *p*-methoxyphenyl. The ^{13}C NMR chemical shifts of the diol protecting group are given for **19**.



Scheme 4. a) DDQ, 4-Å molecular sieves, CH_2Cl_2 :pH 7 buffer (15:1), 0.5 h, 83%; b) DMSO, $(\text{COCl})_2$, CH_2Cl_2 , -78°C , 1.5 h, then NEt_3 , $-78 \rightarrow 0^\circ\text{C}$, 0.25 h; c) **21**, LiOH , Et_2O , 15 min, RT, then addition of **20**, RT, 4 h, 85%; d) $\text{MeOH}:\text{HCl}_{\text{aq}}$ (0.2 M) (5:1), RT, 6 h, 92% (*ds* = 97:3); e) 1. *p*-TsNHNH $_2$, EtOH , 4-Å molecular sieves, reflux, 4 h; 2. NaCNBH_3 , ZnCl_2 , EtOH , reflux, 8 h, 65%; f) $\text{MeOH}:\text{HCl}_{\text{aq}}$ (2 M) (5:1), RT, 4 h, 97%; g) NBS, CH_3CN , 40°C , 12 h, 75%. DMSO = dimethylsulfoxide; *p*-Ts = *p*-toluenesulfonyl; NBS = *N*-bromosuccinimide; TBDPS = *tert*-butyldiphenylsilyl.

cyclization in situ to furnish tetrahydropyran **23**^[11] in satisfactory yield (84%) and high diastereoselectivity (*ds* = 97:3). Reduction of the carbonyl group gave intermediate **24**, which was transformed into arene **25**^[12] by O-deprotection and aromatic bromination.

The 5,9-*cis* configuration of the tetrahydropyran ring in **23** was assigned by 2D NMR experiments (COSY, HSQC, NOESY) (Figure 1, top). To our surprise, a very broad signal for the benzylic (5-H) and the neighboring proton (6-H), as

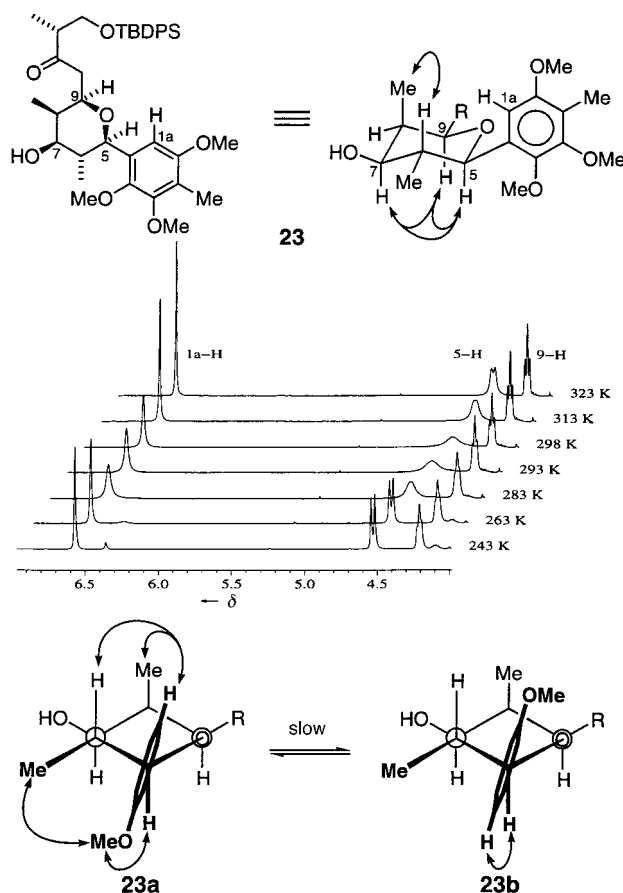


Figure 1. Hindered rotation in compound **23**. Top: structural formulas and indication of the NOEs in the THP unit; middle: NMR spectra of **23** at various temperatures showing the region containing the signals for 1a-, 5-, and 9-H; bottom: NOE interactions of the aromatic *ortho* substituents at -40°C in the main isomer **23a** and the minor isomer **23b**.

well as a slightly less-broad signal for the aromatic proton (1a-H) was found in both compounds **23** and **24**. In addition, the corresponding signals (C1a, C5, and C6) were missing in the ^{13}C NMR spectra of **23** and **24**. These phenomena were attributed to dynamic effects, and indeed, a temperature-dependent ^1H NMR study of compound **23** (Figure 1, middle) revealed that at lower temperature a second set of signals appeared at lower field, while the benzylic proton (5-H) sharpened to the expected doublet with a *trans*-diaxial coupling constant ($J_{5,6} = 10.7\text{ Hz}$). The NOE effects of **23** indicate there is a relatively rigid chairlike conformation, and hence these dynamic effects were assigned to a restricted $\text{sp}^2\text{-sp}^3$ rotation,^[13] with a coalescence temperature between 15 and 25°C . The individual rotamers **23a** and **23b**, in which the middle planes of the THP rings and arene planes are almost perpendicular to one another, could be identified from the NOE experiments. Thus, it was concluded that compound **23** exists as a 93:7 equilibrium mixture of the rotamers **23a** and **23b** at -40°C (Figure 1, bottom). Remarkably, the phenom-

enon is connected with the configuration at C9; the ^1H NMR spectrum of 9-*epi*-**23** showed sharp signals over the entire temperature range. The C9 side chain has to adopt an axial position in 9-*epi*-**23** which could lead to a twist conformation of the dihydropyran ring and hence to a larger degree of rotational freedom for the aryl residue.

The rotational barrier in aryl bromide **25** is considerably increased. Analysis of 2D NMR experiments indicated that **25** exists as a 1:5 mixture of the rotamers **25a** and **25b** (assignment from NOEs, Figure 2, top). In contrast to **23**, the

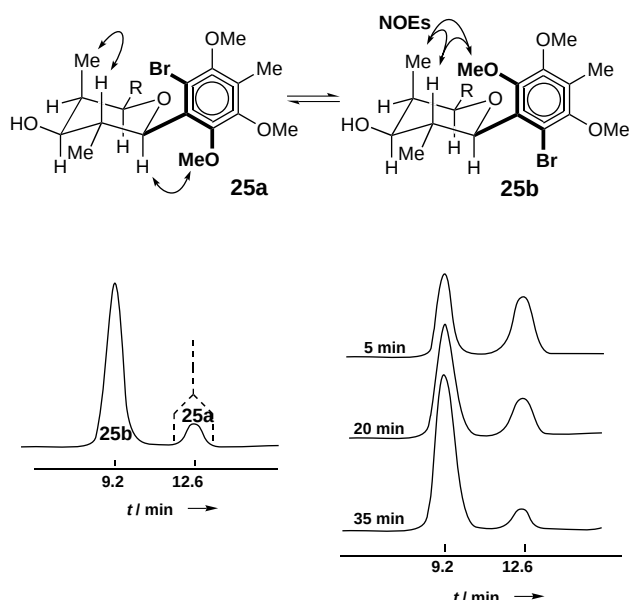


Figure 2. Atropisomerism of compound **25**. Top: NOE interactions in the THP unit of **25a** and **25b**; bottom left: HPLC separation of the rotamers **25a** and **25b**; bottom right: HPLC chromatograms of a pure solution of **15a**, analyzed after the given time intervals.

^1H NMR spectrum of **25** did not change with the temperature (up to 100°C), which suggests that the rotation is too slow to show dynamic NMR effects. Hence, compound **25** should exist as a mixture of two relatively long-lived atropisomers which might be separable by high-pressure liquid chromatography (HPLC). In fact, a base-line separation of **25a** and **25b** was achieved by maintaining the temperature of the HPLC column at 5°C . The minor isomer **25a** was collected, stored at 0°C , and reinjected after intervals of 5, 20, and 35 minutes (Figure 2, bottom) to show that the equilibration of the atropisomers **25a** and **25b** was complete at 0°C within 30 minutes. In view of the abundance of C-aryl glycosides in the realm of natural products^[14] this phenomenon is certainly of interest.^[15, 16]

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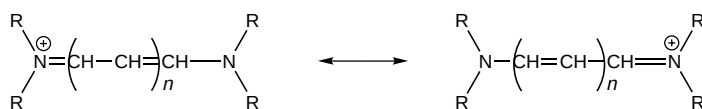
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[11] Analytical data of **23**: ^1H NMR (600 MHz, CDCl_3): δ = 7.61 (dt, J = 1.3, 7.7 Hz, 4H), 7.43–7.38 (m, 2H), 7.37–7.32 (m, 4H), 6.55 (brs, 1H), 4.56–4.25 (brs, 1H), 4.15 (t, J = 5.9 Hz, 1H), 3.83–3.77 (m, 1H), 3.80, 3.77, 3.76 (3s, $3 \times 3\text{H}$), 3.69–3.63 (brs, 1H), 3.61 (dd, J = 5.4, 10.1 Hz, 1H), 2.88 (dd, J = 6.5, 17.4 Hz, 1H), 2.77 (sext., J = 6.3 Hz, 1H), 2.65 (dd, J = 6.3, 17.4 Hz, 1H), 2.11 (s, 3H), 2.09–2.03 (m, 1H), 1.95–1.75 (brs, 1H), 1.06 (d, J = 7.1 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 0.98 (s, 9H), 0.78 (d, J = 6.8 Hz, 3H); ^{13}C NMR (62 MHz, CDCl_3): δ = 211.2, 154.8, 145.9, 135.9, 133.7, 131.2, 130.1, 128.1, 121.0, 66.2, 61.7, 60.7, 56.1, 49.4, 45.4, 38.4, 37.8, 27.2, 19.6, 13.7, 13.4, 9.3, 6.5; IR (film) $\tilde{\nu}$ = 2960, 2930, 2856, 1714, 1486, 1463, 1428, 1404, 1387, 1361, 1260, 1237, 1189, 1129, 1112, 1086, 1034 cm^{-1} ; MS (EI, 70 eV, 190°C): m/z : 648, 351, 223, 195; HR-MS (EI, 70 eV, 190°C): calcd for $\text{C}_{38}\text{H}_{52}\text{O}_7$ Si: 648.3482, found: 648.3469; $[\alpha]_D^{20}$ = +11.5 (c = 0.78, CHCl_3).
[12] Analytical data of **25** [minor atropisomer if assignable in brackets]: ^1H NMR (400 MHz, CDCl_3): δ = 4.70 (d, J = 10.3 Hz, 1H, 5-H) [4.65, (d, J = 10.7 Hz)], 3.86, 3.82, 3.75 (3s, 9H, $3 \times \text{OCH}_3$) [3.79, 3.78, 3.76 (3s)], 3.54–3.59 (m, 4H), 2.58–2.46 (m, 1H, 6-H) [2.74–2.62], 2.23 (s, 3H, ArCH_3), 2.02–1.92 (m, 1H, 8-H), 1.68–1.42 (m, 6H), 1.06 (d, J = 6.8 Hz, 3H, 8- CH_3) [1.13 (d, J = 6.8 Hz)], 0.92 (d, J = 6.7 Hz, 3H, 12- CH_3) [0.91 (d, J = 6.8 Hz)], 0.79 (d, J = 6.5 Hz, 3H, 6- CH_3) [0.72 (d, J = 6.5 Hz)]; ^{13}C NMR (100.6 MHz, CDCl_3): δ = 152.80 (C_{ar}), 151.81 (C_{ar}), 150.23 (C_{ar}), 131.87 (C_{ar}), 127.08 [126.88] (C_{ar}), 115.85 (C_{ar}), 84.10 [78.71] (C_5), 80.42 [80.87] (C_9), 78.14 [78.27] (C_7), 68.53 (C_{13}), 61.63 (OCH_3), 60.57 (OCH_3), 60.48 (OCH_3), 38.86 (C_8), 36.29 [34.46] (C_6), 36.20 (C_{12}), 30.34, 29.45 ($\text{C}_{10}/\text{C}_{11}$), 16.9 (12- CH_3), 13.59 [13.70] (6- CH_3), 10.59 (ArCH_3), 6.00 [6.92] (8- CH_3); IR (film) $\tilde{\nu}$ = 3340 (br), 2938, 1712, 1458, 1400, 1384, 1100, 1029 cm^{-1} ; MS (EI, 120°C): m/z : 476 (20.0), 474 (19.4), 302 (25.0), 300 (24.1), 289 (56), 206 (17), 183 (16), 149 (50), 121 (30), 57 (100); HR-MS (EI, 100°C): calcd for $\text{C}_{22}\text{H}_{35}\text{O}_6\text{Br}$: 474.1617, found 474.1623.
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[16] For a hindered rotation around a C-aryl glycosidic bond, see T. Matsumoto, M. Katsuki, H. Jona, K. Suzuki, *Tetrahedron Lett.* **1989**, 30, 6185, footnote 7.

Nonempirical Calculation of Polymethine Excited States**

Volker Buß,* Marko Schreiber, and Markus P. Fülcher

Polymethine dyes are charged or neutral, usually hetero-substituted, π -conjugated compounds in which the chromophore extends over an odd number of unsaturated centers.^[1] As a consequence of this topological peculiarity, which restricts the perturbation of the π -electrons over the σ -skeleton, the electronic absorption bands of polymethines are narrow and intense and can be shifted by proper choice of the chromophore and substituents from the UV/Vis into the infrared (IR) region, which makes them by far the most important class of organic dyes.^[2–4]

The theoretical treatment of polymethines, the industrial importance of which began with their use as spectral sensitizers in photography, has not improved significantly since Kuhn,^[5] more than fifty years ago, and shortly thereafter Simpson,^[6] treated the π -electrons of the simple streptocyanines (Scheme 1) quantum-mechanically as standing waves



Scheme 1. Mesomeric formulae for polymethines considered, with $n = 0$ –4 for $R = H$ and $n = 1$ –4 for $R = CH_3$.

in a one-dimensional box of appropriate length. These calculations, which are extremely simple by today's standards, were nevertheless able to reproduce the nonconvergent behavior of the dyes, that is, the observation that each vinyl unit added to the chromophore produces a spectral shift of about 100 nm—a feat which modern quantum-mechanical methods have difficulties to achieve. Of these, the semi-empirical Pariser-Parr-Pople (PPP) method has developed into the method of choice for correlating color and constitution of organic dye molecules, despite the only fair agreement between calculated and experimental absorption wavelengths.^[7] Semiempirical schemes at the all-valence-electron level such as CNDO/S and ZINDO/S are also highly parameterized, and the results are useful for practical purposes but of limited theoretical value.

We are not aware of any calculations of polymethine excited states at the nonempirical level, which seems odd considering the recent interest these compounds as so-called functional dyes^[8] for industrial use as laser dyes, in nonlinear optics, or as materials for optical data storage.^[9] Herein, we

report the quantitative agreement with experimental data of the excited-state energies of the simple streptocyanine dyes (Scheme 1, $R = H$ for $n = 0$ to 4, and $R = CH_3$ for $n = 1$ to 4) that we have calculated using an ab initio multiconfigurational wavefunction combined with second-order perturbation theory (CASPT2).^[10] The vinyl shift is quantitatively reproduced, as is the bathochromic shift arising from the methyl substitution at the terminal nitrogen atoms. The agreement with experiment is not the result of any arbitrary and serendipitous choice of the wave function, we have shown this by the systematic expansion of the basis set. Methods which employ single-determinant reference wave functions fail to predict correctly the excitation energies and the vinyl shift.^[11] The calculations open the possibility of computing, at the ab initio level, the influence of electronic, geometric, and solvent effects on the spectroscopic properties of cyanine dyes.

Vertical excitation energies were based on ground-state equilibrium geometries which were optimized by density functional theory (DFT) using the B3LYP exchange correlation functional and the 6-31G** basis set.^[12] The nonalternating bond lengths and the bond angles are scarcely affected by increasing the length of the chromophore; Figure 1 shows as a

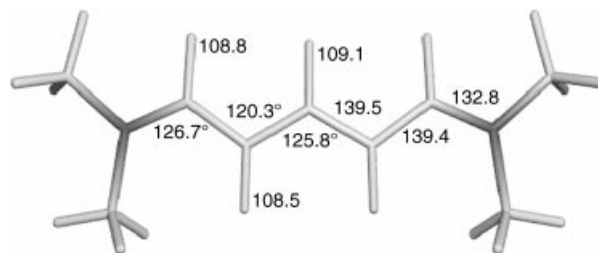


Figure 1. Energy-optimized B3LYP-6/31G** geometry of *N,N'*-tetramethylpentamethine, bond lengths [pm] and bond angles [°].

representative example, the optimized geometry of *N,N'*-tetramethylpentamethine. Along the chain there is a slight, but distinct alternation of the bond angles between around 121° and 125°, which correlates with the higher and lower π -densities at the respective carbon centers. Kulpe et al. have ascribed this alternation, which is clearly manifest in the X-ray structures of the streptocyanines,^[13] to hybridization changes.^[14] In agreement with this interpretation we find that the C–H bond lengths are longer at the carbon centers with higher s-character (i.e. larger C–C–C bond angles) and shorter at the carbon atoms between.

For the excited-state calculations we employed atomic natural orbital (ANO) type basis sets and the contraction scheme 4s3p1d/2s for the heavy atoms and for hydrogen, respectively. We also included, for the unsubstituted species, the more elaborate 4s3p2d/3s1p basis set. The active space for the CASSCF reference function included all π -orbitals (from 3 to 11) and π -electrons (from 4 to 12). State averaging was performed for the three lowest states considered. Energy corrections were calculated by CASPT2, and transition matrix elements by the CASSCF state interaction (CASSI) method, routines which are all provided within the MOLCAS-5^[15] software package. In Table 1, the calculated energies of the first and the second excited states, S_1 and S_2 , relative to the

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Table 1. Calculated^[a] and experimental^[b] energies^[c] and (in parentheses) oscillator strengths of the two lowest polymethine excited states as a function of chromophore length and the substituents (Scheme 1).

R		<i>n</i> = 0	1	2	3	4
H	S ₁	6.5 (0.46)	4.28 (0.87)	3.18 (1.34)	2.52 (1.79)	2.11 (2.23)
H	exp.		4.34	3.28		
H	S ₂	9.75 (0.15)	6.18 (0.05)	4.76 (–) ^[d]	3.68 (–)	3.01 (–)
CH ₃	S ₁		4.17 (0.74)	2.95 (1.16)	2.32 (1.81)	1.93 (2.29)
CH ₃	exp.	5.53	3.99 (0.87)	2.98 (1.12)	2.39 (1.32)	1.98
CH ₃	S ₂		6.12 (0.03)	4.68 (–)	3.56 (–)	2.93 (–)
CH ₃	exp.		5.71	5.51	4.00	3.45

[a] 4s3p2d/3s1p basis set for R = H and 4s3p1d/2s for R = CH₃. [b] Data are from B. Grimm, S. Dähne, G. Bach, *J. Prakt. Chem.* **1975**, 317, 161 (R = H) and from S. S. Malhotra, M. C. Whiting, *J. Chem. Soc.* **1960**, 6, 3812 (R = CH₃). [c] Relative to the S₀ (¹A₁) ground state; in eV. [d] Values smaller than 0.03 have been omitted.

ground state and their oscillator strengths are shown together with experimental results where available.

For the unsubstituted species, which were calculated with four different basis sets, only the results obtained with the 4s3p2d/3s1p basis are shown in Table 1. The energy of the ¹B₂ state calculated with this (the largest) basis and with the smallest basis 3s2p/2s averaged over the five compounds differs by only 0.05 eV, that is, the results are practically independent of the basis set at this level of approximation. The energy difference of the higher ¹A₁ state obtained with these two basis sets is significantly larger (0.34 eV).

The agreement of the S₁ state with the experimental data is excellent. The average deviation is 0.085 eV, which in the 500 nm range corresponds to about 17 nm. Moreover, the agreement is equally good for the short and for the long dye molecules and for the unsubstituted and the methyl substituted species, that is, both the vinyl shift and the bathochromic shift from the methyl groups are correctly reproduced. The agreement with the short axis polarized S₂ state is not quite as good (average deviation 0.55 eV) which is probably a result of experimental difficulties in localizing this weak transition next to the high-intensity S₁ state, but also to the higher calculation sophistication needed to describe higher excited states.

The bathochromic shift exerted by the methyl groups can be traced to a very simple pattern. Analysis of the wave function reveals that compared to the unsubstituted polymethines *all* the π -orbitals are raised in energy, but to a lesser extent as the orbital energies increase. This effect is exactly what one would expect from a perturbation of the π -orbitals of the chromophore by the low-lying p orbitals of each methyl carbon and the appropriate hydrogen 1s combinations, which leads to the decreased HOMO–LUMO gap and the observed red shift of the absorption maximum.

The calculated oscillator strengths of the S₁ state increase linearly with the length of the chromophore, in agreement with the simple electron in a box calculations. They agree closely with experiment for the short systems (*n* = 1 and 2). As the chromophore gets longer, there is a significant drop in the observed intensities relative to the calculated values, which may be an indication of the increasing contamination of the *all-trans*-polymethines by *cis*-isomers.

Configurationaly, the S₁ state is quite pure consisting of 100 % of the HOMO–LUMO single excitation in the case of the monomethine; for the nonamethine, its proportion is still 78 %; the S₂ state always contains doubly excited configurations, the contribution reaching 37 % in the nonamethine.

With respect to their composition, the polymethine excited states resemble conjugated *all-trans*-polyenes, with the S₁ state corresponding to the optically allowed ¹B_u state and S₂ to the forbidden ¹A_g state.^[16] However, in contrast to the polyenes, where the “dark” ¹A_g state drops below the ¹B_u state as the chromophore gets longer (the cross-over point appears to be reached with four conjugated double bonds^[17]), the 2¹A₁ state stays significantly above the ¹B₁ state, at least for the systems considered, though the gap decreases, from 1.9 to 0.9 eV, going from the tri- to the nonamethine. What happens to this gap as the chromophores become twisted or their symmetry is lowered by different end groups remains to be studied.

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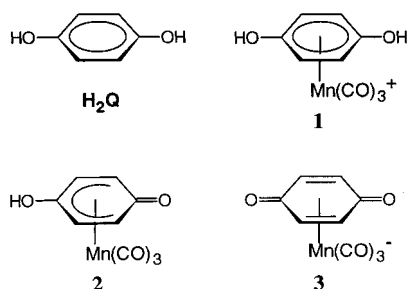
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Metal-Mediated Self-Assembly of π -Bonded Benzoquinone Complexes into Polymers with Tunable Geometries**

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Self-assembly of two or more components into supramolecular nanostructures, networks, and polymers has been used to generate materials that have many potential applications in magnetics, optics, molecular recognition, catalysis, sensing, separations, and general host–guest interactions.^[1–14] The recognition process implicit in the formation of such materials is most commonly based on hydrogen bonding, π – π stacking, or metal–ligand interactions. The use of transition metals in coordination-directed self-assembly of polymers and nanostructures has become a particularly fruitful area of investigation.

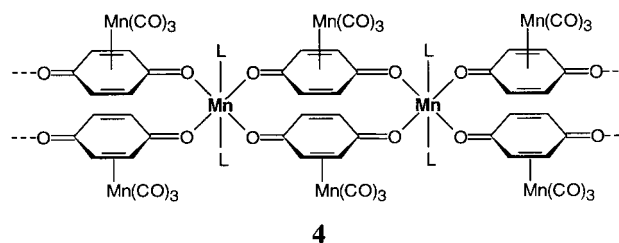
Herein we report that hydroquinone (H_2Q), which is the key building block in many biological proton–electron transfer reactions,^[15] can be induced to self-assemble into one-, two-, and three-dimensional polymers by suitable interaction



with transition metal ions. The type of polymer obtained appears to be readily controllable. The key step is initial coordination of the transition metal fragment $Mn(CO)_3^+$ to the π system in H_2Q , which facilitates proton loss from the OH groups. Subsequent σ complexation of the oxygen atoms to added metal ions is propagated in one, two, or three dimensions in a manner that depends on the geometrical requirements of the metal ion and on the presence of added ligands that function as “spacers”.

While there are many examples of metal coordination through σ bonding from the oxygen atoms in hydroquinone, as well as in the isomeric resorcinol and catechol molecules,^[16–18] metal complexation to the π network is rare.^[19–21] However, η^6 coordination of the $Mn(CO)_3^+$ moiety to afford complex **1** was found to be facile, and to result in greatly increased acidity of the OH groups, so that even a base as mild as Et_3N effects reversible deprotonation of **1** in DMSO to give η^5 -semi-

quinone (**2**) and η^4 -quinone (**3**) complexes.^[20] As shown herein, the oxygen atoms in **3**, generated from **1** or **2**, readily σ bind to metal ions. With divalent metal ions, the charges balance to neutral with each metal being bound to four quinoid oxygen atoms in a polymeric array. For example, when **2** was allowed to stand in DMSO at either 25 °C or 70 °C in the presence of ambient light, the one-dimensional (linear) polymer **4** was formed in good yield. In the absence of light,



no reaction occurred, which suggests that the Mn^{II} bridging ions in **4** originate from a photodissociation process. The need for light could be circumvented by merely adding Mn^{II} directly to **1** or **2** in the form of the acetate, $Mn(OAc)_2$, which automatically provides the base necessary to deprotonate the OH groups.

The X-ray structure of **4** is shown in Figure 1a.^[22] The bridging manganese metal is octahedral, with two axial DMSO ligands (L). The benzoquinone unit in **4** adopts a

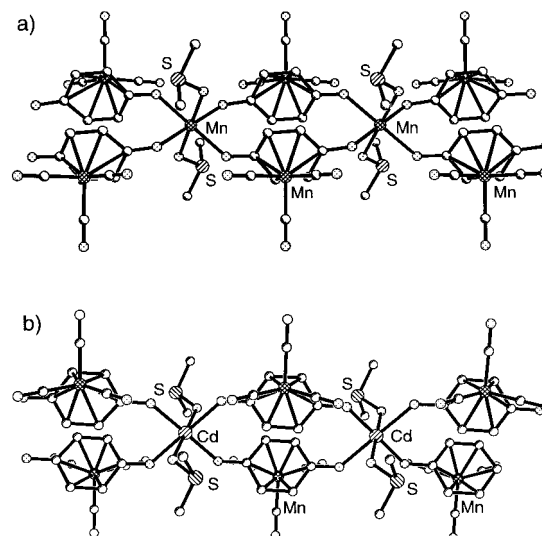


Figure 1. X-ray structure of benzoquinone polymers a) **4** and b) **5**.

boat conformation with the phenolic carbon atoms bent out of the diene plane by about 12°, which results in these carbon atoms being only weakly bonded to the $Mn(CO)_3$ unit. In contrast, the quinoid diene carbon atoms are strongly bonded to the $Mn(CO)_3$, so that the overall structure is best described as an η^4 -quinone. The bridging benzoquinone rings in **4**, which are roughly eclipsed, are separated by a perpendicular distance of 3.30 Å. Measurement of the magnetic susceptibility of polymer **4** at 298 K gave $\mu_{eff} = 5.9 \mu_B$ per monomer unit, as would be expected for a high-spin Mn^{II} bridge.

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Examination of the structure of **4** suggests two obvious ways in which the magnetic, optical, and structural properties could be tuned: 1) replacement of the axial ligand L and 2) replacement of the bridging metal. It was found that substitution of the axial DMSO ligands in **4** by pyridine (py) was readily achieved by performing the polymerization in DMSO containing an excess of free pyridine. An X-ray study of the product confirmed that it consists of polymeric structure **4** with L = py. Similarly, performing the polymerization of **3** with $\text{Cd}(\text{OAc})_2$ instead of $\text{Mn}(\text{OAc})_2$ led to the clean formation of **5**, which is essentially identical to **4** in which octahedral Cd^{II} substitutes for octahedral Mn^{II} . The X-ray structure of **5** is shown in Figure 1b.^[22] Preliminary experiments indicated that other divalent metal ions that prefer octahedral coordination, for example, Co^{II} and Ni^{II} , form analogous polymers.

The polymerization of the quinone complex **3** in the presence of a bridging metal that prefers tetrahedral rather than octahedral geometry would be expected to afford a nonlinear polymer structurally quite different from **4**. This possibility was explored using Zn^{II} , and the result was the three-dimensional polymer **6**. The X-ray structure of **6** (Figure 2) indeed features a bridging zinc ion that is tetra-

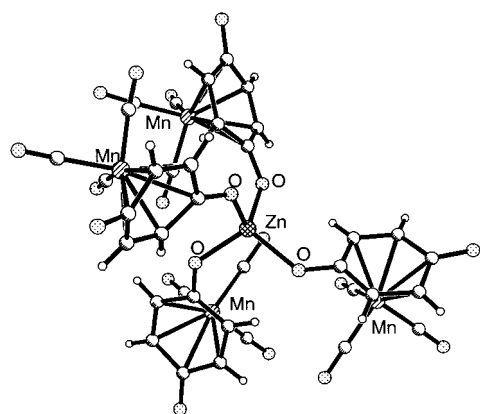
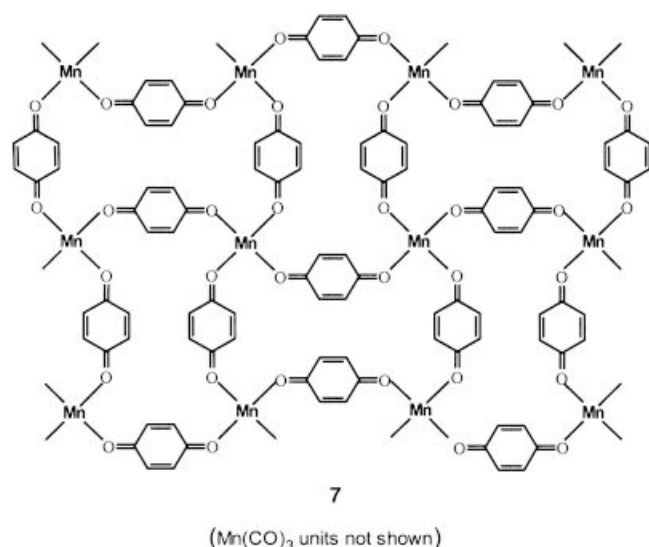


Figure 2. X-ray structure of zinc polymer **6**.



drally coordinated to the quinoid oxygen atoms. The solid-state structure in this case consists of two interpenetrating three-dimensional polymeric networks, as illustrated by the blue and yellow colors in Figure 3a. The polymer crystallizes in a chiral tetragonal space group and the crystals appear to be racemic twins. The chiral nature of the structure is seen in Figure 3b by noting the spiraling around a fourfold screw axis, with the two network spirals threaded together. The basic topology can also be described as two interpenetrating diamondoid networks.

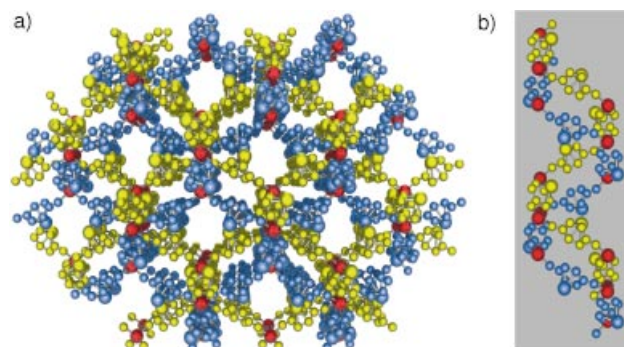
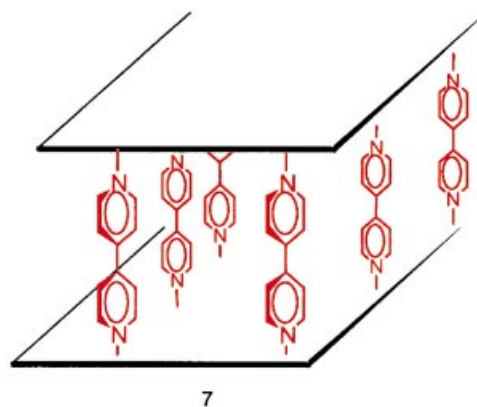


Figure 3. Structure of zinc polymer **6** showing a) the interpenetrating networks in blue and yellow with the zinc atoms shown in red and b) a portion of the structure along the crystallographic c axis showing how the spiral arrangements are threaded together. The CO ligands are omitted for clarity.

An attempt was made to synthesize polymer **4** with 4,4'-bipyridine (4,4'-bipy) as the axial ligand. The expectation was that 4,4'-bipy would serve as a bifunctional spacer^[23] and join together the linear polymer units in **4**. However, when complex **1** or **2** was combined with $\text{Mn}(\text{OAc})_2$ and excess 4,4'-bipy in DMSO, polymerization did not afford a linear product. Rather, two-dimensional pseudo-planar networks joined by 4,4'-bipy spacers were produced (**7**). A comparison of line drawings for **4** and **7** illustrates the different ways in which the quinone molecules **3** are linked to produce one-



dimensional and two-dimensional structures, respectively. The X-ray structure of **7** was determined and is shown in Figure 4.^[22] An intriguing feature of **7** is the presence of rectangular channels or pores that are bounded by the bipyridine spacers and the pseudo-planar quinoid networks. The channels, clearly visible in Figure 4, have a cross section or "opening" of about 12×6 Å. The longitudinal axis of each channel is propagated in the unit cell *b* direction with a repeat distance of 12.3 Å, thus generating an array of molecular boxes. In the crystalline polymer, the channels are filled with two disordered DMSO solvent molecules per molecular box.

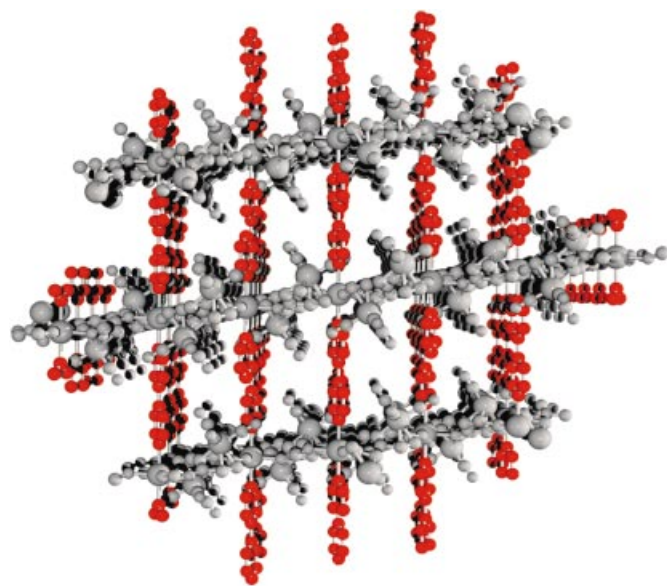


Figure 4. Structure of polymer **7** with the bridging bipyridine ligands shown in red. Each rectangular "box" contains two DMSO molecules (not shown).

The nanoporous polymer **7** appears to be of indefinite thermal stability at room temperature. It is likely that the manganese ion bridging the quinone centers in **7** can be substituted for a variety of metals. The quinoid networks in this material could provide the foundation for the synthesis of polymers with tunable pore size by variation of the bifunctional spacer, which may also allow control of the chemical environment in the nanopores. The attachment of a redox switch to the spacer may permit individual quinoid network planes to be differentially addressed and activated.^[24]

In conclusion, we have shown that π complexation to benzoquinone activates the molecule to metal-mediated polymerization. The resulting materials can be predesigned to have one-, two-, or three-dimensional quinoid structures. Applications of these materials in electrocatalysis are being investigated, as well as possible extension to *ortho*- and *meta*-quinones. The chemistry described herein may also be applicable to the stabilization and utilization of quinone methides, which are reactive species important in biochemical and natural products chemistry, and believed to occur as intermediates in the reactions of some antitumor drugs.^[25–27]

Experimental Section

Synthesis of polymers 4–7: The semiquinone complex **2** (100 mg, 0.40 mmol) and $\text{Mn}(\text{OAc})_2 \cdot \text{tetrahydrate}$ (49 mg, 0.20 mmol) were combined in DMSO (10 mL) under N_2 at room temperature. The reaction mixture was stirred in the absence of light for 24 h. The crystalline polymer **4** that had precipitated over this period was filtered and washed with acetone and diethyl ether to afford a yield of 86%. The cadmium analogue **5** was obtained in 70% yield by the same procedure except that $\text{Cd}(\text{OAc})_2$ replaced the $\text{Mn}(\text{OAc})_2$. The zinc polymer **6** was synthesized in 60% yield by combining **2**, $\text{Zn}(\text{ClO}_4)_2$, and NEt_3 (molar ratio 1:0.6:1) in DMSO and heating to 70 °C for 10 days. The synthesis of **7** was achieved in 78% yield by combining **1** or **2** with $\text{Mn}(\text{OAc})_2$ and excess 4,4'-bipyridine in DMSO (70 °C, 13 h). Finally, it may be noted that the manganese polymer **4** was also obtained in good yield (73%) by exposing a DMSO solution of **2** to room light for several days.

Characterization of polymers 4–7: For **4**: IR (DMSO): $\bar{\nu}_{\text{CO}} = 1997$ (s), 1915 (s) cm^{-1} ; IR (KBr): $\bar{\nu}_{\text{CO}} = 2021$ (s), 1945 (s), 1566 (s), 1521 (s), 1016 (m) cm^{-1} ; elemental analysis calcd (%) for $\text{C}_{18}\text{H}_8\text{Mn}_3\text{O}_{10} \cdot (\text{DMSO})_2$: C 37.46, H 2.86, S 9.09; found: C 37.60, H 2.74, S 8.92. For **5**: IR (DMSO): $\bar{\nu}_{\text{CO}} = 1997$ (s), 1918 (s) cm^{-1} ; IR (KBr): $\bar{\nu}_{\text{CO}} = 2020$ (s), 1948 (s), 1559 (s), 1519 (s), 1010 (m) cm^{-1} ; ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta = 4.83$ (br, 4H); elemental analysis calcd (%) for $\text{C}_{18}\text{H}_8\text{Mn}_3\text{CdO}_{10} \cdot (\text{DMSO})_2$: C 34.64, H 2.64, S 8.41; found: C 34.22, H 2.34, S 7.15. For **6**: IR (DMSO): $\bar{\nu}_{\text{CO}} = 1997$ (s), 1917 (s) cm^{-1} ; IR (KBr): $\bar{\nu}_{\text{CO}} = 2024$ (s), 1945 (s), 1559 (s), 1513 (s) cm^{-1} ; ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta = 4.78$ (br, 4H); elemental analysis calcd (%) for $\text{C}_{18}\text{H}_8\text{Mn}_3\text{ZnO}_{10}$: C 38.64, H 1.44; found: C 37.45, H 1.37. For **7** $\cdot 2\text{DMSO}$: IR (DMSO): $\bar{\nu}_{\text{CO}} = 1998$ (s), 1918 (s) cm^{-1} ; IR (KBr): $\bar{\nu}_{\text{CO}} = 2021$ (s), 1957 (s), 1931 (s), 1603 (w), 1566 (s), 1520 (s), 1058 (m) cm^{-1} ; elemental analysis calcd (%) for $\text{C}_{32}\text{H}_{28}\text{Mn}_3\text{N}_2\text{O}_{12}\text{S}_2$: C 44.61, H 3.28, N 3.25, S 7.44; found: C 43.73, H 2.98, N 2.84, S 7.42.

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- [22] X-ray structural analysis of **4–7**. Crystals of **4–7** suitable for X-ray studies were grown from DMSO solutions. In all cases, X-ray data collection with MoK_α radiation was carried out at 298 K using a Siemens P4 diffractometer equipped with a CCD area detector.

Structures were determined by direct methods and refined on F^2 . Crystal data for **4**: $C_{11}H_{10}Mn_{1.5}O_6S$, $fw = 352.66$, monoclinic, space group $P2_1/c$, $a = 8.3033(5)$, $b = 12.4987(8)$, $c = 12.9551(8)$ Å, $\beta = 90.228(1)^\circ$, $V = 1344.48(14)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.742$ g cm⁻³, $\mu = 1.604$ mm⁻¹, $F(000) = 710$, θ range 2.26 – 26.39° , 180 variables refined with 2757 independent reflections to final R indices [$I > 2\sigma(I)$] of $R_1 = 0.0512$ and $wR_2 = 0.1330$, and $GOF = 1.137$. Crystal data for **5**: $C_{11}H_{10}MnCd_{0.5}O_6S$, $fw = 381.39$, monoclinic, space group $P2_1/c$, $a = 8.4067(9)$, $b = 12.4128(13)$, $c = 12.9325(14)$ Å, $\beta = 90.089(2)^\circ$, $V = 1349.5(2)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.877$ g cm⁻³, $\mu = 1.918$ mm⁻¹, $F(000) = 756$, θ range 2.27 – 25.09° , 219 variables refined with 2383 independent reflections to final R indices [$I > 2\sigma(I)$] of $R_1 = 0.0685$ and $wR_2 = 0.1559$, and $GOF = 1.149$. Crystal data for **6**: $C_{36}H_{16}Mn_4Zn_2O_{20}$, $fw = 1118.99$, tetragonal, space group $P4_3$, $a = 15.4449(13)$, $c = 15.664(2)$ Å, $V = 3736.4(7)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.989$ g cm⁻³, $\mu = 2.663$ mm⁻¹, $F(000) = 2208$, θ range 1.85 – 26.42° , 559 variables refined with 7684 independent reflections to final R indices [$I > 2\sigma(I)$] of $R_1 = 0.0680$ and $wR_2 = 0.1560$, and $GOF = 1.154$. Crystal data for **7**: $C_{16}H_{14}Mn_{1.5}NO_6S$, $fw = 430.75$, monoclinic, space group $P2_1/c$, $a = 11.7056(10)$, $b = 12.3216(10)$, $c = 12.7812(10)$ Å, $\beta = 98.154(2)^\circ$, $V = 1824.8(3)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.568$ g cm⁻³, $\mu = 1.199$ mm⁻¹, $F(000) = 874$, θ range 1.76 – 26.47° , 253 variables refined with 3757 independent reflections to final R indices [$I > 2\sigma(I)$] of $R_1 = 0.0567$ and $wR_2 = 0.1161$, and $GOF = 1.304$. 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-161517 (**4**), CCDC-161518 (**5**), CCDC-161519 (**6**), and CCDC-161520 (**7**). 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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A Large Dendritic Multiporphyrin Array as a Mimic of the Bacterial Light-Harvesting Antenna Complex: Molecular Design of an Efficient Energy Funnel for Visible Photons**

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Photosynthesis is triggered by the absorption of a photon by the light-harvesting antenna system, which has a large absorption cross-section. This process is followed by a rapid and efficient transfer of the excitation energy to the reaction center, where conversion of solar energy into chemical potentials in the form of a charge-separated state takes place.^[1, 2] To mimic the natural photosynthetic systems, artificial light-harvesting antennae consisting of many porphyrin units linked through covalent^[3] and noncovalent^[4] bonds have been synthesized which can provide a large absorption cross-section to capture dilute photons. However, utilization of the light energy requires further molecular design to realize a long-range vectorial transfer of the excitation energy to a designated point. An efficient cooperation of chromophore units is essential if this is to be achieved. Such a molecular design could be inspired by the successful crystal-structure analysis of the light-harvesting antenna complex (LH2) from purple photosynthetic bacterium *Rhodospseudomonas acidophila* strain 10050, where highly efficient light-driven energy migration and transfer are realized by the wheel-like arrays of a large number of bacteriochlorophyll *a* (Bchl *a*) units.^[5]

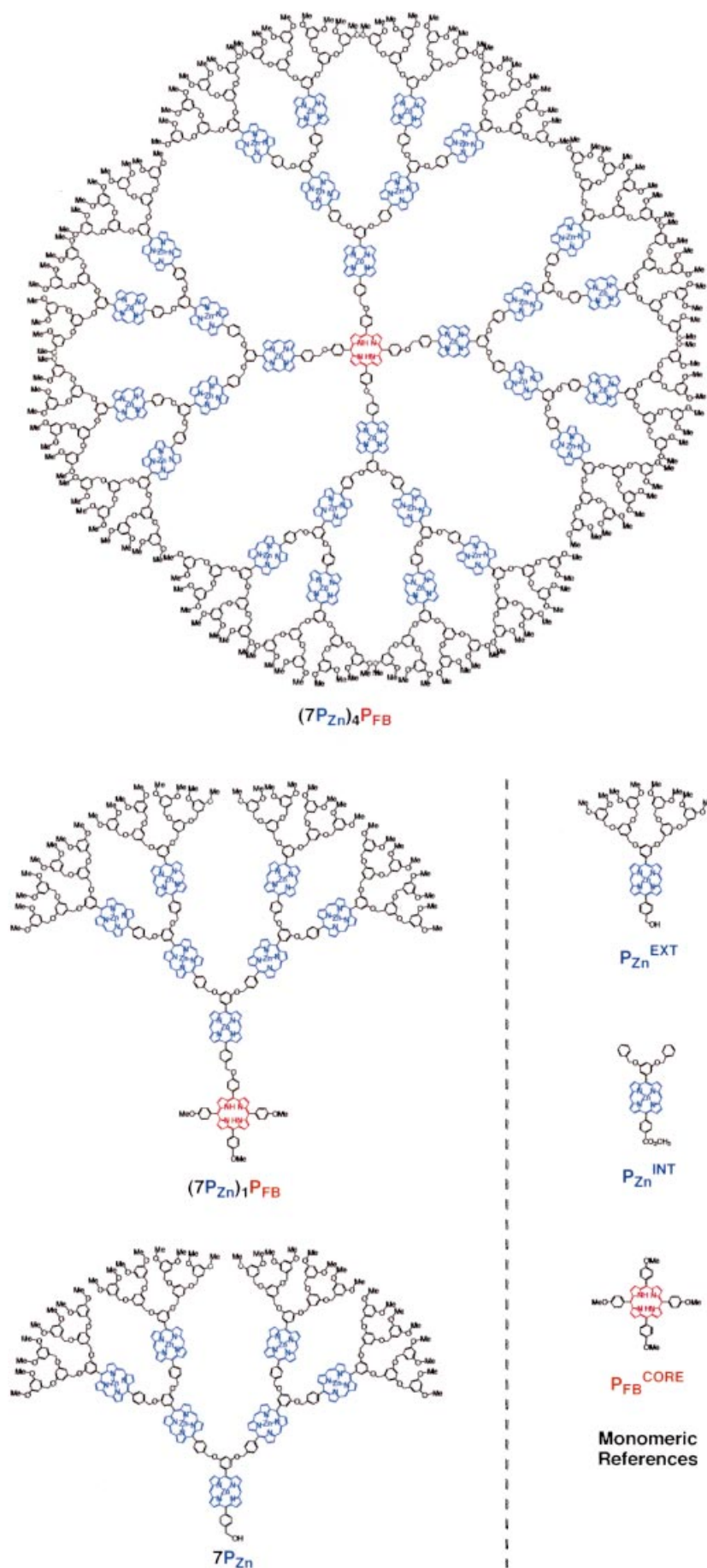
Recently, dendritic architectures have attracted attention as potential scaffolds for the incorporation of many light-absorbing units to enable the efficient capturing of photons.^[6] Herein we report on a designed large multiporphyrin array ($(7P_{Zn})_4P_{FB}$)^[7] consisting of four dendritic wedges of a zinc porphyrin heptamer ($7P_{Zn}$) as the energy-donating units which are anchored by a focal free-base porphyrin unit (P_{FB}) which functions as the energy acceptor. This star-shaped dendritic macromolecule is intended to mimic the unique morphology of the wheel-like chromophore array in LH2, and is expected to operate as an efficient energy funnel for visible

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[+] Time-resolved spectroscopy

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photons in an analogous fashion to the natural antennae. Although the importance of π -electronic conjugation between chromophore units for facilitating energy transfer has been suggested,^[8] our light-harvesting molecule $(7P_{Zn})_4P_{FB}$, in contrast, is devoid of any π -electronic bridges, while the chromophore units are simply suspended by the dendritic scaffold through flexible ether linkers. A point to be highlighted here is how the cooperativeness of the chromophore units in energy migration and transfer is affected by the morphology of the multiporphyrin array.^[9] Thus, conically shaped $(7P_{Zn})_1P_{FB}$ with only a single dendritic wedge of the zinc porphyrin heptamer ($7P_{Zn}$) was synthesized as a morphological reference for star-shaped $(7P_{Zn})_4P_{FB}$.^[7] The presence of poly(benzyl ether) dendritic wedges^[10] at the periphery makes both of these multiporphyrin arrays highly soluble in common organic solvents such as CH_2Cl_2 , $CHCl_3$, and THF.

A solution of $(7P_{Zn})_4P_{FB}$ in THF at 25 °C showed an intense Soret absorption band at 415.8 nm ($\epsilon = 5\,130\,000\text{ L cm}^{-1}\text{ mol}^{-1}$).^[7] This band was red-shifted by 1.3 nm with a slight broadening (FWHM = 19200 cm^{-1}) relative to a noncovalent reference prepared by simply mixing three monomeric porphyrins P_{Zn}^{EXT} , P_{Zn}^{INT} , and P_{FB}^{CORE} at a molar ratio of 16:12:1 ($\lambda_{Soret} = 414.5\text{ nm}$, FWHM = 12600 cm^{-1}). The Q-bands, however, showed neither substantial shift nor broadening, which indicates there is a weak ground-state interaction between the P_{Zn} units in the dendritic array.^[11]

When a solution of the noncovalent reference mixture P_{Zn}^{EXT} , P_{Zn}^{INT} , and P_{FB}^{CORE} (16:12:1) in THF was excited at 544 nm, only a fluorescence from the P_{Zn} units ($Q(0,0) = 589$, $Q(0,1) = 637\text{ nm}$) was detected (Figure 1 A, broken line). In contrast, excitation of star-shaped $(7P_{Zn})_4P_{FB}$ under identical conditions to the above resulted in the detection of an emission predominantly from the focal P_{FB} unit ($Q_X(0,0) = 658$, $Q_X(0,1) = 723\text{ nm}$), with only a weak emission from the P_{Zn} units (Figure 1 A, solid line). This result suggests there is an efficient transfer of energy from the excited singlet states of the dendritic P_{Zn} units to the focal P_{FB} unit. Accordingly, the excitation spectrum of $(7P_{Zn})_4P_{FB}$ monitored at 725 nm (fluorescence from P_{FB}) displayed substantial absorption characteristics of the 28 P_{Zn} units.

Although the absorption spectrum of conically shaped $(7P_{Zn})_1P_{FB}$ in THF at 25 °C was quite similar to that of star-shaped $(7P_{Zn})_4P_{FB}$ ($\lambda_{Soret} = 416.6\text{ nm}$, $\epsilon = 2\,290\,000\text{ L cm}^{-1}\text{ mol}^{-1}$),^[7] the luminescence profile was much different: Excitation of $(7P_{Zn})_1P_{FB}$ at 544 nm resulted in an emission spectrum (Figure 1 B, solid line) in which the fluorescence intensity from the focal P_{FB} unit,

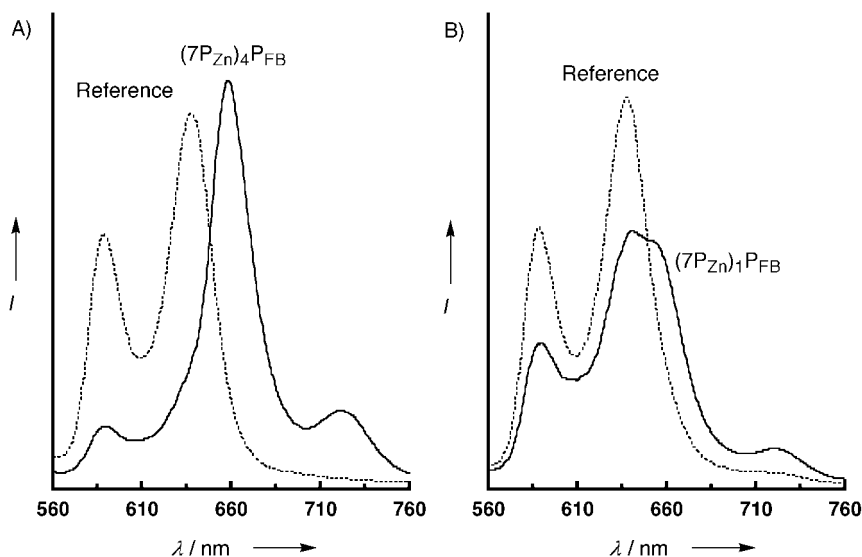


Figure 1. Steady-state fluorescence spectra upon excitation of a solution of star-shaped $(7P_{Zn})_4P_{FB}$ (A; solid line) and conically shaped $(7P_{Zn})_1P_{FB}$ (B; solid line) in THF (25 °C) at 544 nm together with those of their noncovalent references (broken lines) consisting of P_{Zn}^{EXT} , P_{Zn}^{INT} , and P_{FB} at molar ratios of 16:12:1 and 4:3:1, respectively. All the spectra were normalized to a constant absorbance ($A = 0.03$) at the excitation wavelength.

relative to that from the dendritic P_{Zn} units, was much less pronounced than in the case of $(7P_{Zn})_4P_{FB}$. On the other hand, a noncovalent reference of $P_{Zn}^{EXT}:P_{Zn}^{INT}:P_{FB}^{CORE}$ (4:3:1) again showed, under identical conditions to the above, a fluorescence predominantly from the zinc porphyrin components, with only a little contribution from the free-base porphyrin (Figure 1 B, broken line). These observations suggest a much less efficient $P_{Zn} \rightarrow P_{FB}$ energy transfer in conically shaped $(7P_{Zn})_1P_{FB}$ than in star-shaped $(7P_{Zn})_4P_{FB}$.

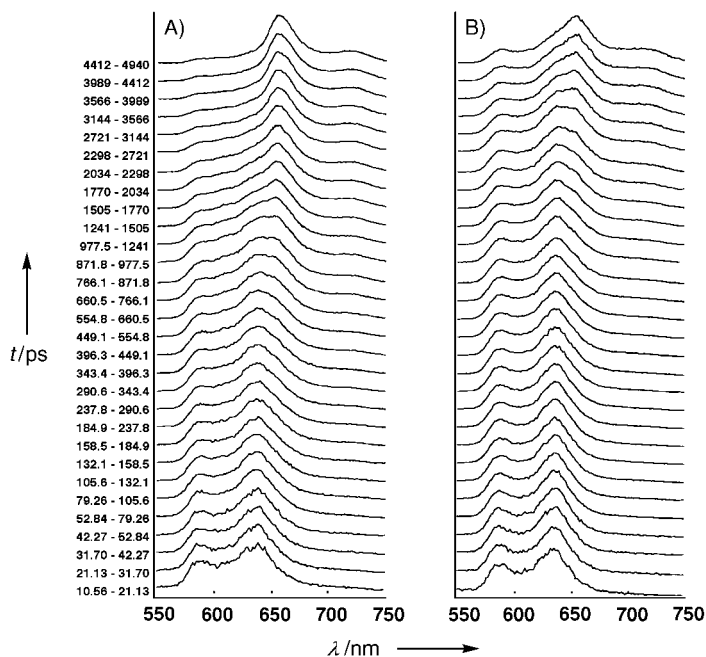


Figure 2. Time-resolved fluorescence spectra upon excitation at 415 nm of star-shaped $(7P_{Zn})_4P_{FB}$ (A) and conically shaped $(7P_{Zn})_1P_{FB}$ (B) in THF at 25 °C.

Picosecond time-resolved fluorescence spectroscopy of $(7P_{Zn})_4P_{FB}$ and $(7P_{Zn})_1P_{FB}$ was conducted to evaluate the intramolecular energy transfer events in a more quantitative manner. When star-shaped $(7P_{Zn})_4P_{FB}$ was excited at 415 nm, the time-resolved fluorescence spectrum (Figure 2 A) initially showed a rise in the fluorescence from the P_{Zn} units (589, 637 nm) which then decayed progressively to allow a rise in a new fluorescence from the P_{FB} core. Although a similar time-resolved spectral change was observed for conically shaped $(7P_{Zn})_1P_{FB}$ (Figure 2 B), the rise in the fluorescence from the P_{FB} units (658, 723 nm) was much slower than in the case of star-shaped $(7P_{Zn})_4P_{FB}$. For comparison, a dendritic zinc porphyrin heptamer $7P_{Zn}^{[7]}$ without the energy-accepting free-base porphyrin (P_{FB}) core showed, upon excitation at 415 nm, a fluorescence decay profile at 585 nm (Figure 3 C), which could be fitted with a single exponential decay for a

lifetime (τ_D) of 2309 ps ($\chi^2 = 1.13$). Since this decay profile was quite similar to that of a monomeric zinc porphyrin such as P_{Zn}^{EXT} ($\tau_D = 2305$ ps, $\chi^2 = 1.28$), self-quenching of the photo-excited P_{Zn} units in the dendritic $7P_{Zn}$ wedges is unlikely to occur. In contrast, $(7P_{Zn})_4P_{FB}$ showed a remarkable quenching signature in the fluorescence decay profile (Figure 3 A), which was analyzed as the sum of three exponential components

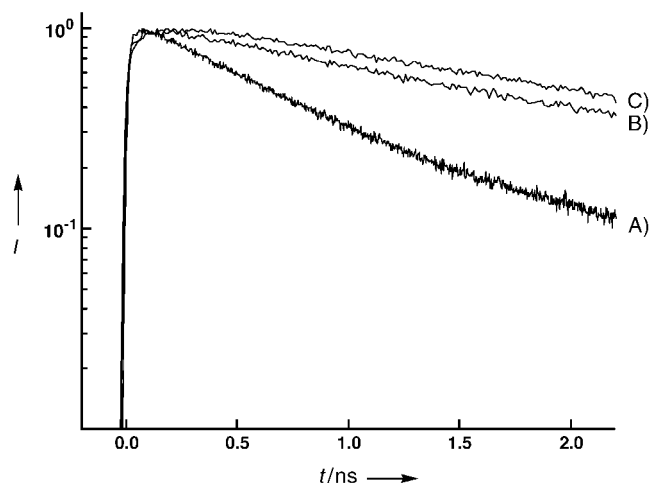


Figure 3. Fluorescence decay profiles upon excitation at 415 nm of star-shaped $(7P_{Zn})_4P_{FB}$ (A), conically shaped $(7P_{Zn})_1P_{FB}$ (B), and reference $7P_{Zn}$ (C) in THF ($A_{ext} < 0.1$) at 25 °C, monitored at 585 nm.

($\chi^2 = 0.99$) with τ_{DA} values of 70 (21 %), 500 (56 %), and 1674 (23 %) ps. The conically shaped $(7P_{Zn})_1P_{FB}$, however, displayed only a little quenching in the fluorescence decay curve (Figure 3 B), which gave three exponential components ($\chi^2 = 1.03$) with τ_{DA} values of 79 (3 %), 640 (25 %), and 2367

(72 %) ps. Since these values are roughly consistent with the fluorescence rise-times of the P_{FB} units in $(7P_{Zn})_4P_{FB}$ and $(7P_{Zn})_1P_{FB}$,^[12] the decay profiles in Figure 3 can be utilized for the kinetic analysis of the transfer of excitation energy in the dendritic multiporphyrin arrays. Thus, the energy-transfer rate constant k_{ENT} for star-shaped $(7P_{Zn})_4P_{FB}$ could be evaluated from the average τ_{DA} value (τ_{DA}^{av}) to be $1.04 \times 10^9 \text{ s}^{-1}$, which is one order of magnitude larger than that for conically shaped $(7P_{Zn})_1P_{FB}$ ($0.10 \times 10^9 \text{ s}^{-1}$). The energy-transfer efficiencies Φ_{ENT} , calculated from these k_{ENT} values, were 71 and 19% for $(7P_{Zn})_4P_{FB}$ and $(7P_{Zn})_1P_{FB}$, respectively.^[13] Such large differences in the k_{ENT} and Φ_{ENT} values for $(7P_{Zn})_4P_{FB}$ and $(7P_{Zn})_1P_{FB}$ clearly demonstrate a significant role of the morphology of the chromophore array in energy transfer.

In the photochemical events in bacterial light-harvesting antenna complex LH2, the excitation energy is considered to be maintained in the wheel-like chromophore array without radiative decay as a result of a superb cooperativity among the chromophore units for energy migration. In order to evaluate the energy migration characteristics in morphologically different $(7P_{Zn})_4P_{FB}$ and $(7P_{Zn})_1P_{FB}$, a steady-state fluorescence depolarization was investigated in a viscous medium of polyethylene glycol (MW=200) at 25 °C, where molecular motions that lead to fluorescence depolarization should be suppressed. In such a case, fluorescence depolarization should occur mostly by energy migration between randomly oriented chromophore units in the dendritic array. Excitation of $(7P_{Zn})_4P_{FB}$ at 544 nm ($A=0.03$) with polarized light resulted in a highly depolarized fluorescence from the P_{Zn} units, where the fluorescence anisotropy (P) was evaluated to be only 0.03. This value is considerably smaller than that of monomeric P_{Zn}^{EXT} ($P=0.19$), used as an isolated chromophore reference. This observation indicates the occurrence of an efficient migration of excitation energy over the dendritic zinc porphyrin array in star-shaped $(7P_{Zn})_4P_{FB}$ before transfer to the focal free-base porphyrin unit.^[9] Conically shaped $(7P_{Zn})_1P_{FB}$ bearing only a single dendritic zinc porphyrin wedge, however, showed a larger P value (0.10) than $(7P_{Zn})_4P_{FB}$. These contrasting trends indicate a possible cooperation of the four dendritic zinc porphyrin wedges in large $(7P_{Zn})_4P_{FB}$ for facilitating the energy migration among the P_{Zn} units.^[14] Such a long-range energy migration in $(7P_{Zn})_4P_{FB}$ is quite interesting when the absence of any π -electronic conjugations among the P_{Zn} units is considered.

In conclusion, by mimicking the natural light-harvesting antenna complex LH2, we designed a novel large multiporphyrin array $(7P_{Zn})_4P_{FB}$ by incorporating 28 light-absorbing zinc porphyrin units into a dendritic scaffold having a focal free-base porphyrin unit as the energy acceptor. The large number of chromophore units in $(7P_{Zn})_4P_{FB}$ allows an efficient capture of visible photons. More importantly, the zinc porphyrin units in star-shaped $(7P_{Zn})_4P_{FB}$ cooperate with one another, to facilitate long-range energy migration and transfer without any assistance of π -electronic conjugation. Thus, the bio-inspired approach reported herein provides a new paradigm for the rational molecular design of highly efficient light-harvesting antennae.

Experimental Section

$(7P_{Zn})_4P_{FB}$, $(7P_{Zn})_1P_{FB}$, and reference $7P_{Zn}$ were synthesized by the convergent approach using a 5,15-diarylporphyrin as the fundamental building block.^[7] Time-resolved fluorescence spectra and decay curves were measured with a picosecond pulse laser and a single-photon timing apparatus. The laser system was a mode-locked titanium-sapphire laser (Coherent, Mira900) and pumped by an Ar ion laser (Coherent, Innova300) combined with a pulse picker (Coherent, Model9200). The decay curve was obtained with an excitation wavelength of 415 nm by using a microchannel-plate photomultiplier (Hamamatsu R2809U-01). The pulse width of the instrumental response function was 30 ps (full width of half maximum height, FWHM). The energy-transfer rate constant (k_{ENT}) was calculated according to: $k_{ENT} = 1/\tau_{DA}^{av} - 1/\tau_D$, where τ_{DA}^{av} represents the average fluorescence lifetime of P_{Zn} units in a dendritic multiporphyrin array, while τ_D denotes the fluorescence lifetime of the P_{Zn} units in $7P_{Zn}$. The energy-transfer efficiency (Φ_{ENT}) was calculated according to: $\Phi_{ENT} = k_{ENT}\tau_{DA}^{av} = 1 - \tau_{DA}^{av}/\tau_D$. The fluorescence anisotropy (P) was evaluated according to: $P = (I_{\parallel} - GI_{\perp})/(I_{\parallel} + GI_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are fluorescence intensities observed through polarizers oriented parallel and perpendicular, respectively, to a vertically polarized excitation light, G is an instrumental correction factor for depolarization effects arising from the instrument and is given by: $i_{\parallel}/i_{\perp}$, where $i_{\parallel}$ and $i_{\perp}$ denote the fluorescence intensities observed through the polarizers.

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Color Indicators of Molecular Chirality Based on Doped Liquid Crystals

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With the rapidly increasing role of combinatorial methods in chemistry and (bio)catalysis there is an ever-growing need for fast screening processes for activity and selectivity.^[1] This is particularly evident in the search for high enantioselectivity in asymmetric catalysis, which is often a time-consuming process. Recently reported methods for enantiomeric excess (*ee*) determination employ,^[2] in an elegant way, UV/Vis spectroscopy,^[3] fluorescence,^[4] mass spectrometry,^[5] and IR thermography.^[6] One ingenious assay involves the different fluorescence emission of enantiomers and using a method based on four synthetic steps including a kinetic resolution.^[7] Traditional techniques for chiral chromatographic analysis are currently being miniaturized to allow rapid screening and, although enantiomer separation is still necessary, new methods such as parallel chiral capillary electrophoresis offer promising alternatives.^[8] Direct visualization of molecular

chirality is a fundamental challenge which ultimately could have considerable practical importance for the rapid screening of libraries of nonracemic compounds. Here we report a simple color test for enantiomeric excess (*ee*) based on the chirality-dependent color generation in doped films of liquid crystals. Our design for a color test of chirality is based on the consideration that a change from a nematic to a cholesteric phase in liquid crystalline (LC) materials can be induced upon doping with suitable chiral guest compounds.^[9] For example, we have shown by using chiral molecular switches as dopants that LC materials can function as amplifiers of chirality.^[10, 11]

The chirality of a cholesteric LC material is indicated by the sign and magnitude of the cholesteric pitch. The pitch is dependent on: 1) the concentration (*c* in wt %) of the dopant; 2) the helical twisting power (β) of the dopant, and 3) the enantiomeric excess (*ee*) of the dopant [Eq. (1)].

$$\text{pitch } (p) = (c\beta ee)^{-1} \quad (1)$$

The helical twisting power is an intrinsic property of any chiral dopant which indicates how efficient this molecule is in inducing a chiral orientation in the LC material.

The pitch, for a given chiral substrate doped in an LC matrix at a fixed concentration, is dependent only on the *ee* value of the dopant. This property, therefore, might be used as a measure for the enantiopurity of any chiral compound that can induce a cholesteric phase.

The pitch is generally determined by the Grandjean–Cano technique,^[12] a method which requires an aligned LC sample to be placed between a plane-convex lens and a flat surface. The pitch can then be obtained from the distances between distinct lines seen through a polarizing microscope. It is evident that for screening purposes this technique for the determination of *ee* values can hardly compete with other techniques because of laborious sample preparation. Cholesteric materials, however, show interesting optical properties when the pitch of the liquid crystal lies in the region of the wavelength of visible light. Liquid crystalline samples which are oriented by a linearly rubbed polyimide-covered glass plate show reflection at a specific wavelength (λ) that is dependent on the angle of the incident light (α) relative to the normal of the surface and on the average refractive index of the material (*n*) [Eq. (2)].^[13]

$$\lambda(\alpha) = n p \cos[\sin^{-1}(\sin \alpha / n)] = n(c\beta ee)^{-1} \cos[\sin^{-1}(\sin \alpha / n)] \quad (2)$$

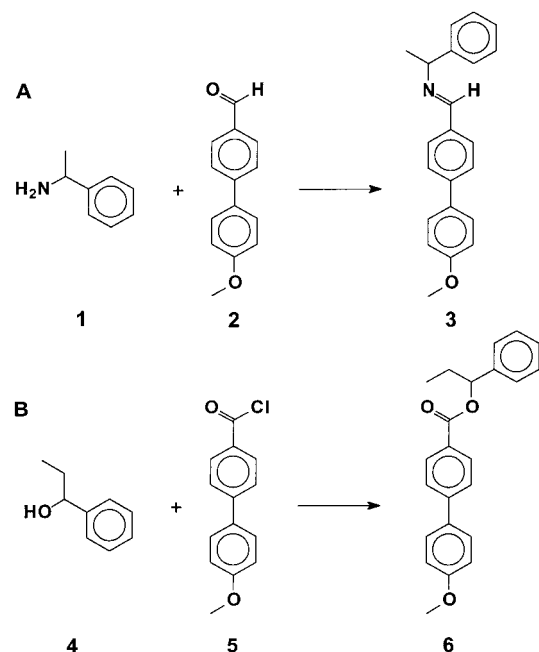
The color, and hence the chiral nature, of such a phase, which is readily prepared, can be instantly assessed by eye. Doped, colored LC materials are therefore promising candidates for color indicators of *ee* values, with potential use in the combinatorial screening of enantioselective catalysts.

The major problem to overcome in the development of such a screening technique is that the helical twisting powers of common products of enantioselective catalysis are negligible (and therefore no cholesteric phase can be induced) or they are very small (only pitches in the range of micrometers can be obtained). Color induction is normally only achieved with specially designed dopants.^[9] We reasoned that compounds that structurally resemble the LC material can be expected to

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show high helical twisting powers as well as high compatibility. The present concept is based on the one-step functionalization of chiral compounds with an achiral mesogenic group to obtain derivatives that resemble the LC material and as a consequence possess high helical twisting powers and high compatibility. To prove this principle, 1-phenylethylamine (**1**; Scheme 1) and 1-phenylpropanol (**4**) were selected as representative examples of two classes of chiral products, namely amines and alcohols. Commercially available E7 was chosen as the LC host material. The structural motif of E7 is a *para*-alkoxy-substituted cyanobiphenyl unit, which is frequent in nematic liquid crystalline materials. In order to structurally resemble this material we designed a *para*-methoxy-substituted biphenyl moiety as a mesogenic unit to be affixed to the chiral amine or alcohol to be analyzed.

In the case of 1-phenylethylamine (**1**), such a mesogenic unit was readily introduced through a fast reaction with carbaldehyde **2** to give the corresponding imine **3** (Scheme 1A).



Scheme 1. Derivatization of chiral amine **1** and alcohol **4** with achiral mesogenic units.

Enantiomerically pure (*S*)-imine **3** showed a very high compatibility with E7 (doping up to at least 20 wt % was possible) and, more importantly, cholesteric textures were readily obtained (the imine has a helical twisting power (β) of $-21.4 \pm 0.9 \mu\text{m}^{-1}$), whereas amine **1** itself induces no cholesteric texture. Enantiomerically pure material in the range from 10 to 19 wt % gives LC phases with colors ranging from red (680 nm) to violet (360 nm), thus covering the entire visible spectrum, as predicted by Equations (1) and (2).

The color of the LC film composed of an 18.9 wt % sample of **3** in E7, which is violet for enantiomerically pure imine, should, according to Equations (1) and (2), show a shift to longer wavelengths with decreasing *ee* values of the dopant. Samples of E7 doped with 18.9 wt % of imine **3** derived from

amine **1** with 100, 90, 80, 60, and 50 % *ee* were prepared and the reflected wavelength was measured with an incident light beam at 45° (Figure 1).^[14]

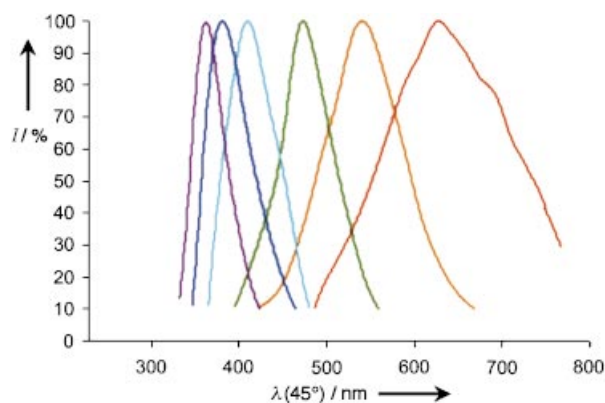


Figure 1. Light intensity versus wavelength of reflected light ($\alpha = 45^\circ$, normalized to 100 %) for E7 doped with imine **3** (18.9 wt %): *ee* values of 100, 90, 80, 70, 60, and 50 % are indicated by the colors purple, dark blue, light blue, green, orange, and red, respectively.

A doped LC matrix is thus seen to function as a visual chirality indicator, with the wavelength of the reflected light increasing as the *ee* values fall. In this way, every LC sample doped with imine **3** with an *ee* value above 50 % can be visualized and those with lower *ee* values remain colorless. From Equation (1) it is evident that the lower limit of the *ee* value that is visually detected can be shifted by varying the concentration of the dopant, if desired.

A slight change in the mesogenic unit is necessary if the same concept is to be used for chiral alcohols. Acid chloride **5** was tested for the visual determination of the *ee* value of **4** (Scheme 1B). 1-Phenylpropanol is an important compound in terms of combinatorial catalysis since it is the product of the extensively studied enantioselective catalytic addition of diethylzinc to benzaldehyde.^[15] A simple esterification reaction of alcohol **4** with acid chloride **5** results in carboxylate **6**.

The enantiomerically pure (*S*)-ester **6** shows very high compatibility with E7, and dopant concentrations of at least 23 wt % could be obtained. The helical twisting power of this ester ($\beta = -28.8 \pm 0.7 \mu\text{m}^{-1}$) is significantly higher than that of imine **3**. The doping of E7 with 14.8 wt % of ester **6** leads to colored LC phases that range from violet for 100 % *ee* to red for 50 % *ee*, with reflection spectra of **6** similar to those of imine **3** (Figure 2). The measurement of the wavelength of maximum reflection by absorption spectroscopy allows quantitative determination of the *ee* values, where a change in the *ee* value of only 1 % results in a readily detectable shift of at least 3.6 nm in the wavelength of the maximum reflection. The results for both compounds are summarized in Figure 2.^[16]

Again, all the colors can also be observed instantaneously by eye. Figure 3 shows the colors of the doped LC phases with different *ee* values of the dopant. Samples with *ee* values lower than 50 % do not show any color. In this way, potential enantioselective catalysts can be screened rapidly and the enantiomeric excess of the products can then be further quantified by measurement of the wavelength of reflection as discussed above.

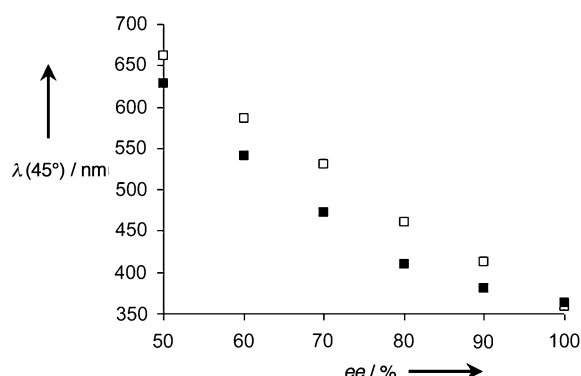


Figure 2. Wavelength of the maximum reflection ($\lambda(45^\circ)$) versus the enantiomeric excess for both imine **3** (■, 18.9 wt %) and ester **6** (□, 14.8 wt %) in E7 (the wavelengths of reflection are independent of whether the *S* or the *R* enantiomer is used in excess).

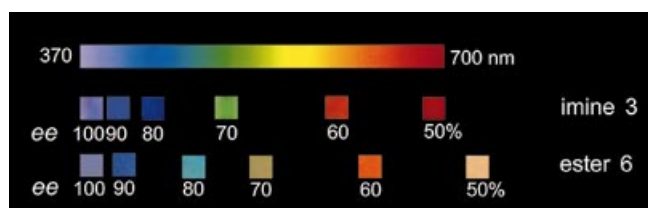


Figure 3. Color of the reflection ($\lambda(0^\circ)$) of doped LC samples with different enantiomeric excesses for both imine **3** and ester **6**. The colors shown are photographs of the aligned LC films taken perpendicular to the surface.

The main criterion for substrates to be amenable to analysis by this method is that the combination of the helical twisting power and maximum dopant concentration of the derivative in an LC phase results in pitches in the visible range. A change in the helical twisting power going from one substrate to another, structurally related, substrate can be readily balanced by changing the dopant concentration. If the current method is used for the direct screening of the *ee* values of products of enantioselective catalysis, for example, in a combinatorial fashion, one should realize that the color of the obtained liquid crystal is not solely dependent on the *ee* value of the product (or the enantioselectivity of the catalyst) but is also dependent on the concentration of the product (for example, the conversion of the catalytic reaction and thus the activity of the catalyst). The dependency of the generated color on the conversion and *ee* value is particularly useful for the initial screening of chiral catalysts where a set lower limit of both conversion and enantioselectivity is to be detected.^[17]

The presented method requires no chiral auxiliaries, only microgram quantities of chiral products, and has excellent prospects for automation in view of recent advances in liquid crystal technology.^[18] Since a variety of mesogenic units bearing suitable functional groups for coupling to the analyte can be readily prepared and a large number of nematic LC materials are available it should be possible to “custom design” these color indicators for numerous chiral products.

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- [17] Every sample with (% conversion × % *ee*) ≥ 5000 can, in principle, be visualized [Eq. (2)]. The method, however, does not discriminate between conversion and the *ee* value. Since a good chiral catalyst should give high activity as well as enantioselectivity one can use the current method for the rapid screening of both quantities simultaneously. In general, the lower the wavelength of reflection of the doped LC phase the more suitable the catalyst.
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Flexible *meso*-Bis(sulfinyl) Ligands as Building Blocks for Copper(II) Coordination Polymers: Cavity Control by Varying the Chain Length of Ligands**

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Crystal engineering of coordination polymers with fascinating structural topologies has attracted a great deal of attention in recent years because of their potential as functional materials.^[1] Concurrent with this has been the development of multidimensional networks based primarily on the linking of metal centers by rigid bridging components.^[2] Far less common has been the use of flexible bridging units in the construction of extended networks,^[3] this approach is attractive because the flexibility and conformational freedom of such ligands offer the possibility of generating unique frameworks with useful properties.

Herein we report the synthesis of three novel Cu^{II} complexes with unique macrometallacyclic networks formed by using flexible *meso*-bis(sulfinyl) ligands (**1**–**3**; Figure 1)^[4] as building blocks. The underlying principles of our design are

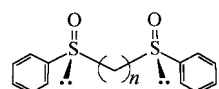


Figure 1. Ligands **1** ($n=2$), **2** ($n=3$), and **3** ($n=4$).

as follows: a) the ditopic coordination sites of bis(sulfinyl) may allow coordination polymers with higher dimensions; b) the pyramidal S atom is an asymmetric center to introduce acentric sites into the resulting crystals; c) the dimensions and cavity sizes of the frameworks with such ligands

may be easily controlled by ligand modifications; d) bis(sulfinyl) ligands, the chemistry of which is still undeveloped, are likely to be applicable to a vast variety of metal moieties.

The two-dimensional (2D) polymers [Cu₂(ClO₄)₂]_∞ (**4**), [Cu₃(ClO₄)₂]_∞ (**5**), and the three-dimensional (3D) polymer [Cu₃(ClO₄)₂]_∞ (**6**), were prepared from Cu(ClO₄)₂ and the corresponding purified *meso* form ligand. These polymers are insoluble in most standard solvents but slightly soluble in

DMF. The IR peaks for $\nu_{\text{S=O}}$ in **4**–**6** are shifted to lower frequencies compared to those in the free ligands.^[5] Thermogravimetric analysis (TGA) studies of **4**–**6** revealed that they decompose at 216, 207, and 227 °C, respectively. Attempts to exchange ClO₄[−] for NO₃[−], BF₄[−], or PF₆[−] gave only noncrystalline complexes, the structural motifs of which were not clear.

In **4**, each Cu^{II} center is six-coordinated by four O atoms of the S=O groups of four different **1** ligands in equatorial positions (Cu–O bonds: 1.918(3), 1.942(3) Å), and two other O atoms from ClO₄[−] ions in axial positions (Cu–O bond: 2.518 Å); while in **5** and **6**, each Cu^{II} is six-coordinated by the O atoms of the S=O groups of six distinct bis(sulfinyl) ligands (Cu–O bonds: 1.980(3), 1.987(3), 2.311(3) Å in **5**; 1.965(4), 1.970(4), 2.362(5) Å in **6**). It is clear that every Cu^{II} center in **4**–**6**^[7,8] is in an axially elongated pseudo-octahedral coordination geometry with normal Cu–O bonds in the equatorial plane and longer Cu–O bonds in the axial positions because of the Jahn–Teller effect.

Figure 2 shows the lamellar network of **4**, in which the adjacent Cu^{II} ions are linked by **1** in two directions to form a 28-membered rhombic macrometallacyclic unit with dimen-

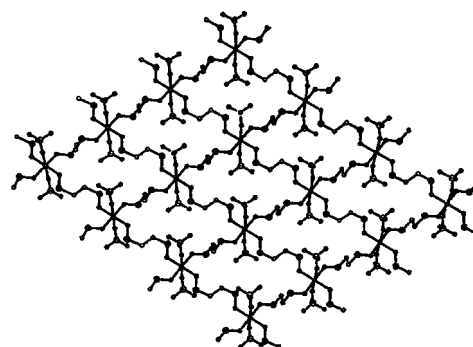


Figure 2. The lamellar structure of **4**. Phenyl groups and H atoms are omitted for clarity.

sions of 8.05×9.16 Å and the repeating units fused to form a 2D rhombohedral array. The ClO₄[−] ions coordinate to the Cu^{II} centers rather than occupying the rhombus cavity, probably because the $-\text{CH}_2\text{CH}_2-$ chain in **1** is too short to form a cavity with a large enough void to accommodate a ClO₄[−] ion.

As shown in Figure 3, **5** forms an unprecedented lamellar molecular-square array. The adjacent Cu^{II} ions are linked by **2** in two essentially orthogonal directions and there are two distinct ligand conformations (**A** and **B**) in a 2:1 ratio. Two ligands with the **A** conformation coordinate to Cu^{II} centers in equatorial positions to form a smaller 16-membered Cu₂2₂ ring. The ligands with **B** conformation link adjacent Cu^{II} ions in the axial positions to form a 32-membered macrometallacyclic square domain containing Cu^{II} centers at the corners. Adjacent rings are fused to a unique 2D network, with the smaller rings disposed along the *a* direction. Unlike **4**, the square cavity with dimensions of 9.44×10.96 Å can accommodate a ClO₄[−] ion. Other ClO₄[−] ions are sandwiched between layers to maintain the electric balance.

In **6**, the adjacent Cu^{II} ions are linked by **3** in two orthogonal directions and the third direction, to form a 3D structure with three types of channels running in three directions

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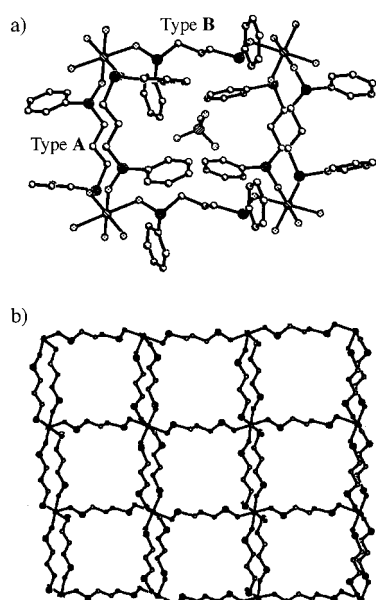


Figure 3. a) View of the repeating unit of **5** showing the 16- and 32-membered macrometallacycles and the **A** and **B** ligand conformations. b) The molecular-square network of **5**.

(Figure 4), which is a key difference to **4** and **5**. Compound **6** is composed of three kinds of 36-membered Cu_4S_4 macrocycles. There are three types of ligand conformations in a 1:1:1 ratio in the unit cell. Two carbon atoms of **3** on the *c* axis are disordered. A feature of **6** is the nature of the polymeric array resulting from the linking of adjacent Cu^{II} ions with $\text{M-O}\cdots\text{O-M}$ bridges in three directions. The basic repeating unit with dimensions of $11.2 \times 11.8 \times 11.0 \text{ \AA}$ encapsulates two ClO_4^- ions.

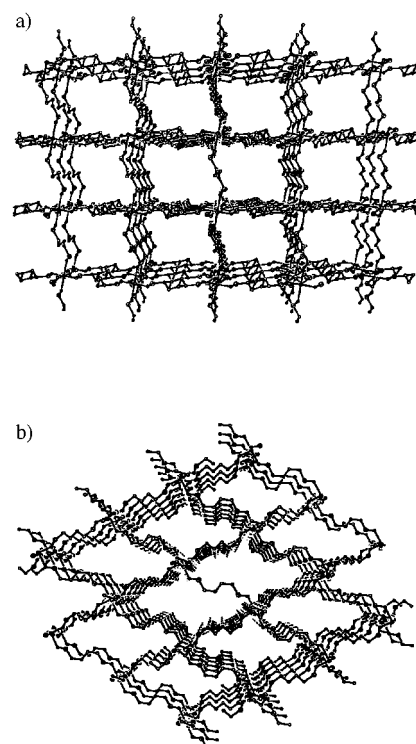


Figure 4. Projection of **6** a) along *a* axis showing the square channels and b) along *c* axis showing the rhombic channels.

The space-filling diagrams of the repeating units of **4–6** (Figure 5) clearly show that the cavity in **4** is too small to accommodate a ClO_4^- ion, and is big enough to fully encapsulate a ClO_4^- ion in **5**, and because of the longer spacer units in **6** the octahedral cavity can encapsulate two ClO_4^- ions. Therefore, varying the chain length of ligands controls the cavity sizes of such networks.

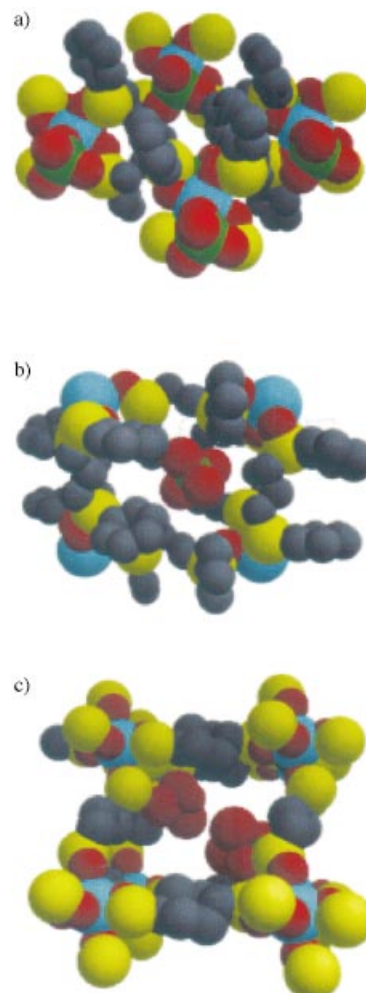


Figure 5. The space-filling diagrams of the cavities for the repeating units of a) **4**, b) **5**, and c) **6**. H atoms are omitted for clarity, and the phenyl groups in **6** are omitted to reveal the ClO_4^- ions; O: red, Cl: green, Cu: turquoise, S: yellow.

The magnetic behavior of **4–6** was studied, and Figure 6 shows the temperature dependence of $\chi_{\text{M}}T$ for **4** and (insert) the field dependence for the reduced molar magnetization ($M/N\beta$). With the decreasing of temperature, the observed $\chi_{\text{M}}T$ values of **4** are constant until around 15 K, ($0.433 \text{ cm}^3 \text{ mol}^{-1} \text{ K}$, typical for Cu^{II}). At very low temperature, $\chi_{\text{M}}T$ decreases, characteristic of a system with a net weak antiferromagnetic interaction;^[6a] the behavior of **5** and **6** was similar. The susceptibility data were fitted by the expansion series of Lines^[6b] for an $S = 1/2$ antiferromagnetic quadratic layer, assuming only one *J* value. The best fit is given by the superexchange parameter $J = -0.012 \text{ cm}^{-1}$, $g = 2.13$ ($R = 2.6 \times 10^{-5}$). The curve of the reduced magnetization versus *H* (Figure 6, insert) at 2 K follows the Brillouin formula. At

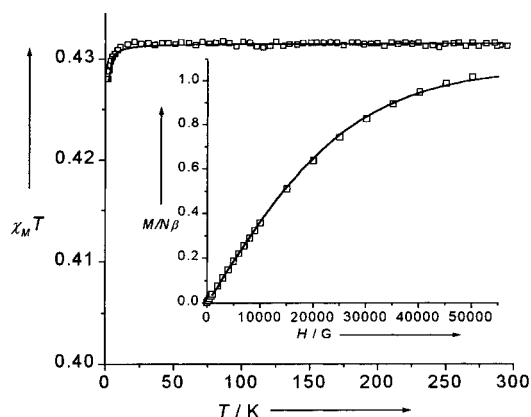


Figure 6. Temperature dependence of $\chi_M T$ [$\text{cm}^3 \text{mol}^{-1} \text{K}$] for **4** (**5** and **6** have similar patterns). Insert: Field dependence for the reduced magnetization at 2 K ($M/N\beta$, number of electrons per formula unit).

50 000 G, the $M/N\beta$ value tends to the saturation value (one electron, $S = 1/2$). This feature corroborates the weak anti-ferromagnetic character shown in the $\chi_M T$ curve. Evidently, there is a possible interlayer contribution, the J value of which is impossible to calculate because of the features of the experimental curves.

In conclusion, **4–6** are rare examples of pseudo-octahedral metal organic frameworks which are noninterpenetrating and made up of macrometallacyclic arrays with controllable cavity sizes. The presence of the ligand phenyl groups and the need for counterions may play a key role in the generation of such noninterpenetrating structures. This implies that the interpenetration of coordination polymers might be controlled by ligand modification. Although **1–3** are very similar in structure, they form quite different metal–organic networks. This suggests that subtle variations of the ligands may result in quite different networks. The lattice dimensions in **4–6** are rather large, however, the ligand phenyl groups and counterions occupy the major parts of their voids. As *meso* ligands with *R,S* configurations were used, the networks of **4–6** are all mesomeric. Studies using *S,S* or *R,R* bis(sulfinyl) for construction chiral networks and systematic studies on such ligands by further varying their backbones are under way.

Experimental Section

4: A solution of $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (1 mmol) in acetone (10 mL), DMF (0.5 mL), and diethoxyethane (10 mL) was heated at reflux for 15 min, and stirred at 40 °C for further 1.5 h. Then **1** (3 mmol) dissolved in CHCl_3 (10 mL) was added to the solution and heated at reflux for 2 h. After cooling to room temperature, the solution was filtered and the filtrate was allowed to slowly evaporate to obtain the single crystals of **4** in about 30 % yield. **5** and **6** were prepared in the same manner. IR (KBr pellet) $\tilde{\nu}$: **4**: 2924, 1444, 1092, 1035, 998, 966, 751, 691, 623 cm^{-1} ; **5**: 2947, 1444, 1085, 1042, 996, 959, 744, 690, 624 cm^{-1} ; **6**: 2956, 1479, 1092, 1072, 999, 972, 759, 695, 624 cm^{-1} .

Magnetic susceptibility data were collected with a Quantum Design SQUID magnetometer between 2–300 K. From room temperature to 4 K, these measurements were repeated with a pendulum-like MANICS-DMS8 susceptometer. The results were the same. The output data were corrected for the diamagnetism of the samples and holder. Magnetization measurements were performed at 2 K in the ± 5 T range.

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- [8] Crystal data for **6**: $\text{C}_{48}\text{H}_{54}\text{Cl}_2\text{CuO}_{14}\text{S}_6$, green, $M_r = 1181.71$, $0.50 \times 0.40 \times 0.37 \text{ mm}^3$, triclinic, space group $P\bar{1}$, $a = 11.191(3)$, $b = 11.750(4)$, $c = 11.802(3) \text{ \AA}$, $\alpha = 86.92(3)^\circ$, $\beta = 82.65(2)^\circ$, $\gamma = 62.90(2)^\circ$, $V = 1370.1(6) \text{ \AA}^3$, $F(000) = 613$, $Z = 1$, $\mu = 0.79 \text{ mm}^{-1}$, $\rho = 1.432 \text{ g cm}^{-3}$, $T = 293 \text{ K}$, $\theta_{\text{max}} = 25.00^\circ$. Of 5596 measured data 4789 were observed ($R_{\text{int}} 0.0409$) with $I > 2\sigma(I)$. Final R , R_w , and S are 0.0401, 0.0507, and 1.066, respectively. The largest residual peak and hole are 0.897 and $-0.619 \text{ e \AA}^{-3}$, respectively. Semi-empirical absorption correction. Siemens P4 diffractometer with $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). The structure was solved by direct methods using the SHELXS 94 program.^[9b] Crystallographic data (excluding structure factors) for the structures **4–6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-149507–149509. 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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Magnetic Nanosensors for the Detection of Oligonucleotide Sequences

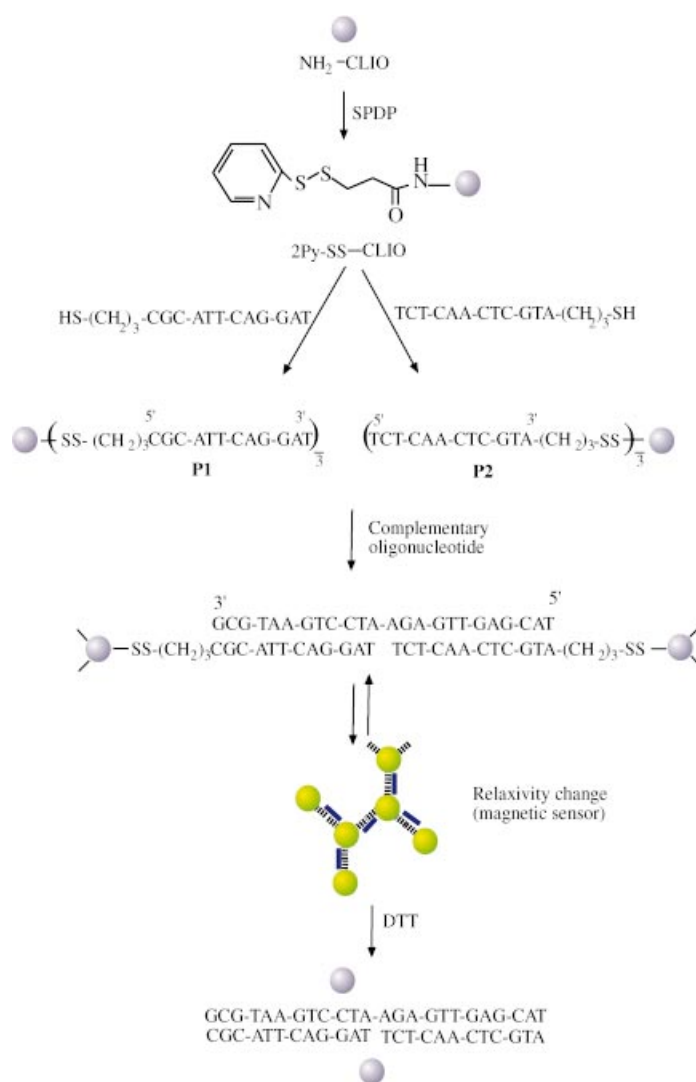
Lee Josephson, J. Manuel Perez, and Ralph Weissleder*

The rapid detection and read-out of specific nucleic acid sequences has become a fundamental necessity in medicine and biotechnology. Photosensitive reporters such as fluorophores,^[1, 2] gold particles,^[3] and chemiluminescent probes^[4] bound to complementary oligonucleotides have now largely replaced older detection systems based on radioisotopes. A number of biological applications, however, dictate fast sample throughput, simplified sample preparation, and measurements in turbid/obscure tissue-type media. As one method, bioelectronic DNA chip technology has recently been developed which relies on the detection of electrical current generated by oxidation and reduction of labeled nucleic acid targets.^[5] We report here on a novel magnetic nanosensor technology as an alternative method for the detection of specific oligonucleotides.

The starting material consisted of monodisperse, fluid-phase nanoparticles containing an icosahedral core of superparamagnetic, crystalline $\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$ (3 nm), caged by epichlorohydrin cross-linked dextran and functionalized with amine groups (CLIO-NH_2).^[6, 7] We then coupled thiolated oligonucleotides to CLIO-NH_2 using *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) as a linker (Scheme 1). The physical properties of the conjugates **P1** ($\text{CLIO}-(\text{SS}-(\text{CH}_2)_6\text{-CGC-ATT-CAG-GAT})_3$) and **P2** ($((\text{TCT-CAA-CTC-GTA}-(\text{CH}_2)_3\text{-SS})_3\text{-CLIO})$) are summarized in Table 1. **P1** and **P2** each had an average of three oligonucleotides per particle based on a single crystal per particle, and 2064 iron atoms per crystal.^[7] They could be stored at room temperature or 4 °C for several weeks without precipitation.

Mixtures of **P1** and **P2** were incubated with a complementary oligonucleotide to examine the ability of **P1** and **P2** to hybridize. The sample became slightly turbid within 3–4 hours at room temperature, with a brown precipitate forming after 16 hours (Figure 1 B). The precipitate moved to the side of the tube on application of a hand-held magnet. Tubes containing the **P1/P2** mixture alone (Figure 1 A), the **P1/P2** mixture with noncomplementary oligonucleotide (Figure 1 C), and the **P1/P2** mixture with half-complementary oligonucleotide (Figure 1 D) did not show turbidity or precipitate formation even after several weeks at room temperature.

Gel electrophoresis was performed to further elucidate the interaction of **P1/P2** with a complementary oligonucleotide. Under nondenaturing conditions and without 1,4-dithiothreitol (DTT) the precipitate (Figure 1 B) remained at the top of the gel (Figure 2 A, lane 1). Treatment with DTT



Scheme 1. Alkanethio-substituted oligonucleotides were treated with SPDP-activated nanoparticles to form the **P1** and **P2** nanosensors. **P1** and **P2** hybridize with complementary oligonucleotide and result in oligomerization and changes in the magnetic relaxivity. Treatment with DTT breaks the bond between the nanoparticle and alkanethio-substituted oligonucleotide.

Table 1. Size and relaxivities before and after hybridization.

Compound	Hybridization ^[a]	Size [nm]	$R1^{[b]}$ [$\text{s}^{-1}\text{mm}^{-1}$]	$R2^{[b]}$ [$\text{s}^{-1}\text{mm}^{-1}$]	$R2/R1$
P1	no	53 ± 11	27.7 ± 0.3	75 ± 2	2.7 ± 0.1
P2	no	53 ± 11	26.7 ± 0.3	71 ± 10	2.6 ± 0.4
P1 + P2 + oligo noncomplement.	no	65 ± 25	25.8 ± 0.4	67 ± 1	2.6 ± 0.1
P1 + P2 + oligo complement.	yes	215 ± 19	23.0 ± 1.0	128 ± 3	5.6 ± 0.2

[a] Determined by gel electrophoresis. [b] Relaxivities are the slopes of plots of relaxation rate ($1/T$, s^{-1}) against nanosensor concentration (Fe, mM); values are plotted as means ± standard deviation from three determinations. Size refers to the unimodal size distribution as determined by light scattering, mean ± standard deviation over six measurements.

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(Figure 2 A, lane 2) resulted in a single band of hybridized oligonucleotide. Under denaturing conditions and with DTT added (Figure 2 B) two bands were observed, the slower one consisting of complementary oligonucleotide and the faster one of a mixture of 3' and 5' oligonucleotides.

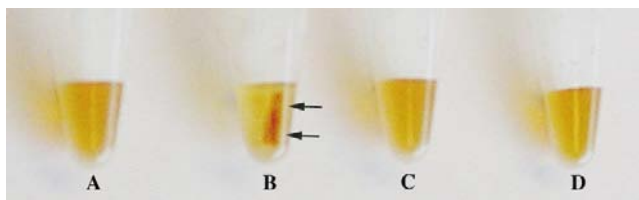


Figure 1. Effect of incubating nanoparticle probes with oligonucleotides: A) **P1** and **P2**; B) **P1** and **P2** plus complementary oligonucleotide; C) **P1** and **P2** plus noncomplementary oligonucleotide; D) **P1** and **P2** and half-complementary oligonucleotide.

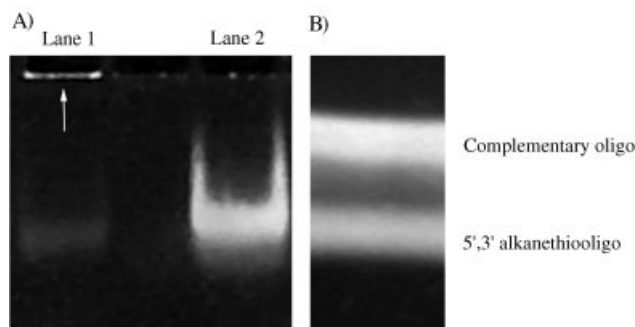


Figure 2. Gel electrophoresis of **P1/P2**/oligonucleotide precipitate: A) Nondenaturing conditions: lane 1) no DTT, lane 2) with DTT. B) Denaturing conditions with DTT.

The nanoparticles **P1** and **P2** are potent enhancers of the spin–spin and spin–lattice relaxation processes (Table 1). Interestingly, the spin–spin relaxation was significantly enhanced by oligonucleotide hybridization, and rendered the particles potential “magnetic nanosensors”. Figure 3 shows the effect of adding oligonucleotide to an aqueous

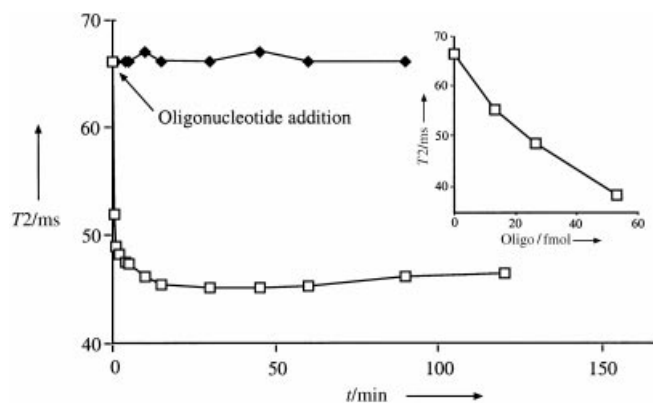


Figure 3. Temporal change of water T_2 relaxation times with (□) and without complementary oligonucleotide (◆). The insert shows the effect of increasing concentrations of complementary oligonucleotide on the T_2 value. All data points shown represent the average of three measurements with standard deviations ranging between 0.4–0.6 ms for the T_2 values (too small to graph).

solution of **P1/P2** on the relaxation times T_2 . The value of T_2 decreased from 63 ms to 45 ms within several minutes, and this effect persisted for the period of observation (2 h). Table 1 summarizes the concentration-independent relaxivities R_1 and R_2 before and after hybridization. Hybridization primarily affected R_2 with a doubling of the R_2/R_1 ratio being

observed. Concomitant laser light scattering indicated a significant size increase of the hybridized conjugates, which presumably affected the spin–spin relaxation. Similar changes in relaxivity were also observed in turbid and tissue-like samples (data not shown).

We next investigated the effect of temperature cycling on the hybridization of the nanosensors by measuring changes in the T_2 values (Figure 4). Hybridization was minimal at 80 °C and changes in the T_2 values were small. Representative changes in the T_2 values were observed during multiple cycles of heating and cooling. Furthermore, the oligonucleotides were cleaved from the nanosensors upon addition of DTT, and the T_2 value did not change during further temperature cycling.

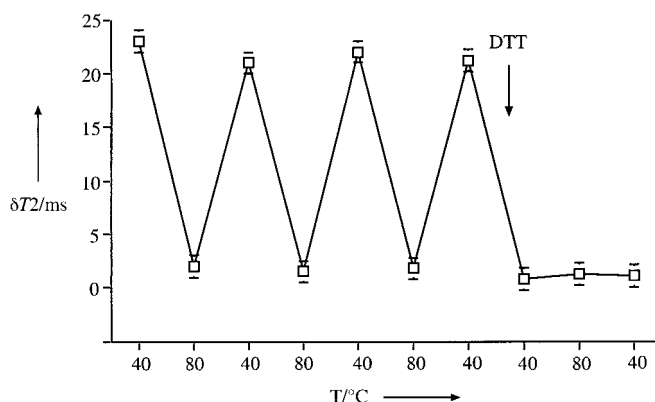


Figure 4. Changes in the T_2 values of an aqueous solution of **P1/P2**/complementary oligonucleotide as a function of temperature cycling.

The above results indicate that oligonucleotide hybridization efficiently changes the spin–spin relaxation time of adjacent water protons, that these effects occur within minutes, that the magnetic effects are fully reversible, and that the oligonucleotides are recoverable from the sensors. We believe that the described nanosensors may have considerable applications in biotechnology and biomedicine. Reduced sample sizes required by modern magnetic bench-top readers should enable the attomole detection of oligonucleotides at high throughputs, while detection systems with higher sensitivity, such as magnetic force microscopy or sensitive oscillating magnetic field readers, should allow detection of single strands of hybridized oligonucleotides.^[8] Finally, since the developed nanosensors are essentially nontoxic to mammalian cells,^[9] nondegradable oligonucleotide analogues (for example, peptide nucleic acid (PNA)) may be coupled to nanoparticles and used to image sequences of nucleic acids *in vivo*.^[10]

Experimental Section

Synthesis of magnetic nanoparticles: Biocompatible, fluid-phase magnetic nanoparticles (CLIO-NH₂) were synthesized as described in more detail elsewhere^[6] and treated with *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) to yield 2Py-SS-CLIO (Scheme 1).^[6] The 5'-alkanethiol oligonucleotide (HS-(CH₂)₆-CGC-ATT-CAG-GAT) and 3-alkanethiol oligonucleotide (TCT-CAA-CTC-GTA-(CH₂)₃-SH) were synthesized at a 1 μmol scale using standard phosphoramidite chemistry. The sulfhydryl

groups were protected with a thioalkyl linker. The oligonucleotides were deprotected with DTT immediately before reaction with 2Py-SS-CLIO.^[11]

Synthesis of magnetic oligonucleotide nanoparticles: Complementary (⁵TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG³), half-complementary (⁵TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT³), and noncomplementary (⁵ATG-CTA-AAT-GAC-GAC-TGC-CCA-CAT³) oligonucleotides were synthesized using standard phosphoramidite chemistry (underlined bases will hybridize). Either 5'- or 3'-alkanethio-oligonucleotide (550 µg) was added to of 2Py-SS-CLIO (1.1 mL; 3 mg of Fe in 0.1M phosphate buffer, pH 8.0), and the mixture incubated overnight at room temperature. The mixture was purified using an LS+ high-gradient magnetic separation column (Miltenyi Biotec, Auburn, CA) equilibrated with 0.1M phosphate buffer, pH 7.5. The number of oligonucleotides attached per particle was determined by treatment with DTT, followed by separation of the iron and oligonucleotide using a microconcentrator as described.^[6] The oligonucleotide concentration was then determined from absorbance spectroscopy at 260 nm using an extinction coefficient of $1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. The probes are denoted **P1** (CLIO-SS-(CH₂)₆-CGC-ATT-CAG-GAT) and **P2** (TCT-CAA-CTC-GTA-(CH₂)₃-SS-CLIO).

Hybridization: For Figure 1 equal volumes (25 µL) of **P1** and **P2** (both at 550 µg Fe mL⁻¹) were mixed with a solution of 1M NaCl and 0.1M phosphate at pH 7.5 (14 µL). Various oligonucleotides (2 µL, 400 ng) were then added. The mixture was heated to 50 °C for 5 min and allowed to react at room temperature overnight. The precipitate shown in Figure 2B was obtained after overnight incubation of **P1/P2** with complementary oligonucleotide; the precipitate was washed with 0.1M NaCl and 0.1M phosphate buffer, and re-suspended in the same buffer (300 µL). The sample was split into two portions (15 µL) and examined by electrophoresis without DTT (lane 1) or with 4 mM DTT (lane 2) under nondenaturing conditions (Figure 2A) or denaturing conditions (Figure 2B).

Gel electrophoresis: Nondenaturing gels (10% polyacrylamide) and denaturing gels (20% polyacrylamide) were used. Gels were stained with SYBR Gold dye (Molecular Probes, Eugene OR).

Determination of proton relaxation times: Relaxation time measurements were performed at 0.47 T and 40 °C or 80 °C (Bruker NMR Minispec, Billerica, MA). To determine the effect of hybridization on the T2 values of water, equal amounts of **P1** and **P2** (5 µL) were diluted in a solution of 1M NaCl and 0.1M phosphate buffer at pH 7.5 (1 mL) to give a total iron content of 10 µg mL⁻¹. T2 values were obtained before and after addition of 1 µL (390 ng) of complementary, half-complementary, or noncomplementary oligonucleotides and plotted as a function of time. The relaxivity was determined by plotting 1/T2 and 1/T1 values of water as a function of the iron concentration. The size of the conjugates was determined by light scattering (Coulter N4, Hialeah, FL).

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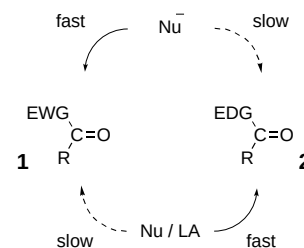
Do More Electrophilic Aldehydes/Ketones Exhibit Higher Reactivity toward Nucleophiles in the Presence of Lewis Acids?

Naoki Asao, Toru Asano, and Yoshinori Yamamoto*

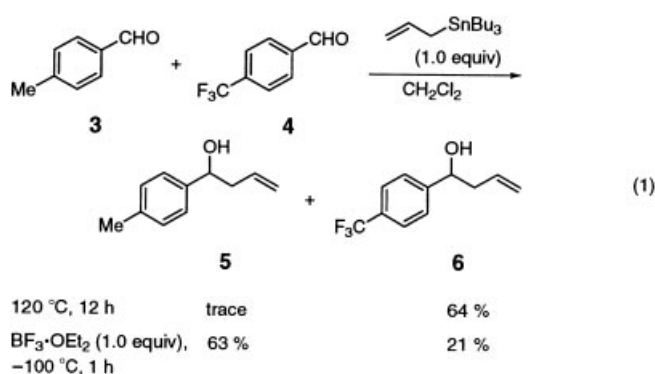
Lewis acid mediated electrophilic reactions of carbonyl compounds are among the most fundamental and important reactions in modern organic synthesis.^[1] It is well known that the coordination of carbonyl groups to Lewis acids exerts a dramatic effect on the rates and selectivities of reactions at the carbonyl centers. While much research into the Lewis acid mediated stereoselective or regioselective reactions has been carried out, less attention has been paid to the chemoselective reactions in the presence of Lewis acids.^[2–4] It is evident for organic chemists that more electrophilic aldehydes and ketones **1** react with nucleophiles (Nu⁻) much faster than less electrophilic analogues

2. We found that the reverse is the case in the Lewis acid mediated reactions: more electrophilic aldehydes and ketones **1** react much slower than the less electrophilic analogues **2** in the presence of Lewis acids (Scheme 1), with a chemoselectivity that is not attainable under ordinary conditions.

For many years, we have researched the Lewis acid mediated reaction of allyl stannanes and related organometallic compounds.^[5] An interesting observation was made in the reaction of allyltributylstannane with aldehydes. The reaction of a 1:1 mixture of *p*-tolualdehyde (**3**) and *α,α,α*-trifluoro-*p*-tolualdehyde (**4**) with one equivalent of allylstannane at 120 °C gave the homoallylic alcohol **6** derived from **4**, in 64% yield, along with trace amounts of the homoallylic alcohol **5** derived from **3** [Eq. (1)]. This is an expected result,



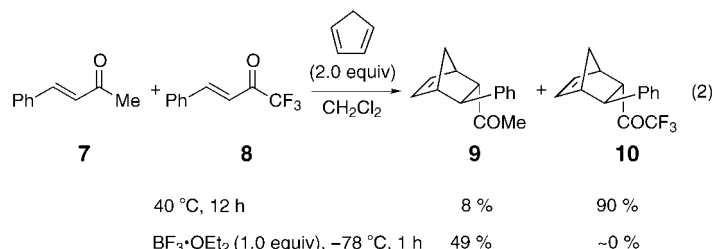
Scheme 1. Chemoselective reactions of equimolar mixtures of **1** and **2**. EWG = electron-withdrawing group, EDG = electron-donating group.



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since **4** is more electrophilic than **3** as a result of an electron-withdrawing CF₃ group at the *para* position. On the other hand, the BF₃·OEt₂-mediated reaction at –100 °C gave **5** in 63 % yield together with 21 % of **6**. This was an unexpected result for us. A similar observation was also made in a Diels–Alder reaction. The thermal reaction of a 1:1 mixture of **7** and **8** with cyclopentadiene at 40 °C afforded the [4+2] cycloadduct **10** derived from **8** in 90 % yield, along with a small amount (8 %) of **9** derived from **7** [Eq. (2)]. This is also an



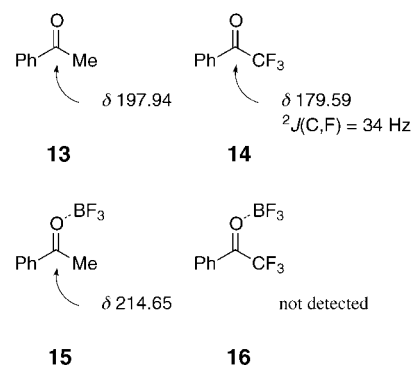
expected result, since **8**, which has an electron-withdrawing CF₃ group, is a better dienophile than **7**. However, the BF₃·OEt₂-mediated reaction at –78 °C gave **9** as the sole product in 49 % yield.^[6]

The dramatic reversal of the chemoselectivity in the thermal and Lewis acid mediated reactions is understandable if the coordination ability of more electrophilic carbonyl groups to Lewis acids **11** is weaker than that of less electrophilic analogues **12**. This means that the observed chemoselectivities would be ascribed to a higher equilibrium constant for the formation of complex **12** rather than **11**.

To confirm this proposal, we carried out the ab initio study of the BF₃ complexes of acetaldehyde and trifluoroacetaldehyde by using the GAUSSIAN94 program,^[7] and the energies for all possible stable conformations of each complex were calculated (see Supporting Information).^[8] A large difference in energy between CF₃CHO·BF₃ and CH₃CHO·BF₃ complexes were calculated at all levels of theory that were employed (3-21G, 6-31G*, and MP2/6-31G*). At the MP2/6-31G* level of theory, the most stable conformer of the CF₃CHO·BF₃ complexes was 6.46 kcal mol^{–1} less stable than the most stable conformer of the CH₃CHO·BF₃ complexes. In other words, the basicity of the oxygen atom of more electrophilic carbonyl groups is lower than that of less electrophilic analogues, making the formation of **12** much easier.^[9]

The preferred formation of complex **12** rather than of **11** was also supported by the low-temperature ¹³C NMR spectroscopic experiments of ketones **13** and **14** in the presence of BF₃·OEt₂. The original signals of the carbonyl carbon atoms of acetophenone **13** and trifluoroacetophenone **14** appeared at δ = 197.94 and 179.59, respectively. When **13** was treated with one equivalent of BF₃·OEt₂ in CD₂Cl₂ at –78 °C to form complex **15**, a significant downfield shift occurred, and the signal for the carbonyl carbon atom was observed at δ = 214.65; the integration ratio of **13**:**15** was 3.0:1.^[10] In contrast, no signal for **16** was detected at all when **14** was treated with

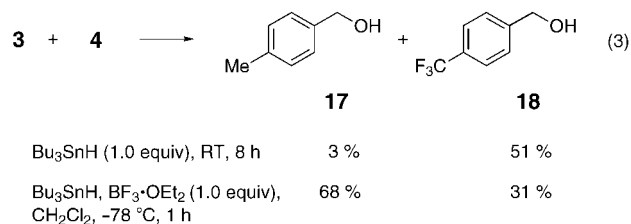
BF₃·OEt₂, and only the original peak of **14** was observed (Scheme 2). Next, the equilibrium study of an equimolar mixture of **13** and **14** with one equivalent of BF₃·OEt₂ was carried out. As expected, complex **16** was not observed, and



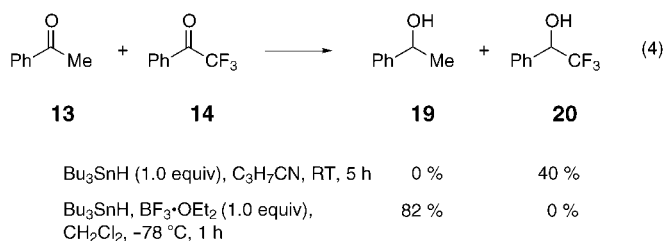
Scheme 2. ¹³C NMR chemical shifts of **13**–**15** at –78 °C in CD₂Cl₂.

the peak for the carbonyl carbon atom of **15** appeared at δ = 214.65 (**13**:**15** 3.0:1). Even when an additional amount of BF₃·OEt₂ (two equivalents in total) was added to this mixture, the complex **16** was not formed and only an enhancement of the ratio of **15** (**13**:**15** 1.8:1) was observed. These results clearly imply that a carbonyl compound bearing an electron-donating group can form the complex **12** with a Lewis acid much more readily and can also be activated selectively.

The aldehydes and ketones were reduced chemoselectively based on the above concept. The reduction of a 1:1 mixture of **3** and **4** with Bu₃SnH (1.0 equivalent) at room temperature gave **18** in 51 % yield along with 3 % of **17**, whereas the reduction with Bu₃SnH–BF₃·OEt₂ (1.0 equivalent)^[11] at –78 °C afforded **17** in 68 % yield together with 31 % of **18** [Eq. (3)]. Similarly, the reduction of a 1:1 mixture of **13** and **14**



with Bu₃SnH (1.0 equivalent) in butyronitrile gave **20** in 40 % yield as the sole product,^[12] whereas the reduction with Bu₃SnH–BF₃·OEt₂ (1.0 equiv) afforded **19** in 82 % yield as the sole product [Eq. (4)].



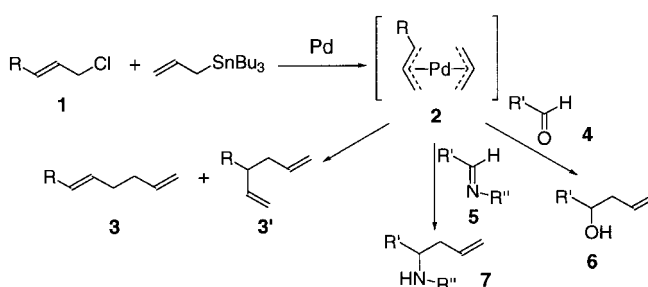
It is now clear that the chemoselectivities in the Lewis acid promoted reactions of certain aldehydes and ketones are opposite to those in the reactions in the absence of Lewis acid. By proper choice of the reaction conditions, either by carrying out the reaction in the presence or in the absence of Lewis acids, secondary and primary alcohols, homoallylic alcohols, and Diels–Alder adducts can be synthesized chemoselectively. A further extension of the above concept is being investigated in our laboratories.

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The Fate of Bis(η^3 -allyl)palladium Complexes in the Presence of Aldehydes (or Imines) and Allylic Chlorides: Stille Coupling versus Allylation of Aldehydes (or Imines)

Hiroyuki Nakamura, Ming Bao, and Yoshinori Yamamoto*

Palladium-catalyzed Stille coupling between allylic chlorides **1** and allyltributyltin proceeds through bis(η^3 -allyl)palladium intermediates **2** to give the allylic–allylic 1,5-hexadiene coupling products **3** and **3'** in moderate to high yields (Scheme 1).^[1] Recently, we found that **2** (R = H), generated



Scheme 1. Different reaction pathways of bis(η^3 -allyl)palladium intermediates **2**.

from allyl chloride and allyltributyltin in the presence of a palladium catalyst, reacts with aldehydes **4** and imines **5** to produce the corresponding homoallyl alcohols **6** and amines **7**, respectively, in high yields.^[2, 3] Also, we found that unsymmetrical bis(η^3 -allyl)palladium complexes **2** can selectively transfer the unsubstituted allyl group to aldehydes and imines.^[4] Important questions are what factors control the reaction pathways of bis(η^3 -allyl)palladium intermediates **2**, and why does the Stille coupling of **2** cease in the presence of aldehydes **4** or imines **5**?

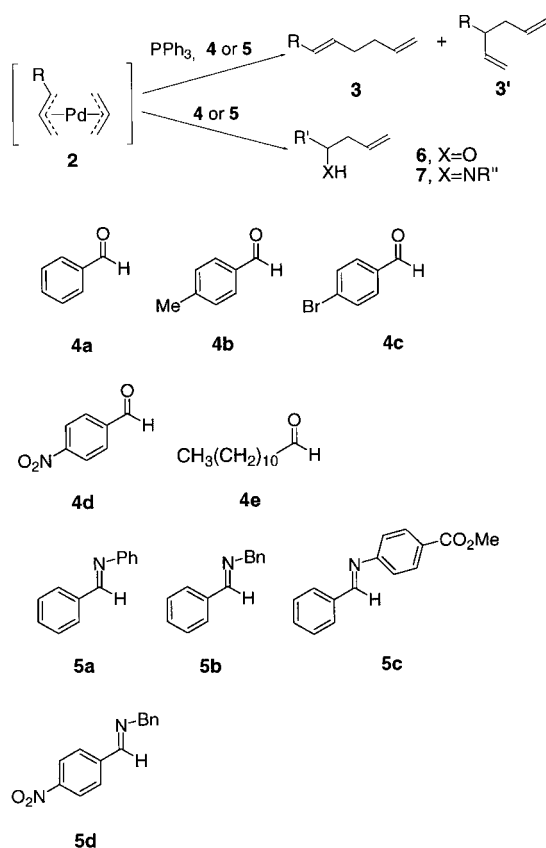
We now report that phosphine ligands play a key role in this process; the Stille coupling reaction takes place in the presence of triphenylphosphine,^[5] even if aldehydes **4** and imines **5** are present, whereas the allylation of aldehydes and imines occurs in the absence of the phosphine, even in the presence of allylic chlorides **1** (Scheme 2).

The palladium-catalyzed reactions of various aldehydes **4** and imines **5** with allylic chlorides were investigated in the presence or absence of PPh₃ (Table 1). In the absence of a phosphine ligand, the reaction of **1a** and allyltributyltin with benzaldehyde (**4a**) gave the homoallyl alcohol **6a** in 94% yield, and cinnamyl chloride **1a** was recovered in essentially

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Scheme 2. The chemoselectivities of bis(η^3 -allyl)palladium complexes in the presence of aldehydes (or imines) and allylic chlorides. **4a–4e** and **5a–5d** were used for the reactions shown in Table 1.

quantitative yield (entry 1), whereas in the presence of one equivalent of PPh_3 (relative to Pd), the reaction gave a 2/1 mixture of **6a** and **3a' + 3a'**. When two equivalents of PPh_3 were used, the ratio of **6a** to **3a' + 3a'** became 1/1. With 3–5 equiv of PPh_3 , only the Stille coupling reaction took place; using four equivalents of PPh_3 gave the best result: a 92/8 mixture of **3a** and **3a'** in 90% yield, and benzaldehyde was recovered quantitatively (entry 1).^[6] Similar observations were made in the reactions of **1b**, **1c**, and **1a** (entries 2–4). β -Elimination took place with the aliphatic substrate **1d** in the presence of PPh_3 to give 1,3-tetradecadiene (entry 5).^[7] A key role of PPh_3 in controlling the chemoselectivity was also observed in the reactions of the imines. In the absence of the phosphine ligand, the reaction of **1a**, allyltributyltin, and **5a** gave the allylation product **7a** in 82% yield, and **1a** was recovered, whereas in the presence of 4 equiv of PPh_3 , a 92/8 mixture of **3a** and **3a'** was obtained in 91% yield, and the imine **5a** remained unchanged (entry 6). Similar results were obtained in the other cases (entries 7–9). Thus, highly chemoselective reactions can be accomplished by means of the presence or absence of PPh_3 .

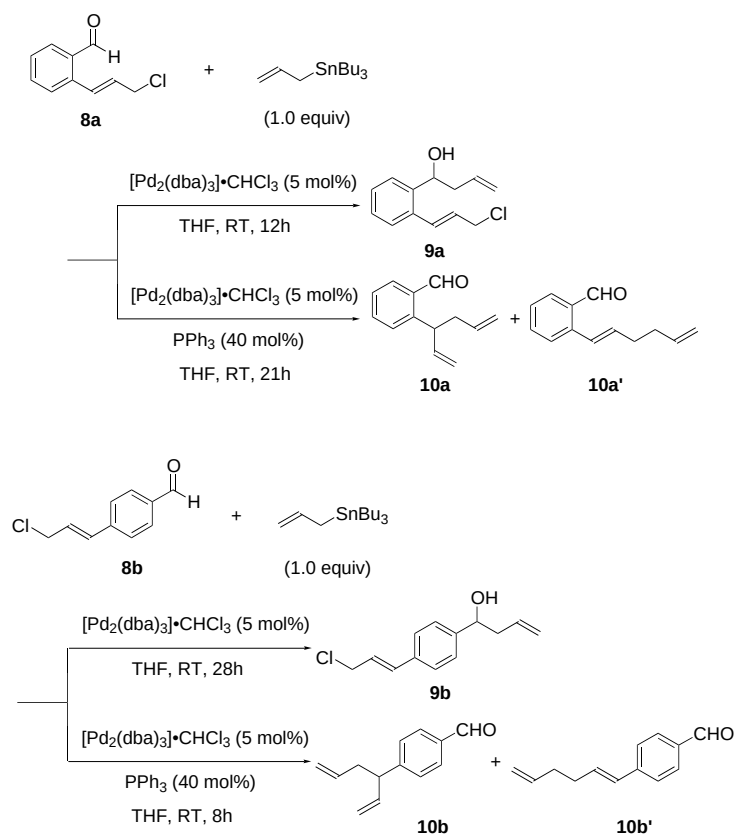
We investigated the chemoselectivity on substrates **8a** and **8b**, which contain an allyl chloride unit and an aldehyde group in the same molecule (Scheme 3). In

Table 1. Palladium-catalyzed reaction of allylic chlorides **1** and allyltributyltin with aldehydes **4** or imines **5** in the presence or absence of PPh_3 .^[a]

Entry	1 , R	4 or 5	Ligand/ time [h]	Yield [%] of 6 or 7 ^[b]	Yield [%] of 3 + 3' (3/3') ^[b,c]
1	1a , Ph	4a	none/11 PPh_3 /19	94 –	– 90 (92/8)
2	1b , <i>p</i> -MeC ₆ H ₄	4b	none/37 PPh_3 /13	83 –	– 78 (100/0)
3	1c , <i>p</i> -BrC ₆ H ₄	4c	none/22 PPh_3 /22	98 –	– 80 (83/17)
4	1a , Ph	4d	none/26 PPh_3 /22	93 –	– 88 (92/8)
5	1d , CH ₃ (CH ₂) ₁₀	4e	none/29 PPh_3 /17	71 –	– – ^[c]
6	1a , Ph	5a	none/2 PPh_3 /4	82 –	– 91 (92/8)
7	1a , Ph	5b	none/64 PPh_3 /4	78 –	– 82 (91/9)
8	1a , Ph	5c	none/132 PPh_3 /5	92 –	– 79 (91/9)
9	1a , Ph	5d	none/132 PPh_3 /21	73 –	– 80 (91/9)

[a] All reactions were carried out in the presence of 5 mol% of $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$ in THF at room temperature (dba = dibenzylideneacetone). In the Stille coupling, 40 mol% of PPh_3 was added. The aldehydes **4** and imines **5** are shown in Scheme 2. [b] Yields of isolated products based on **1**, **4**, or **5**. [c] β -Elimination took place to give the diene product CH₃(CH₂)₉CH=CHCH=CH₂ (89%, *cis/trans* = 1/1).

the absence of the phosphine ligand, the *ortho* isomer **8a** reacted with allyltributyltin to give the allylation product **9a** in 88% yield, and the allyl chloride unit remained unchanged.



Scheme 3. Investigation of chemoselectivity on substrates **8a** and **8b**, which contain an allyl chloride unit and an aldehyde group in the same molecule.

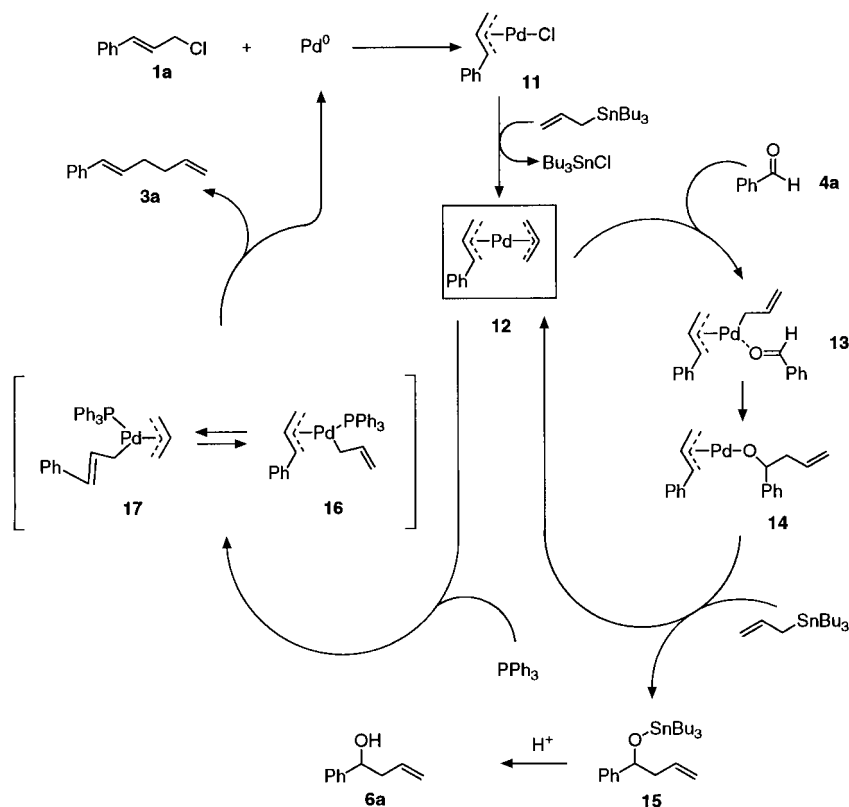
However, in the presence of 4 equiv of PPh_3 , only the Stille coupling reaction took place to give a 96/4 mixture of **10a** and **10a'** in 73% yield, and the aldehyde group remained unchanged. Similarly, the *para* isomer **8b** gave **9b** in 83% yield, whereas in the presence of PPh_3 a 90/10 mixture of **10b** and **10b'** was produced in 74% yield.

A plausible mechanism is shown in Scheme 4. Oxidative addition of **1a** to palladium(0) gives the η^3 -allyl(chloro)palladium complex **11**, and transmetalation of **11** with allyltributyltin produces the bis(η^3 -allyl)palladium complex **12**. In the

presence of PPh_3 (1 equiv) to give **3a**, whereas only a trace of **3a** was obtained in the absence of PPh_3 . Therefore, the coordination of PPh_3 to the complex **12** in the reaction is essential for the Stille coupling process.

In conclusion, the fate of bis(η^3 -allyl)palladium complexes in the presence of aldehydes (or imines) and allylic chlorides has been clarified for the first time. The PPh_3 ligand plays a key role in controlling the chemoselectivity. Now, we can perform chemoselective allylation of allylic halides and aldehydes (imines).

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Scheme 4. Proposed mechanism for the chemoselective allylation in the presence of cinnamyl chloride **1a** and benzaldehyde **4a**.

absence of PPh_3 , benzaldehyde (**4a**) coordinates to palladium(II) to produce the homoallyloxypalladium complex **13**. The transmetalation of **13** with allyltributyltin produces the corresponding homoallyloxytin compound **15** and regenerates the bis(η^3 -allyl)palladium intermediate **12**. In the allylation reaction, the unsubstituted η^3 -allyl group of **12** undergoes nucleophilic addition to benzaldehyde, while the phenyl-substituted allyl group acts as a nontransferable η^3 -allyl ligand.^[4b] Therefore, only catalytic amounts of cinnamyl chloride **1a** react initially with allyltributyltin to afford the bis(η^3 -allyl)palladium intermediate **12**, and large amounts of **1a** remain in the reaction medium. However, in the presence of PPh_3 , the phosphine ligand coordinates to **12** to give **16** and/or **17**.^[8] Reductive elimination from **16** and/or **17** gives the Stille coupling product **3a** and regenerates the palladium(0) catalyst. Indeed, the Stille coupling reaction of allyltributyltin with the dimer of complex **11**, which was synthesized according to a literature procedure,^[9] proceeded in the

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High Nuclearity $\text{Zn}^{\text{II}}/\text{MeCO}_2^-/(\text{C}_5\text{NH}_4)_2\text{CO}_2^{2-}$ Clusters by “Depolymerization”: Conversion of a Three-Dimensional Coordination Polymer Containing Hexameric Units into Its Constituent Hexanuclear Complex**

Nikolia Lalioti, Catherine P. Raptopoulou, Aris Terzis, Abil E. Aliev, Ioannis P. Gerothanassis*, Evy Manessi-Zoupa*, and Spyros P. Perlepes*

One of the goals in polynuclear coordination chemistry is to control the nature of the final product and to escape from the reliance on spontaneous self-assembly. The elegant research of some groups,^[1] which include those of Christou (coupling or linking smaller manganese fragments to make larger assemblies)^[2] and Winpenny (oligomerizing low nuclearity clusters by desolvation),^[3] supplied rare examples of such designed syntheses. In most cases, the precise nuclearity and structure of the reaction products could not be predicted from the reagents and synthetic conditions employed, even though the reactions were certainly directed toward the formation of higher nuclearity species. All these syntheses of higher nuclearity clusters used either covalent linking of lower nuclearity complexes or aggregation of “building blocks”. An alternative attractive approach would be the cleavage, in a controlled manner, of coordination polymers $(\text{M}_x)_n$ containing recognizable high nuclearity M_x units (“polymers of clusters” or “polymers of aggregates”) to obtain the discrete M_x clusters. Herein, we report our successful realization of this idea for $\text{M} = \text{Zn}^{\text{II}}$ and $x = 6$. The cleavage of coordination polymers into *mononuclear* complexes by donor solvents is well-known in inorganic chemistry.^[4]

The reaction of $\text{Zn}(\text{O}_2\text{CMe})_2 \cdot 2\text{H}_2\text{O}$ with di-2-pyridyl ketone, $(\text{C}_5\text{NH}_4)_2\text{CO}$, in a 3:1 molar ratio led to a colorless solution, from which the three-dimensional (3D) polymeric complex **1** containing doubly deprotonated anions of the

gem-diol form $(\text{C}_5\text{NH}_4)_2\text{C}(\text{OH})_2$ as ligands could be isolated; the presence of dianions is a consequence of the high MeCO_2^- to $(\text{C}_5\text{NH}_4)_2\text{C}(\text{OH})_2$ ratio (6:1) used in the reaction. The X-ray crystal structure analysis of **1** revealed repeating hexameric units (Figure 1)^[5]; there is a twofold axis passing through C6

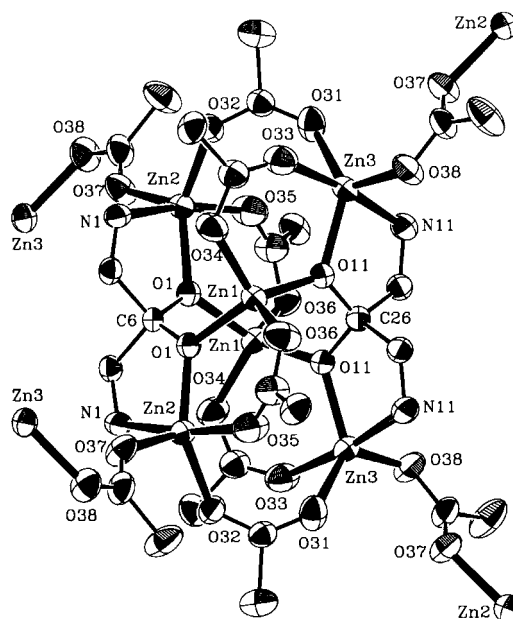
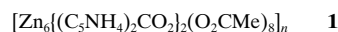


Figure 1. A portion of the 3D polymeric structure of **1** showing one $[\text{Zn}_6\{(\text{C}_5\text{NH}_4)_2\text{CO}_2\}_2(\text{O}_2\text{CMe})_8]$ cluster unit, four extra Zn^{II} atoms belonging to four different units (peripheral Zn2, Zn2, Zn3, Zn3) and two extra *syn,anti*- μ_2 - η^1 : η^1 acetate ligands; the extra metal ions and acetate ligands have been drawn to emphasize the intercluster connections. Atoms generated symmetrically by the twofold axis that runs through C6 and C26 are identically labeled. Most of the carbon atoms of the pyridine rings have been omitted. Intracluster distances [Å]: Zn...Zn 3.38–6.56, Zn-O 1.92–2.12, Zn-N 2.11–2.13. The intercluster distance Zn2...Zn3 is 5.014(1) Å. The open lines indicate weak bonds (2.50–2.75 Å).



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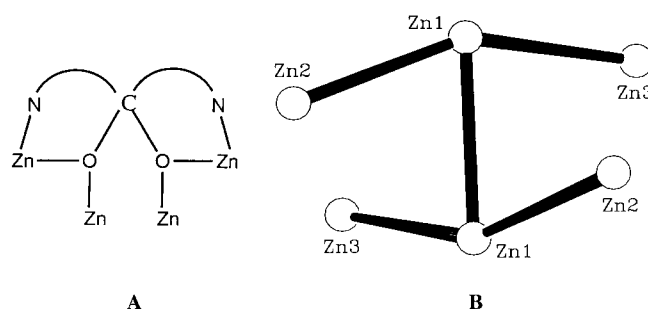
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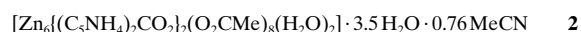
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and C26. Each cluster unit has two μ_4 : η^1 : η^2 : η^2 : η^1 $(\text{C}_5\text{NH}_4)_2\text{CO}_2^{2-}$ dianions (**A**), six *syn, syn*- μ_2 : η^1 : η^1 MeCO_2^- ions and two *syn, anti*- μ_2 : η^1 : η^1 MeCO_2^- ions as ligands; the



latter provide the intercluster covalent linking. Inspired by the remarkable structure of **1** and considering the $\text{Zn} \cdots \text{Zn}$ distances of less than 3.7 Å, we refer to the cluster unit as a “twin anchor” (**B**). To the best of our knowledge, **1** is only the second example of a zinc(II) polymer containing hexameric repeating units; the other example is $[\{(\text{ZnCl})_6(\mu_2\text{-Cl})_8\}^{2-}]_n$, which was isolated with $[(\eta\text{-C}_5\text{Me}_5)_3\text{Nb}_3\text{Cl}_3\text{O}_3]^+$ as the counterion.^[6]

The presence of well-defined Zn_6 cluster units in **1** suggested that solvation, that is, reaction with terminal donor solvent molecules, might cause polymer cleavage and formation of the constituent hexanuclear complex. Since the hexanuclear units in **1** are connected by the four metal ions $\text{Zn}2$, $\text{Zn}3$, and their symmetry partners, we reasoned that these sites might be suitable for the attachment of donor solvent molecules, which has, indeed, turned out to be the case. Treatment of a slurry of **1** in MeCN with MeOH and few drops of H_2O led to a homogeneous solution, from which the discrete hexanuclear cluster **2** slowly crystallized in good yield. The purity of the bulk material **1** used to form **2** was confirmed by comparison of the observed and calculated X-ray powder diffraction (XRPD) patterns.



Complex **2** (Figure 2)^[5] has no symmetry elements and is held together by two terminal aqua ligands, two asymmetrical chelating MeCO_2^- ions, four *syn,syn*- $\mu_2:\eta^1:\eta^1$ MeCO_2^- ions, two $\mu_2:\eta^1:\eta^2$ acetate ions and two $(\text{C}_5\text{NH}_4)_2\text{CO}_2^{2-}$ dianions, which also adopt (as in **1**) the coordination mode $\mu_4:\eta^1:\eta^2:\eta^2:\eta^1$ (**A**); this coordination mode is very rare and has been observed only in a dodecanuclear copper(II) cluster.^[7a] The six Zn^{II} atoms of **2** adopt the same topological arrangement **B**

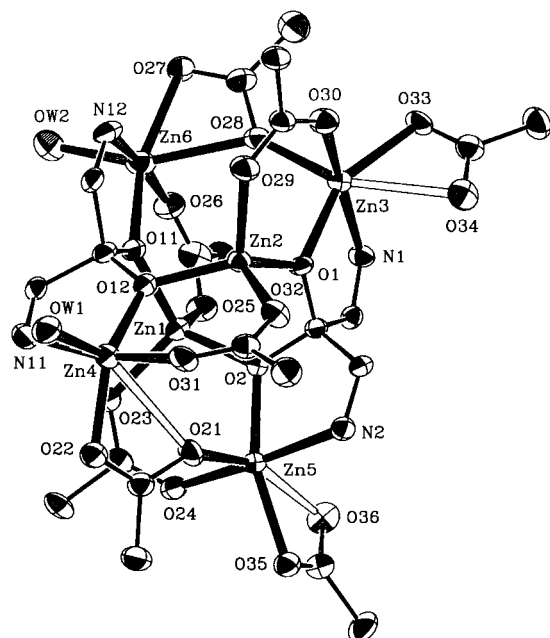
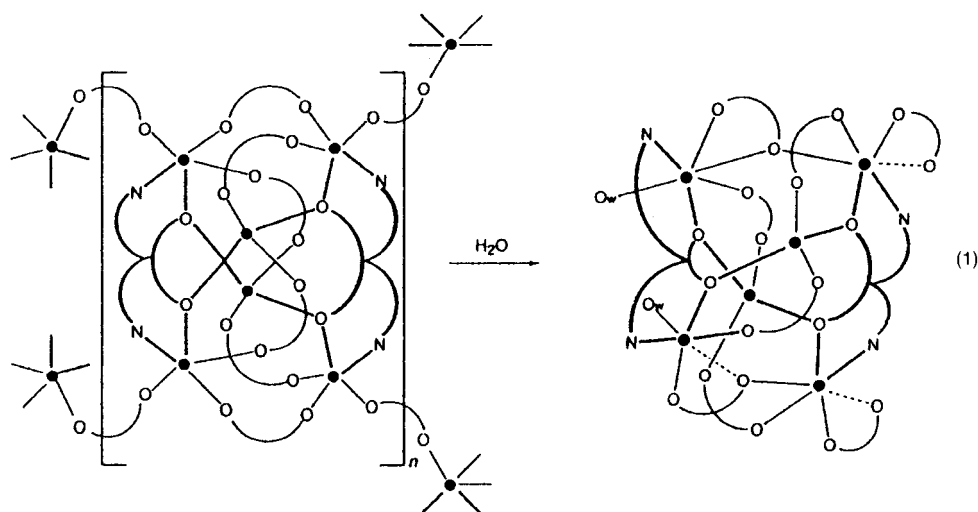


Figure 2. Molecular structure of **2**. Solvent molecules in the lattice are omitted. Most of the carbon atoms of the pyridine rings have been omitted for clarity. Interatomic distances [Å]: $\text{Zn} \cdots \text{Zn}$ 3.39–6.66, $\text{Zn}-\text{O}$ 1.91–2.75, $\text{Zn}-\text{N}$ 2.10–2.12.

found in **1**. The hexanuclear molecules are connected through a complex system of intermolecular hydrogen bonds involving both the aqua ligands, all the lattice molecules, and the acetate oxygen atoms O25, O29, O30, O35, and O36. Complex **2** is the sixth structurally characterized member of a group of polynuclear 3d metal complexes with mixed $(\text{C}_5\text{NH}_4)_2\text{CO}_2^{2-}/\text{RCO}_2^-$ ligation, the other examples being Cu_7^{II} ,^[7a] $\text{Cu}_{12}^{\text{II}}$,^[7a] and Co_9^{II} ^[7b,c] clusters, and it is only the second example of a discrete hexanuclear Zn^{II} species with exclusively O and/or N ligation.^[8]

The conversion of **1** into **2** [Eq. (1)] can be rationalized as follows: coordination of two H_2O molecules cleaves the polymer at two Zn^{II} sites per repeating unit; these sites are converted into six-coordinate ones by two new intracuster linkages ($\text{Zn}6-\text{O}28$ and $\text{Zn}4 \cdots \text{O}21$ in **2**); the two remaining monodentate acetate ligands (formerly *syn,anti*- $\mu_2:\eta^1:\eta^1$) at

the other two, formerly 5-coordinate, Zn^{II} sites become chelating. Thus, the four metal sites from which polymerization could start are coordinatively saturated (six-coordinate) in **2**, which contributes to the stability of the hexanuclear cluster.

The conversion of **1** into its constituent Zn_6 cluster does not depend on the volume ratio of the donor solvents H_2O and MeOH. Thus, dissolution of **1** in H_2O and addition of few drops of MeOH also led to a homogeneous solution from which the discrete cluster $[\text{Zn}_6\{(\text{C}_5\text{NH}_4)_2\text{CO}_2\}_2(\text{O}_2\text{CMe})_8(\text{H}_2\text{O})_2] \cdot 7\text{H}_2\text{O} \cdot \text{MeOH}$ crystallized. Single-crystal X-ray diffraction revealed^[9] that the hexanuclear molecule of this complex is essentially superimposable on that of **2**, the only difference being the nature of the lattice molecules. Moreover, it appears that the $(\text{Zn}_6)_n \rightarrow \text{Zn}_6$ conversion is not limited by the nature of the donor solvent. For example, dissolution of **1** in the minimum volume of DMF and addition of Et_2O yielded a microcrystalline solid that, according to elemental analyses, IR spectroscopy, and thermal techniques (TG/DTA, DTA), can be formulated as $[\text{Zn}_6\{(\text{C}_5\text{NH}_4)_2\text{CO}_2\}_2(\text{O}_2\text{CMe})_8(\text{DMF})_2] \cdot 2\text{DMF} \cdot 0.5\text{H}_2\text{O}$.

Apart from the above-mentioned structural changes (coordination of H_2O molecules and formation of the $\text{Zn}3-\text{O}34$, $\text{Zn}5-\text{O}36$, $\text{Zn}4-\text{O}21$, and $\text{Zn}6-\text{O}28$ bonds in **2**), the

$[\text{Zn}_6\{(\text{C}_5\text{NH}_4)_2\text{CO}_2\}_2(\text{O}_2\text{CMe})_8(\text{H}_2\text{O})_2]$ molecules of **2** and the $[\text{Zn}_6\{(\text{C}_5\text{NH}_4)_2\text{CO}_2\}_2(\text{O}_2\text{CMe})_8]$ cluster units in **1** are essentially identical with respect to their formulation, gross structural arrangement, and the coordination mode of the $(\text{C}_5\text{NH}_4)_2\text{CO}_2^{2-}$ ions.

The utility of our “depolymerization” approach is shown by the fact that direct reaction between three equivalents of $\text{Zn}(\text{O}_2\text{CMe})_2 \cdot 2\text{H}_2\text{O}$ and one equivalent of $(\text{C}_5\text{NH}_4)_2\text{CO}$ in MeOH or MeOH/H₂O gave a mixture of **2**, $[\text{Zn}_4\{(\text{C}_5\text{NH}_4)_2\text{C}(\text{OH})\text{O}\}_4(\text{O}_2\text{CMe})_3(\text{H}_2\text{O})] \cdot (\text{MeCO}_2) \cdot 7.5\text{H}_2\text{O}$ ^[9] (the identity of this cubane cluster has been established by single-crystal X-ray diffraction), and an as-yet unidentified product.

The depolymerization procedure described above can be monitored by solid-state ¹³C NMR spectroscopy; we^[10a] and others^[10b,c] have shown that this form of spectroscopy is an important complementary technique to X-ray crystallography for diamagnetic carboxylatometal complexes. The high-resolution solid-state ¹³C NMR spectrum of **1** (recorded at 75.5 MHz) contains four signals for both the methyl ($\delta = 24.40\text{--}21.51$) and the carboxylate carbon atoms ($\delta = 181.78\text{--}177.26$); this is consistent with the four crystallographically independent acetate ions present in the unit cell. The corresponding regions in **2** ($\delta = 24.31\text{--}20.53$, $184.55\text{--}175.20$) are more complicated, reflecting the presence of eight crystallographically inequivalent acetate ions. It might be suggested that the signals at $\delta = 184.55$ in the carboxylate region and at $\delta = 20.53$ in the methyl region, which are not present in the spectrum of **1**, belong to the two *asymmetrically* chelating acetate ligands; this deshielding effect has been interpreted in terms of an increased polarization of the carboxylate bond which makes the carbon atom more positive.^[10c]

The present work represents a successful “test of feasibility” for our depolymerization approach, and the described procedure may be generally applicable because coordination polymers containing high nuclearity repeating units are not rare.^[11] We recognize that the formation of **2** from **1** relies on the acetate ligand and its flexibility in converting from *syn,anti*- $\mu_2\text{:}\eta^1\text{:}\eta^1$ into chelating (η^2) and from *syn,syn*- $\mu_2\text{:}\eta^1\text{:}\eta^1$ into $\mu_2\text{:}\eta^1\text{:}\eta^2$ coordination. Although there was no chance to predict the exact formula of the product (for example, the reaction could have given $[\text{Zn}_6\{(\text{C}_5\text{NH}_4)_2\text{CO}_2\}_2(\text{O}_2\text{CMe})_8(\text{H}_2\text{O})_4]$ with monodentate instead of chelating acetate ligands) and the precise form of the intracuster linkage, we are encouraged by the fact that it was possible to isolate a hexanuclear cluster without significantly altering the structure of the hexameric repeating unit of the polymer.

Three years ago, Ranford et al.^[12a] reported the irreversible dehydration of discrete dinuclear zinc(II) complexes stabilized by noncovalent hydrogen bonds, which led to the construction of a 3D covalent polymer with the same basic architecture; their approach, which has been highlighted by Zaworotko,^[12b] may be regarded as the opposite of the approach described here. Recently Yaghi et al. employed the concept of secondary building units (SBUs),^[13] often used by zeolite chemists, for the design of highly porous and robust metal–organic carboxylate frameworks. SBUs are molecular clusters with ligand coordination modes and metal coordination environ-

ments which can be utilized in their transformation into extended crystalline porous networks using polytopic linkers. In a sense, if one follows Yaghi’s approach, complex **2** is the SBU for **1**. Preliminary experiments, supported by TG/DTG, DTA, and XRPD data, revealed that **2** can be converted into **1** by heating.

Experimental Section

1: Solid $\text{Zn}(\text{O}_2\text{CMe})_2 \cdot 2\text{H}_2\text{O}$ (0.66 g, 3.0 mmol) was dissolved under gentle reflux in a stirred solution of $(\text{C}_5\text{NH}_4)_2\text{CO}$ (0.18 g, 1.0 mmol) in MeCN (30 mL). Slow evaporation of the solvent from the resulting solution at room temperature gave colorless crystals of **1**, suitable for X-ray crystallography. The crystals were collected by filtration, washed with cold EtOH (2×5 mL) and Et₂O (2×5 mL), and dried in air, yield 80%; elemental analysis calcd for $\text{C}_{38}\text{H}_{40}\text{N}_4\text{O}_{20}\text{Zn}$ (%): C 36.1, H 3.2, N 4.4, Zn 31.0; found: C 36.2, H 3.4, N 4.3, Zn 30.8.

2: A slurry of **1** (0.25 g, 0.2 mmol) in MeCN (15 mL) was treated with a mixture of MeOH (6 mL) and H₂O (0.6 mL). The solid polymer dissolved rapidly. The solution was allowed to slowly concentrate by evaporation at room temperature to give colorless crystals, suitable for X-ray crystallography, which were collected by filtration, washed with a little cold EtOH and Et₂O, and dried in air; the formulation **2** was determined crystallographically, but the dried solid was analyzed as MeCN-free, yield 40%; elemental analysis calcd for $\text{C}_{38}\text{H}_{31}\text{N}_4\text{O}_{25.5}\text{Zn}_6$ (%): C 33.4, H 3.8, N 4.1, Zn 28.8; found: C 33.2, H 3.9, N 4.1, Zn 29.4.

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- Crystal data for **1**: Crystal dimensions $0.10 \times 0.20 \times 0.30$ mm, orthorhombic, space group *Fdd2*, $a = 16.627(1)$, $b = 27.141(2)$, $c = 28.356(2)$ Å, $V = 12796.4(2)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.313$ g cm⁻³, $2\theta_{\text{max}} = 130^\circ$, $\text{CuK}\alpha$ ($\lambda = 1.54180$ Å), θ - 2θ scan, $T = 298$ K, 3812 measured reflections, 3812 independent reflections ($R_{\text{int}} = 0.0000$) included in the refinement. Lorentzian, polarization, and ψ -scan absorption corrections were applied, $\mu = 2.998$ mm⁻¹, $[\Delta\sigma]_{\text{max}} = 0.026$, 318 parameters refined, $R1 = 0.0540$ (for 3190 reflections with $I > 2\sigma(I)$), $wR2 = 0.1520$ (on $|F^2|$). Max./min. residual peaks in the final difference map $0.883/-0.429$ e Å⁻³. Crystal data for **2**: Crystal dimensions $0.10 \times 0.30 \times 0.50$ mm, monoclinic, space group *P2₁*, $a = 13.780(6)$, $b = 14.801(6)$, $c = 15.386(7)$ Å, $\beta = 114.77(1)^\circ$, $V = 2849(2)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.626$ g cm⁻³, $2\theta_{\text{max}} = 50^\circ$, $\text{MoK}\alpha$ ($\lambda =$

0.71073 Å), θ -2 θ scan, $T=298$ K, 10115 measured reflections, 9702 independent reflections ($R_{\text{int}}=0.0339$) included in the refinement. Lorentzian, polarization, and ψ -scan absorption corrections were applied, $\mu=2.568$ mm⁻¹, $[A/\sigma]_{\text{max}}=0.046$, 734 parameters refined, $R1=0.0477$ (for 8333 reflections with $I>2\sigma(I)$), $wR2=0.1231$ (on $|F^2|$). Max./min. residual peaks in the final difference map 0.794/−0.599 e Å⁻³. Crystals of **1** and **2** were mounted in capillaries filled with drops of the mother liquor. The structures were solved by direct methods with SHELXS 86 and refined by full-matrix least-squares techniques on F^2 using SHELXL 93. For both structures, almost all non-hydrogen atoms were refined anisotropically; the MeCN solvate and two lattice H₂O molecules of **2** were refined isotropically with occupation factors fixed at 10.76 and 10.50, respectively. All hydrogen atoms in **1** were introduced at calculated positions as riding on bonded atoms. Hydrogen atoms of the methyl groups of the acetate ligands and those of C9, C23, C24, C28, C29, C30, and C31 in **2** were introduced at calculated positions as riding on bonded atoms, the remaining hydrogen atoms were located by difference map calculations and refined isotropically; no hydrogen atoms for the H₂O and MeCN solvate molecules were included in the refinement. 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-147434 and CCDC-147435. 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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Core–Shell–Corona Micelles with a Responsive Shell**

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Supramolecular assemblies formed by self-associating block copolymers have raised considerable interest in the scientific literature.^[1] In most cases, amphiphilic diblock copolymers are dissolved in a solvent selective for one constituent, which results in spherical micelles that consist of a core formed by the insoluble blocks surrounded by a shell of the solvated blocks. Although ABC triblock copolymers self-organize into a wide variety of supramolecular structures in the bulk,^[1] their association in selective solvents has scarcely been studied. “Three-layer” micelles were reported in water,^[2] although other micelle structures can be formed in organic solvents, as demonstrated by the so-called “Janus” micelles,^[3] which consist of a cross-linked polybutadiene core and a corona with a “northern” polystyrene and “southern” poly(methyl methacrylate) hemisphere. These Janus micelles have to be distinguished from three-layer micelles, because their structure is generated in the bulk and persists in solution.

Herein we report on the formation of aqueous three-layer micelles from a polystyrene-*block*-poly(2-vinyl pyridine)-*block*-poly(ethylene oxide) triblock (PS-*b*-P2VP-*b*-PEO), these micelles will be referred to as core–shell–corona (CSC) micelles. The molecular weight of each block is 20000 for PS, 14000 for P2VP, and 26000 for PEO. CSC micelles consisting of a PS core, an intermediate P2VP layer, and a PEO corona are expected to be formed. Because the water-solubility of the central P2VP block depends on the degree of ionization,^[4] the CSC micelles should be pH sensitive. Structurally they are reminiscent of the so-called “onion” micelles prepared by mixing an aqueous micellar solution of PS-*b*-P2VP diblock with P2VP-*b*-PEO chains dissolved in water.^[5] At pH > 10, the P2VP blocks of the two copolymers coprecipitate, and three-layer onion micelles, that consist of a PS core, a P2VP shell, and a PEO corona are formed. The effect of pH on the onion micelles is dramatically different from that on the CSC micelles, the onion micelles disintegrate into the two constituent diblocks as the pH is decreased, which is not the case for their CSC counterparts which remain intact. Moreover, the structural features of the CSC micelles can be better controlled than those of the onion micelles.

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Dynamic light scattering (DLS) was used to measure the mean hydrodynamic diameter (D_h) of the CSC micelles. Because of the glassy PS core, the micelles are expected to be kinetically frozen in water at room temperature.^[6] At pH > 5, a D_h of 75.4(±0.5) nm and a size polydispersity (PDI) of 0.087(±0.017) were measured. At pH < 5, D_h and PDI were shifted to 135.2(±1.2) nm and 0.146(±0.021), respectively. The very low values of PDI must be noted, which indicates the formation of nearly monodisperse micelles. The increase in D_h at pH < 5 is consistent with the protonation of the P2VP blocks, which results in intra- and intersegmental electrostatic repulsion. The reversibility of this phenomenon has been ascertained by cycling the pH several times from basic to acidic values (Table 1). The constancy of D_h at pH > 5 is noteworthy, whereas a decrease in D_h is observed at pH < 5 as the number of cycles is increased. This effect results from the increase in the solution ionic strength because NaCl is formed with each pH-variation cycle, which decreases the strength of the electrostatic repulsion between the charged P2VP blocks.

The morphology of the CSC micelles has been observed by transmission electron microscopy (TEM). The PS and P2VP blocks have been selectively stained with RuO₄, and Fig-

ure 1 a confirms that low polydispersity spherical micelles are formed. The diameter of the core plus shell (D_{CS}) has been determined from the TEM pictures and found to be ≈35 nm. Although this value is characteristic of the “dried” micelles, it should be comparable for the micelles in water at pH > 5, because the PS core and the P2VP corona are basically unswollen. TEM observations at higher magnification do not provide more detail on the internal micellar structure, that is, the core cannot be discriminated from the shell, consistent with a PS core uniformly surrounded by a dense P2VP shell. At pH < 5 an increase in D_{CS} is observed (from ≈35 to ≈50 nm; Figure 1 b), in agreement with the protonation of the P2VP blocks. Moreover, Figure 1 b shows the internal structure of the micelles, and allows the diameter of the PS core (D_C ≈ 20 nm) to be measured. A diffuse P2VP layer is observed around the PS core.

The structure of the CSC micelles in the dried state has also been investigated by atomic force microscopy (AFM) in the tapping-mode. These observations complement the TEM micrographs. At pH > 5, highly regular, low polydispersity spherical micelles are observed, in agreement with DLS data and TEM observations (Figure 2 a). The diameter of the CSC

micelles in the dried state (D_{CSC}) has been measured (D_{CSC} ≈ 60 nm). This value is smaller than D_h , because the PEO corona is now desolvated but it is higher than the D_{CS} value estimated by TEM because the PEO corona was not stained in Figure 1 a and was thus not detected. At pH < 5 the AFM picture of the CSC micelles shows that they have characteristic dimensions (Figure 2 b, D_C ≈ 30 nm and D_{CS} ≈

Table 1. Changes in D_h ^[a] for the CSC micelles triggered by repeated pH variations.^[b]

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
D_h “acid” sample	132.6 ± 1.7	124.6 ± 0.2	120.4 ± 0.6	116.3 ± 1.5	110.1 ± 0.9
D_h “basic” sample	74.2 ± 0.5	72.6 ± 0.3	73.5 ± 1.0	75.5 ± 1.5	74.8 ± 0.7

[a] D_h given in nm. [b] An initial solution with 0.26 μmol of copolymer was prepared (D_h = 71.1 ± 1.0 nm). For each cycle, 15 μmol of HCl was added (“acid” samples), R_h was measured, then 15 μmol of NaOH was added (“basic” samples) and R_h was measured again.

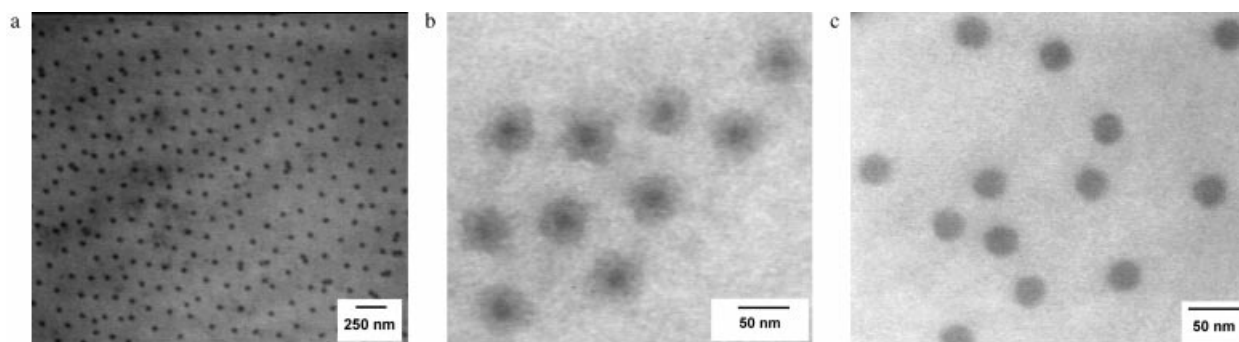


Figure 1. TEM images of the CSC micelles at pH > 5 (a), at pH < 5 (b), and after HAuCl₄ loading and reduction (c).

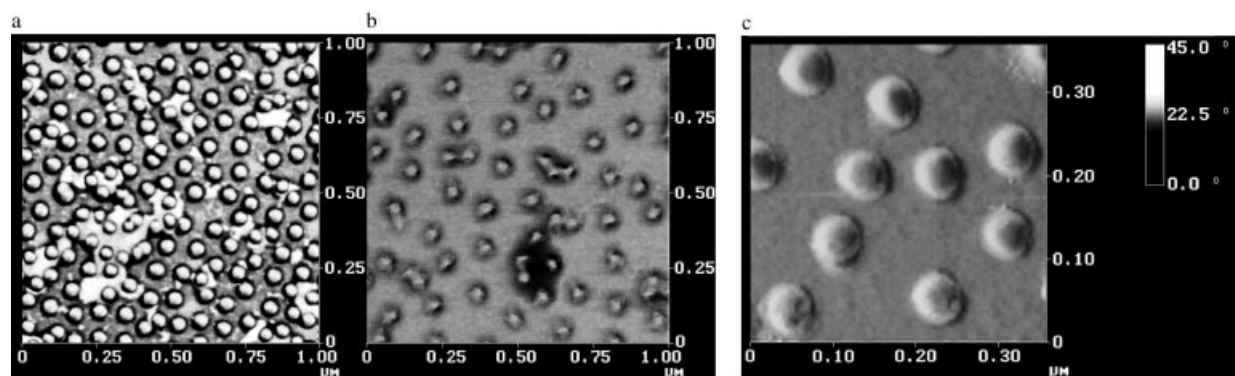


Figure 2. AFM phase-contrast pictures of the CSC micelles at pH > 5 (a), pH < 5 (b), and after HAuCl₄ loading and reduction (c).

40 nm) which are in qualitative agreement with those estimated from the TEM picture (Figure 1b). The CSC micelles are observed at $\text{pH} < 5$ by AFM to have irregular contours. The reason might be found in the electrostatic interactions between the charged P2VP blocks and the charged substrate used, a silicon wafer with a surface oxide layer positively charged at $\text{pH} < 3.8$. In case of TEM observation, the substrate is neutral (Formvar), and the micelles are not significantly deformed.

Metal or semiconductor nanoparticles have raised great interest because of their unique size-dependent chemical and physical properties, which make them ideal candidates for electronic and optical nanodevices.^[7] Moreover, polymer-coated metal and semiconductor nanoparticles exhibit increased stability, that results in protection against oxidation^[8a] and much higher catalytic activity.^[8b] Such nanoparticles can be synthesized in confined reaction vessels such as the micellar core of amphiphilic block copolymers in apolar solvents^[9a,b] and in water,^[9c] with the drawback that the nanoparticle size, which is fixed by the dimensions of the micellar core, can only be varied in a limited range of a few nanometers. In sharp contrast, the P2VP blocks of the CSC micelles can be used as a template for the production of metal nanoshells, the thickness of which can be tuned by the molecular weight of the P2VP block. Moreover, CSC micelles would allow the synthesis of metal or semiconductor capsules in a size range different to that of previously reported systems.^[10]

The P2VP shell has been selectively loaded with AuCl_4^- ions, made possible by the selective interaction of the ions with the protonated 2VP units. The excess of non-interacting AuCl_4^- ions was eliminated by dialysis, followed by the reduction of Au^{3+} into Au^0 centers either by electron irradiation^[9b] or by NaBH_4 .^[9c] Gold-loaded micelles have a uniform structure (Figure 1c and the AFM picture of Figure 2c). Moreover, TEM and AFM observation of a centrifugated micellar solution show that all the CSC micelles have the same gold-loading density. Finally, formation of gold nanoparticle has been monitored by UV/Vis spectroscopy. Before reduction of the gold precursor, the micellar solution is pale-yellow and an absorption is observed at 313 nm, consistent with the HAuCl_4 loading.^[11] After reduction, the micellar solution is wine-red and the previously observed absorption disappears in favor of a broad absorption in the 400–600 nm range, that was assigned to the surface plasmon resonance of gold nanoparticles.^[11] The broadness of this absorption indicates that individual gold particles persist in the P2VP shell and that capsules with a continuous gold wall are not formed. Further work is needed to optimize both the HAuCl_4 loading of the CSC micelles and the reduction conditions.

The PS(20000)-*b*-P2VP(14000)-*b*-PEO(26000) triblock copolymer forms well-defined micelles. The P2VP shell can serve as a reactor for the synthesis of gold nanoparticles. Moreover, the pH-sensitive P2VP shell makes this system useful for encapsulation and/or release of active species. Finally, the hydroxyl end-group of the PEO blocks provides a unique opportunity to functionalize the CSC micelles at their periphery and to provide them with additional responsive or sensing properties.

Experimental Section

The triblock copolymer was prepared in THF at -78°C by sequential living anionic copolymerization of styrene and 2-vinylpyridine, initiated by secondary butyl lithium in the presence of LiCl, then termination with ethylene oxide. The terminal hydroxyl group was treated with naphthalene potassium and ethylene oxide was then added and polymerized at 0°C . $\bar{M}_w/\bar{M}_n$ of the final triblock copolymer was 1.1. The micelles were prepared by first dissolving the copolymer (0.1 g) in a mixture of bidistilled water (0.5 g) and dimethylformamide (DMF; 4.4 g). DMF was then eliminated by dialysis against bidistilled water.

Gold nanoparticles were prepared by adding an excess of HAuCl_4 to the aqueous micellar solution. Unreacted HAuCl_4 was eliminated by dialysis of the loaded micelles against acidic water. Reduction of the gold containing anion was carried out either by electron irradiation during TEM observation^[9b] or by addition of an excess of aqueous NaBH_4 solution (1 g L^{-1}).^[9c] Unreacted NaBH_4 was eliminated by dialysis.

DLS measurements were performed on a Brookhaven Instruments Corp. BI-200 goniometer equipped with a BI-2030 digital correlator. The D_h and PDI of the micelles were obtained by a cumulant analysis of the experimental correlation function.

TEM pictures were recorded on a Philips CM 100 microscope equipped with a Gatan 673 CCD camera, and transferred to a computer equipped with Kontron KS 100 software. Samples were prepared by dipping a Formvar-coated copper grid into 0.2 wt % aqueous copolymer solution and contrasted by RuO_4 vapor.

AFM observations were carried out at the Centre of Microscopy of the University of Liège with a Digital Instrument Inc. Nanoscope III microscope operated at room temperature in the Tapping Mode in air. The instrument with the Extender Electronics Module provided height and phase cartography simultaneously. The samples were prepared by casting 0.004 wt % aqueous copolymer solution onto a silicon wafer covered by a 2 nm thick native silicon oxide layer.

UV/visible spectra were recorded on a Hitachi U3300 spectrometer.

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Homo- and Heterochiral Supramolecular Tapes from Achiral, Enantiopure, and Racemic Promesogenic Formamides: Expression of Molecular Chirality in Two and Three Dimensions**

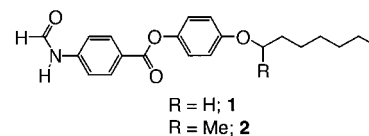
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Understanding how a stereogenic center influences conformations at the molecular scale and organization at the supramolecular level is an elusive and intriguing challenge in a number of scientific disciplines.^[1] A very interesting challenge is to prepare self-assembling systems and to study how the chiral nature of the component compounds in combination with the self-assembly process affects the ordering in two and three dimensions. In both two- and three-dimensional (2D and 3D, respectively) systems, pure enantiomers form enantiomorphous structures.

But what happens when equimolar mixtures of enantiomers are crystallized or are physisorbed at a surface? Will the racemate resolve spontaneously into a racemic conglomerate, or will both enantiomers co-crystallize or co-adsorb forming racemic 3D or 2D crystals? In 3D (liquid) crystals, conglomerate formation is the exception^[2, 3] rather than the rule. In 2D monolayers at the air/water interface, both the controlled separation of enantiomers and racemate formation have been reported from grazing incidence X-ray diffraction studies.^[4, 5] In 2D monolayers on solid supports, modern tools such as atomic-force microscopy (AFM)^[6] and scanning tunneling microscopy (STM) provide compelling evidence for spontaneous segregation.^[7] The use of STM is especially appealing for the visualization of (sub)monolayer structures, since it can reveal with near-atomic resolution the ordering in two dimensions.^[8] The technique has shown that both chiral^[9–11] and achiral^[12, 13] molecules self-assemble into chiral arrays, the former enantiospecifically and the latter as 2D conglomerates. Experimental data available so far suggest a spontaneous

resolution,^[9a, 10, 11a–d] although one report has noted differences in the way molecules discriminate through their chirality depending upon their absorption site.^[14]

The compounds we have targeted to address these questions are the achiral formamide **1** and the chiral formamide **2**.^[15] Here we report the X-ray crystal structure and STM imaging of the self-assembled monolayers of the enantiopure and racemic **2**, as well as the STM data for **1**, and compare the expression of molecular chirality in two and three dimensions.



The X-ray crystal structure^[16, 17] of the enantiopure compound (*R*)-**2** (Figure 1) has a unit cell which contains molecules with four different conformations, all of which form chains (in which the molecules are linked through N–H···O=C and other weaker hydrogen bonds) that unite head-to-head through C–H···O=C hydrogen bonds between formyl groups to generate supramolecular tapes. There are

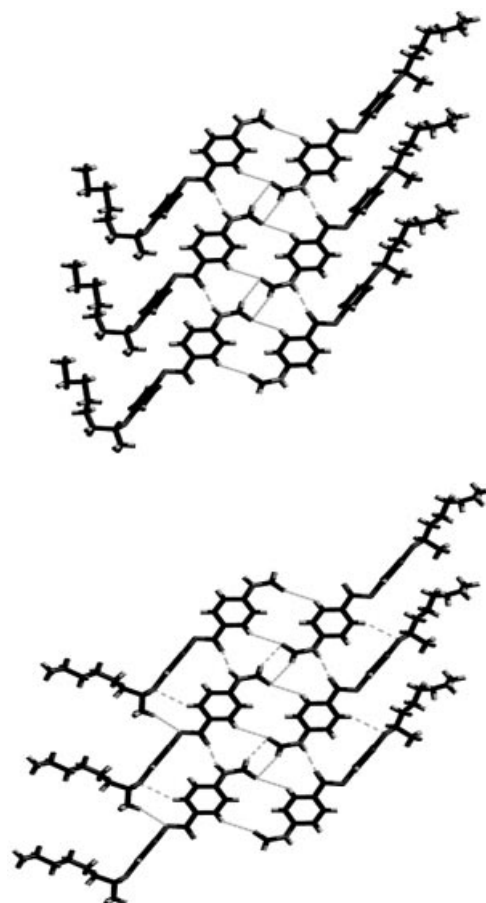


Figure 1. Views of the two hydrogen-bonded tapes formed by the four conformational diastereomers of (*R*)-**2** in its crystals. Intrachain N–H···O distances: 2.072 and 1.940 Å; angles: 167.4 and 157.9°; N···O distances: 2.864 and 2.857 Å; interchain C=O···H–C distances: 2.685, 2.693, 2.675, and 2.686 Å; C–H···O angles: 147.5, 147.4, 146.8, and 145.3°; C···O distances: 3.514, 3.521, 3.500, and 3.500 Å.

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two types of gross conformations among the four molecules: one in which the aliphatic chain extends in the plane of the phenyl group to which it is attached, and one in which it is oriented approximately perpendicularly to this plane. None of the alkyl chains have the all-*trans* conformation, but instead contain portions with cisoid geometry. The reason for this distortion may result from the packing of the tapes, which allows only minimal interdigitation of the aliphatic chains.

The X-ray crystal structure^[17, 18] of racemic **2** has a unit cell comprised of two centrosymmetrically related molecules. These molecules form homochiral chains (Figure 2) connected through various hydrogen bonds to give a very similar

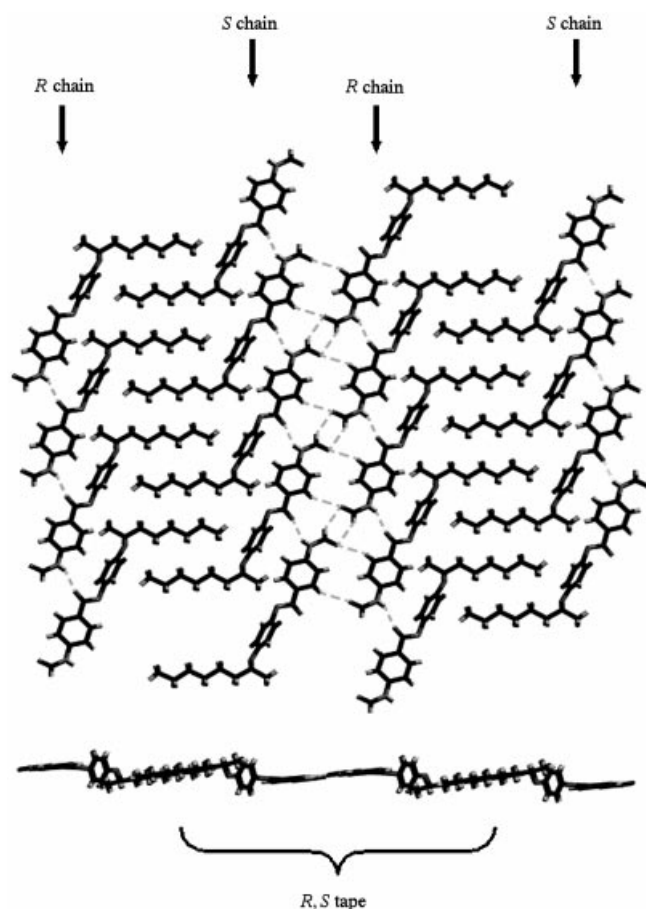


Figure 2. Views (top) perpendicular to a sheet of *rac*-**2** in its crystals showing the homochiral chains and heterochiral tapes and (bottom) parallel to the sheets showing their stepped nature with respect to the alkyl chain long axes. Intrachain N–H...O distance: 2.174 Å, angle: 149.8°, N...O distance: 2.888 Å, interchain C=O...H–C distance: 2.493 Å, C–H...O angle: 148.2°, C...O distance: 3.329 Å.

chain assembly as that of the enantiopure compound. These homochiral chains form heterochiral tapes. The alkyl chains (which are in the all-*trans* conformation) of neighboring chains interdigitate, with a minimum spacing between carbon atoms of adjacent chains of approximately 3.7 Å, to form sheets of molecules (Figure 2, top). There is strict *R*-*S*-*R*-*S* alternance within the sheets, which are not completely flat but undulate (Figure 2, bottom). Thus, there is no spontaneous

resolution into enantiopure crystals in three dimensions but instead, racemic crystals are formed.

The self-assembly of the formamides **1** and **2** (in *S*, *R*, and *rac* forms) in two dimensions has been studied with STM at the 1-heptanol/graphite interface. All form monolayers with domains (areas of parallel tapes) which are related by the C_3 symmetry of the surface, and are characterized by areas of low tunneling current (observed as darker bands in the images) which correspond to the location of the alkyl chains (which are interdigitated) and brighter zones which coincide with the location of the phenyl benzoate moieties. Only in a few cases are the alkyl chains visible, which indicates there is a relatively high mobility. All the formamides self-assemble into tapes reminiscent of those in the crystal structures, with a distance between the same point in adjacent molecules within each chain of 8.60 ± 0.20 Å. The corresponding distance in the tapes of (*R*)-**2** and *rac*-**2** in their crystals are 8.66 and 8.86 Å, respectively. The repeat distance perpendicular to the tape direction is 29.0 ± 1.0 Å in the physisorbed monolayers, compared with 27.70 Å in the sheets formed in crystals of *rac*-**2**.

The images of monolayers of enantiopure formamides (*S*)-**2** and (*R*)-**2** (Figure 3) are enantiomorphous, while *rac*-**2** shows a very distinct structure. The chiral induction phenomenon for

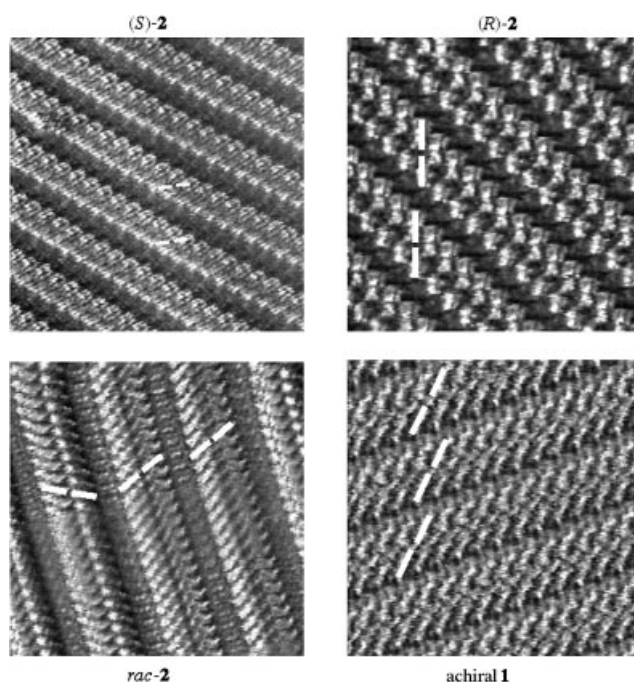


Figure 3. STM images of (*S*)-**2** (16.1×16.1 nm², 1.0 nA, –0.51 V; top left), (*R*)-**2** (12.0×12.0 nm², 1.0 nA, –0.73 V; top right), *rac*-**2** (13.6×13.6 nm², 1.0 nA, –0.54 V; bottom left), and **1** (11.3×11.3 nm², 1.0 nA, –0.83 V; bottom right) at the 1-heptanol/graphite interface. The orientation of some of the phenyl benzoate groups is indicated by a bar.

(*S*)-**2** and (*R*)-**2** in pure form is expressed by the orientation of the phenyl benzoate moiety with respect to the normal on the propagation direction of the tapes (the tape normal): the phenyl benzoate moieties are rotated clockwise for (*S*)-**2** and counter-clockwise for (*R*)-**2**. In order to judge the influence of the graphite substrate, the registration of the observed

monolayers with respect to the surface has been determined and is expressed by the angle θ , that is, the angle between the propagation direction of the tapes and the reference axis along the [0010] direction of the graphite surface (Figure 4), which is almost parallel to the propagation direction of the tape. The angle θ takes negative and positive values for (*S*)-**2** and (*R*)-**2**, respectively (between 0° and 12° with a bias for larger values). Thus the molecular chirality of enantiopure **2** is expressed by both the orientation of the adlayer with respect to the symmetry of the underlying graphite lattice and by the orientation of the phenyl benzoate moieties with respect to the tape normal.

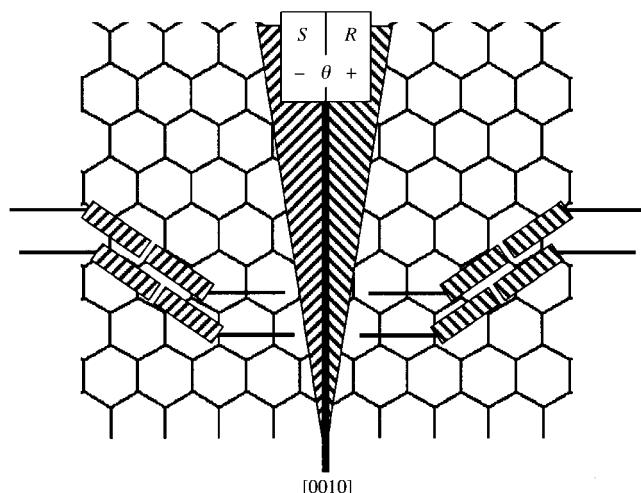


Figure 4. A schematic representation of the orientations of the molecules of (*S*)- (left) and (*R*)-**2** (right) at the graphite/1-heptanol interface.

The racemic mixture *rac*-**2** physisorbed from 1-heptanol on graphite (Figure 3) does not display the generally observed spontaneous resolution on surfaces. The angle θ is always found to be $0 \pm 1^\circ$, which suggests there is a different packing of the tapes to those formed by the pure enantiomers. In addition, the phenyl benzoate groups within a domain are rotated both clockwise and anticlockwise from the tape normal, as can be appreciated clearly in the image shown in Figure 3. In a few cases it was possible to correlate details in the STM images with intermolecular features in great detail. For example, in a high-resolution image, such as Figure 5, the STM contrast in the upper area suggests that one of the phenyl rings of the phenyl benzoate groups is lying flat on the graphite surface while the other one is tilted. In general, the specific STM contrast on a (sub)molecular scale does not allow the chiral nature of a domain to be characterized. However, the two observations mentioned above, that is, the registry of the molecules with respect to the graphite surface and the orientation of the molecules with respect to the tape normal, do show that the domains formed by the racemate have the characteristics of neither the *R* nor *S* enantiopure domains, but are racemic themselves: domains formed from a racemate solution are not enantiomerically pure. To the best of our knowledge this is the first time that this finding has been reported for 2D adlayers adsorbed on a solid surface and studied by STM.

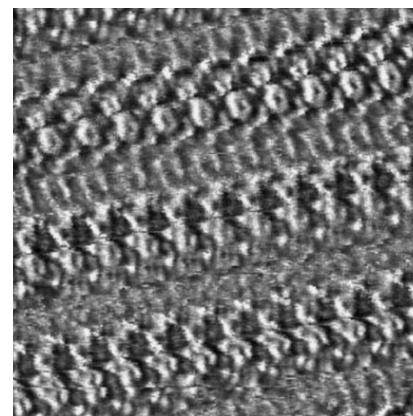


Figure 5. A high-resolution STM image of *rac*-**2** ($9.6 \times 9.6 \text{ nm}^2$, 1.0 nA, -0.58 V) at the 1-heptanol/graphite interface.

Two possibilities have to be kept in mind when considering the composition of the individual tapes in a racemic domain, firstly the tapes are enantiopure. In this case, although there is no segregation at the level of domains, there would be segregation at the tape level. From the orientation of the aromatic groups in Figure 3 (bottom left) the sequence of the chirality of the tapes can then be described as being (from left to right) *S-S-R-R-R-R* when our findings for the enantiopure compounds are taken into account. Secondly the tapes are racemic, as in the 3D crystals. To achieve this packing the *R* and *S* enantiomers have to lie head-to-head, and the long axes of the aromatic groups have to be oriented along the same line. The resulting arrangement is similar to the one shown in Figure 2. This centrosymmetrical arrangement requires that one enantiomer has a methyl group (attached to the stereogenic center) pointing towards the graphite surface, which is energetically unfavorable.^[19]

A final result which is important to highlight is that, in stark contrast to racemic **2**, the achiral compound **1**^[20] exhibits enantiomorphous domains that always consist of only one of the two possible orientations of the aromatic groups with respect to the tape axis (Figure 3, bottom right) and the angle θ takes positive or negative values (never 0°), that is, this achiral compound shows segregation and the formation of chiral domains.

The type of structural resemblance between X-ray structures and physisorbed monolayers, as seen here for *rac*-**2**, is rare,^[21–23] but provides important clues regarding the roles of each structural feature of the molecule in self-assembly processes. The conformational mobility of the chiral alkyl chains in this interesting family of compounds allows the formation of different unions between the hydrogen-bonded tapes in the crystals and on the graphite substrate; the subtle interactions of the latter with the tapes must surely play an important role. The tapes described here are clearly robust “supramolecular synthons”,^[24] both on the graphite surface and in the crystals. It has been shown that a racemic mixture does not show segregation in two dimensions but forms a racemate. This observation might have important implications concerning the expression of stereochemistry at surfaces and its transfer into three dimensions.

Experimental Section

Compounds **1** and **2** were prepared according to the published method.^[15] The STM images were acquired in the variable current mode (constant height) under ambient conditions with the tip immersed in the liquid. For details see ref. [9].

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Mononuclear $\eta^2(4e)$ -Bonded Phosphaalkyne Complexes; Selective Formation of a 1,2-Diphosphacyclobutadiene Tantalum Complex**

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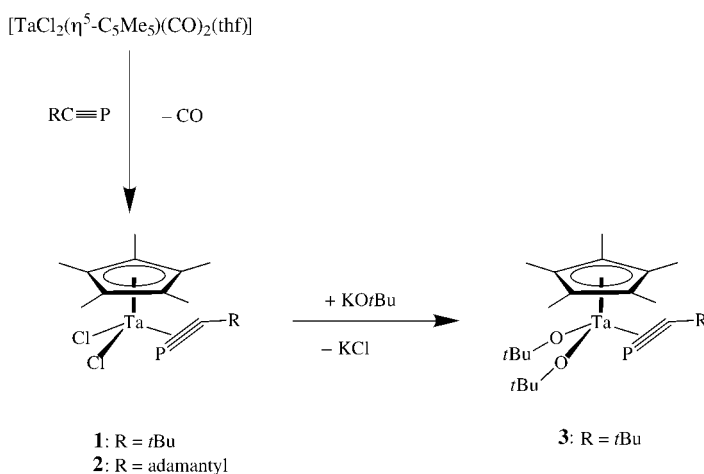
Dedicated to Professor Paul Binger

An interest in $\eta^2(4e)$ -bonded alkyne complexes^[1] together with the recognition that the chemistry of coordinated $t\text{BuC}\equiv\text{P}$ frequently mirrors that of alkynes^[2] led us to explore the possibility that phosphaalkynes might also adopt a $\eta^2(4e)$ bonding mode in mononuclear complexes. Here we describe the synthesis and structural characterization of the d² tantalum complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\eta^2(4e)\text{-}t\text{BuC}\equiv\text{P}\}]$ and its reaction with an excess of $t\text{BuC}\equiv\text{P}$ to selectively form the 1,2-diphosphacyclobutadiene complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\sigma,\sigma\text{-}1,2\text{-P}_2\text{C}_2t\text{Bu}_2\}]$.

In seeking to establish whether phosphaalkynes ($\text{RC}\equiv\text{P}$) can adopt a $\eta^2(4e)$ bonding mode involving $\pi_{\parallel}\rightarrow\text{M}(\sigma)$, $\text{M}(\text{d}_{\pi})\rightarrow\pi_{\parallel}^*$, $\pi_{\perp}\rightarrow\text{M}(\text{d}_{\pi})$, and possibly $\text{M}(\text{d}_{\delta})\rightarrow\pi_{\perp}^*$ orbital interactions, we sought to prepare analogues of $\eta^2(4e)$ -bonded mononuclear alkyne complexes in which the alkyne is replaced by a η^2 -bonded $t\text{BuC}\equiv\text{P}$ ligand. We reasoned that a comparison of the structural data for the two complexes would provide an insight into the nature of the bond between the metal center and the phosphaalkyne. In this study we focused on the report that the well-characterized (NMR, X-ray) d² complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\eta^2(4e)\text{-PhC}_2\text{Ph}\}]$ ^[3] can be synthesized by reaction of $\text{PhC}\equiv\text{CPh}$ with the labile complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)(\text{CO})_2(\text{thf})]$.^[4] This suggested that the tantalum dicarbonyl complex might also react with $t\text{BuC}\equiv\text{P}$ to form a novel $\eta^2(4e)$ -bonded phosphaalkyne complex.

When $t\text{BuC}\equiv\text{P}$ (1.1 molequiv) was added at room temperature to a stirred solution of $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)(\text{CO})_2(\text{thf})]$ in THF, both CO ligands were readily (5 min) displaced (IR),

and the reaction mixture changed color from yellow to deep purple. Removal of the solvent in vacuo and extraction of the residue with hexane afforded the air-sensitive complex **1** (Scheme 1) in 85% yield as a deep purple powder, which



Scheme 1. Synthesis of $\eta^2(4e)$ -bonded phosphaalkyne complexes.

could be recrystallized from hexane or pentane at -30°C . Complex **1** was characterized by elemental analysis, NMR spectroscopy, and single-crystal X-ray crystallography as the phosphaalkyne-substituted d² complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\eta^2(4e)\text{-}t\text{BuC}\equiv\text{P}\}]$. The corresponding reaction with the adamantyl-substituted phosphaalkyne afforded in a similar fashion **2** in high yield (Scheme 1), and it was also found that KO*t*Bu readily (RT, 12 h) reacted in toluene with **1** to form in good yield the bis-alkoxy complex **3** (Scheme 1).

An X-ray crystallographic study^[5] on a suitable single crystal of **1** obtained from pentane (-30°C) established the three-legged piano-stool structure illustrated in Figure 1, which closely resembles that reported for $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\eta^2(4e)\text{-PhC}_2\text{Ph}\}]$. In **1** an η^2 -bonded $t\text{BuC}\equiv\text{P}$ replaces the alkyne ligand: both PhC_2Ph and $t\text{BuC}\equiv\text{P}$ adopt the same orientation in these complexes. Since a characteristic feature of $\eta^2(4e)$ -bonded alkyne complexes is a short metal–carbon bond^[6] it is especially interesting that in complex **1** the Ta–C1 distance is also short (2.079(9) Å) and is of similar length to the Ta–C(alkyne) bond length of 2.067(6) Å in $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\eta^2(4e)\text{-PhC}_2\text{Ph}\}]$. In contrast, a long Ti–C–(phosphaalkyne) distance (2.187(6) Å) is observed in the d² complex $[\text{Ti}(\eta^5\text{-C}_5\text{H}_5)_2\{\eta^2(2e)\text{-}t\text{BuC}\equiv\text{P}\}(\text{PMe}_3)]$,^[7] in which the metal center has 18 electrons and cannot accept more than two electrons from the phosphaalkyne triple bond.

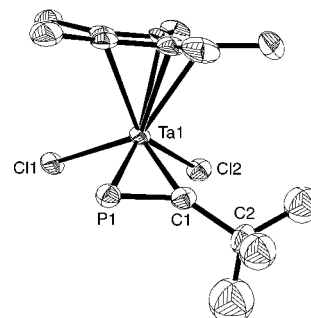


Figure 1. ORTEP representation of **1**. Thermal ellipsoids shown at the 30% probability level. Selected bond lengths [Å] and angles [°]: P1–C1 1.694(10), P1–Ta1 2.438(3), C1–Ta1 2.079(9), Ta1–Cl1 2.346(2), Ta1–Cl2 2.378(2); P1–C1–C2 131.5(7).

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Similarly, there is a difference in the P–C bond lengths and P–C–R “bend-back” angles, which are larger in the Ta complex (1.694(10) Å, 131.5(7)°) than in the titanium complex (1.636(2) Å, 133.4(7)°). All of these observations, but especially the short Ta–C(phosphaalkyne) bond, are consistent with involvement of the phosphaalkyne $\pi_{\perp}$ orbital in bonding to the tantalum atom in **1**.

This conclusion is also supported by Extended Hückel MO (EHMO) calculations^[8] using the bond parameters found for complex **1**. The complex was divided into Ta(η^5 -C₅Me₅)Cl₂ and *t*BuC≡P fragments, and the orbital interactions between the two were examined. The frontier orbitals on the *t*BuC≡P ligand^[9] corresponding to $\pi_{\parallel}$, $\pi_{\perp}^*$, and $\pi_{\perp}$ all showed significant interactions with the TaCl₂(η^5 -C₅Me₅) fragment; the electron populations of these orbitals were 1.81, 1.02, and 1.85 respectively, and the population of the $\pi_{\perp}^*$ orbital of 0.04 implies little or no interaction with the metal center. These results compare well with those reported (EHMO calculation) by Curtis et al.^[10] for [NbCl₂(η^5 -C₅H₅){ η^2 (4e)-HC≡H}] ($\pi_{\parallel}$ 1.74, $\pi_{\perp}^*$ 0.81, $\pi_{\perp}$ 1.68, and $\pi_{\perp}^*$ 0.02) and indicate that, as in the alkyne complex, the phosphaalkyne ligand in **1** is a four-electron donor. A similar calculation on [Ti(η^5 -C₅H₅)₂{ η^2 (2e)-*t*BuC≡P}(PMe₃)] showed orbital populations consistent with the proposed bonding mode of the phosphaalkyne ($\pi_{\parallel}$ 1.87, $\pi_{\perp}^*$ 1.02, $\pi_{\perp}$ 1.94, and $\pi_{\perp}^*$ 0.04).

Further insight into the nature of complex **1** was provided by ab initio calculations on the model complexes [TaCl₂(η^5 -C₅H₅){ η^2 (4e)-RC≡P}] (R = H (**A**), Me (**B**)) with the Gaussian 98 package of programs; the structure depicted in Figure 2 was obtained.^[11–13] The geometrical parameters of **A** and **B** are in reasonable agreement with those of **1** in the crystal. Due to a low barrier to rotation of the phosphaalkyne ligand (< 20 kJ mol^{–1}; see Supporting Information) various geometries were considered in the GIAO calculation of the NMR parameters.^[14, 15] Based on ECP/DFT calculations on **B**, ³¹P NMR chemical shifts of δ = 492, when the phosphaalkyne lies parallel to the Cl–Cl vector, and of δ = 525, when it is perpendicular to the Cl–Cl axis with the methyl group away from the η^5 -C₅H₅ ligand, are predicted. This is in agreement with the experimental values for **1** and **2** (δ = 509.0 and 515.3 in C₆D₆, respectively). The ¹³C chemical shifts of the phosphaalkyne C atom in **B**, calculated at the same level of theory, were δ = 299 (P–C parallel to Cl–Cl) and δ = 327 (P–C perpendicular to Cl–Cl). The difference of $\Delta\delta \approx 10$ –37 between δ_{calcd} (¹³C) and δ_{exp} (¹³C) of the corresponding carbon atoms in **1** and **2** could be due to the different substituents.

The observed and calculated low-field ³¹P NMR chemical shifts are consistent with our earlier finding that the complex [Mo(η^5 -C₅H₅)₂{ η^2 (4e)-*t*BuC≡P}]{ η^2 -P(OMe)₂OBF₂OP(OMe)₂}]^[16] shows a low-field (δ = 467.8) ³¹P chemical shift for the coordinated phosphaalkyne, and it was argued on the basis

of isolobal analogies that such a shift is characteristic of a η^2 (4e) bonding mode. In contrast, higher field ³¹P chemical shifts were reported for the d² titanium (δ = 122.7) and zirconium (δ = 196.9) complexes [M(η^5 -C₅H₅)₂{ η^2 (2e)-*t*BuC≡P}(PMe₃)] (M = Ti, Zr),^[7] in which the phosphaalkyne cannot donate more than two electrons to the metal center. Similarly, the ³¹P chemical shift of the phosphaalkyne P atom in [Pt(η^2 (2e)-*t*BuC≡P)(PPh₃)₂]^[16, 17] is δ = 84.1, characteristic of a 2e donor and to much higher field than the signals of the tantalum complexes **1**, **2**, and **3**. The ³¹P chemical shift (δ = 416.3) of complex **3** can be rationalized by a decrease in $\pi_{\perp}$ donation from the phosphaalkyne ligand and implies that the two *t*BuO ligands donate more π -electron density to the tantalum atom than the chloro ligands in **1** and **2**.

Whereas the ³¹P chemical shifts exhibit a diagnostically useful wide range (δ = 84–515) which spans the η^2 (2e) to η^2 (4e) phosphaalkyne bonding modes, the corresponding ¹³C chemical shifts, in contrast to those of η^2 (2e)- to η^2 (4e)-alkynes,^[6] show a much narrower and hence less useful range (δ = 240–337) for two- to four-electron donor phosphaalkynes. Thus, the ¹³C{¹H} NMR spectra of **1**, **2**, and **3** recorded in C₆D₆ at room temperature showed signals at δ = 337.2 (d, ¹J_{PC} = 113.3 Hz, P≡C), δ = 335.6 (d, ¹J_{PC} = 110.5 Hz, P≡C), and δ = 326.5 (d, ¹J_{PC} = 108.5 Hz, P≡C), respectively, which are similar to the signal of [Mo(η^5 -C₅H₅)₂{ η^2 (4e)-*t*BuC≡P}]{ η^2 -P(OMe)₂OBF₂OP(OMe)₂}] (δ = 328.5, d, ¹J_{PC} = 114 Hz, P≡C),^[16] but only to slightly lower field than those of [M(η^5 -C₅H₅)₂{ η^2 (2e)-*t*BuC≡P}(PMe₃)] (M = Ti: δ = 298.6, ¹J_{PC} = 83.4 Hz; M = Zr: δ = 310.6, ¹J_{PC} = 97.7 Hz)^[7] and [Pt(η^2 (2e)-*t*BuC≡P)(PPh₃)₂] (δ = 240.8).^[18]

The lack of reactivity of the complexes [MCl₂{ η^2 (4e)-alkyne}(η^5 -C₅Me₅)] (M = Nb, Ta) towards alkynes has been rationalized in terms of the large (2 eV) HOMO–LUMO gap.^[10] A similar EHMO calculation on **1** also showed a HOMO–LUMO gap of 2 eV, which implies that this complex would also be unreactive towards alkynes. Interestingly **1** is reactive towards an excess of *t*BuC≡P.

A solution of **1** in C₆D₆ was treated with *t*BuC≡P (ca. 1.6 molequiv), and the subsequent reaction was monitored by ³¹P{¹H} NMR spectroscopy. Over a period of three weeks the signal at δ = 509.0 for **1** decreased in intensity and was replaced by a new singlet at δ = 31.8, with no evidence for any phosphorus-containing intermediates. The color of the solution changed from purple to red. Removal of the C₆D₆ in vacuo and extraction with hexane gave a deep red solution of complex **4** [Eq. (1)]. Concentration of the solution and cooling to –20 °C afforded crystals of **4**, but due to the high solubility of **4** in hexane, the amount isolated was

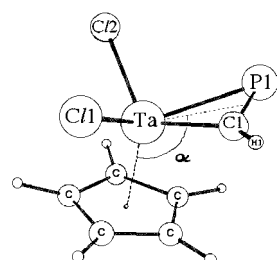
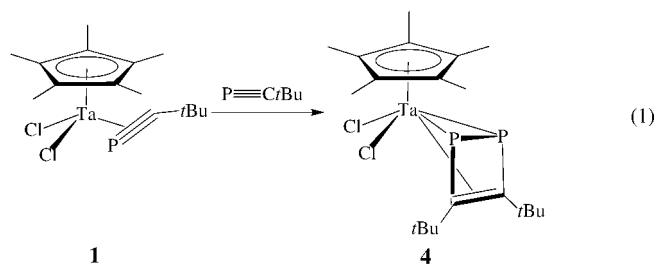


Figure 2. Calculated lowest energy geometry for complex **A**. Selected bond lengths [Å] and angles [°]: P1–Cl1 1.672, P1–Ta 2.486, Cl1–Ta 2.086, Ta–Cl1 2.339, Ta–Cl2 2.337, Cl1–H1 1.099; α = 114.8, P1–Cl1–H1 135.73.



relatively low, even though the conversion of **1** to **4** was essentially quantitative. Complex **4** was characterized by elemental analysis, NMR spectroscopy, and single-crystal X-ray crystallography as the 1,2-diphosphacyclobutadiene complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)(\sigma,\sigma,\pi\text{-1,2-P}_2\text{C}_2\text{tBu}_2)]$.^[19] This is especially interesting since there is only one previously reported example of a 1,2-diphosphacyclobutadiene complex: $[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-1,2-P}_2\text{C}_2\text{tBu}_2)]$ was formed (30% yield) along with $[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-1,3-P}_2\text{C}_2\text{tBu}_2)]$ (30% yield) in an unselective

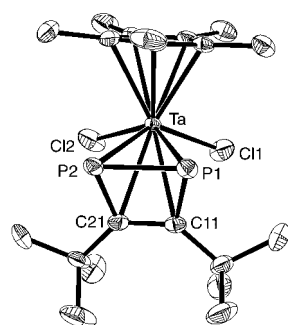


Figure 3. ORTEP representation of complex **4**. Thermal ellipsoids shown at the 30% probability level. For selected bond lengths and angles, see Table 1.

reaction (1/1) between $\text{tBuC}\equiv\text{P}$ and $[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-C}_8\text{H}_8)]$.^[20] In all other studies on the formation of diphosphacyclobutadiene ligands, only the 1,3-isomer has been observed, even though calculations on the free ligand have predicted that the 1,2-isomer is thermodynamically more stable.^[21, 22]

The single-crystal X-ray structure of **4** (from hexane, -20°C) is shown in Figure 3, and selected bond parameters, along with those calculated (ECP/DFT geometry optimi-

zation) for the model complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{H}_5)(\eta^4\text{-1,2-P}_2\text{C}_2\text{H}_2)]$ and reported for the titanium complex $[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-1,2-P}_2\text{C}_2\text{tBu}_2)]$ are listed in Table 1. Examination of these data shows that the bonding mode adopted by the 1,2- $\text{P}_2\text{C}_2\text{tBu}_2$ ligand in the tantalum complex **4** is different from

(1.827(6) Å) in the 1,2-dihydro-1,2-diiododiphosphete.^[23] The corresponding calculated bond lengths for the model complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{H}_5)(1,2\text{-P}_2\text{C}_2\text{H}_2)]$ show the same trend with an even longer P–P bond and shorter C–C bond. An obvious interpretation of these findings is that the 1,2- $\text{P}_2\text{C}_2\text{tBu}_2$ ligand in complex **4** adopts a σ,σ,π bonding mode, as illustrated in Equation (1).

As might be expected, the NMR parameters of the 1,2- $\text{P}_2\text{C}_2\text{tBu}_2$ ligand in **4** differ from those of the corresponding Ti complex. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **4** showed one singlet at $\delta = 31.8$ to high field of that of $[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-1,2-P}_2\text{C}_2\text{tBu}_2)]$ ($\delta = 133.6$), and the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **4** exhibited an apparent doublet of doublets at $\delta = 168.5$ ($J_{\text{PC}} = 23.9$ Hz, $^2J_{\text{PC}} = 18.4$ Hz) not dissimilar to that reported for the Ti complex at $\delta = 153.9$ (app t, $J_{\text{PC}} = 49.5$ Hz) and in the region typical for sp^2 carbon atoms. Again the data are consistent with a σ,σ,π bonding mode for complex **4**.

Neither the selective or the stepwise $[\mathbf{1} \rightarrow \mathbf{4}]$ formation of a 1,2-diphosphacyclobutadiene has been previously observed. In agreement with our previous DFT calculations, it is noteworthy that the recent calculations also show that the observed product has the lowest energy and is the most stable isomer. Therefore, the reaction might be considered to be thermodynamically controlled. However, so far only a tilted 1,3-diphosphabicyclo[1.1.0]butanediyl^[22] has been identified as a potential intermediate in the reaction. It is hoped that the progressive replacement of the chloro ligands in **1** followed by a study of the reaction of the resulting phosphaaalkyne complexes with further $\text{tBuC}\equiv\text{P}$ might enable us to tune the system and thus allow a deeper understanding of this interesting reaction.

In conclusion, single-crystal X-ray crystallography, NMR spectroscopy, and theoretical (EHMO, DFT) studies show that the phosphaaalkyne ligand in $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)\{\eta^2\text{-tBuC}\equiv\text{P}\}]$ (**1**) adopts a $\eta^2(4e)$ bonding mode. Surprisingly, **1** reacts with an excess of $\text{tBuC}\equiv\text{P}$ to form the complex $[\text{TaCl}_2(\eta^5\text{-C}_5\text{Me}_5)(1,2\text{-P}_2\text{C}_2\text{tBu}_2)]$; the X-ray data indicate a σ,σ,π bonding mode for the 1,2-diphosphacyclobutadiene ligand. This is only the second time that the formation of a 1,2-diphosphacyclobutadiene has been observed, but in this case there is no evidence for the formation of the 1,3-isomer.

Experimental Section

Full experimental details (both syntheses and calculations) are provided in the Supporting Information.

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Table 1. Observed and DFT-calculated (in square brackets) bond parameters (bond length [Å], angle $^\circ$) for the 1,2-diphosphacyclobutadiene ligand.

Bond or angle	Complex 4	$[\text{TaCl}_2(\eta^5\text{-C}_5\text{H}_5)(\eta^4\text{-1,2-P}_2\text{C}_2\text{H}_2)]$	$[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-1,2-P}_2\text{C}_2\text{tBu}_2)]^{[11]}$
P1–P2	2.204(2)	[2.250]	2.175(1)
P1–C11	1.820(5)	[1.808]	1.815(5)
P2–C21	1.840(5)	[1.809]	1.806(2)
C11–C21	1.410(6)	[1.385]	1.429(3)
M–P1	2.4992(13)	[2.554]	2.487(1)
M–P2	2.5116(13)	[2.552]	2.494(1)
M–C11	2.684(5)	[2.456]	2.392(2)
M–C21	2.655(5)	[2.454]	2.381(2)
C21–C11–P1	103.0(3)	[103.8]	102.0(1)
C11–P1–P2	77.7(2)	[76.2]	77.8(1)
P2–C21–C11	102.1(3)	[103.9]	101.8(1)
P1–P2–C21	77.3(2)	[76.2]	78.4(1)

that reported for $[\text{Ti}(\eta^8\text{-C}_8\text{H}_8)(\eta^4\text{-1,2-P}_2\text{C}_2\text{tBu}_2)]$, which was described as a η^4 -bonded delocalized π system. Thus, the P1–P2 distance of 2.204(2) Å in **4** is essentially the same as the P–P single-bond length in 3,4-di-*tert*-butyl-1,2-dihydro-1,2-diiododiphosphete^[23] (2.192(4) Å), and perceptibly longer than that in the Ti complex (2.175(1) Å). Although the C11–C21 bond length (1.410(6) Å) in **4** is apparently shorter than that in the Ti complex (1.429(3) Å), the difference is not statistically significant, and the P–C bond lengths (1.820(5) Å, 1.840(5) Å) in **4** are close to the P–C single-bond length

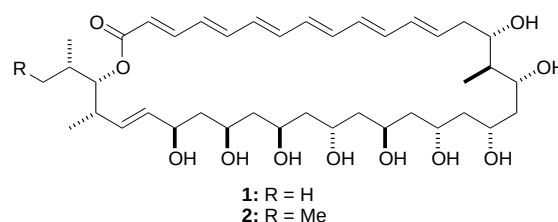
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Total Synthesis of Dermostatin A**

Christopher J. Sinz and Scott D. Rychnovsky*

By virtue of their biological activity and structural complexity, the polyene macrolides have attracted a great deal of interest from the synthetic community.^[1] We have been engaged in the development of broadly applicable methodology for the stereochemical elucidation and the total synthesis of highly oxygenated natural products. Pursuant to these goals, a recent report from our group described a new approach to the rapid stereochemical assignment of polyol-containing natural products in which 2D- ^{13}C acetamide analysis allowed for the stereochemical elucidation of dermostatin A (**1**) and B (**2**).^[2] Herein, we disclose studies which have culminated in the first total synthesis of dermostatin A (**1**).



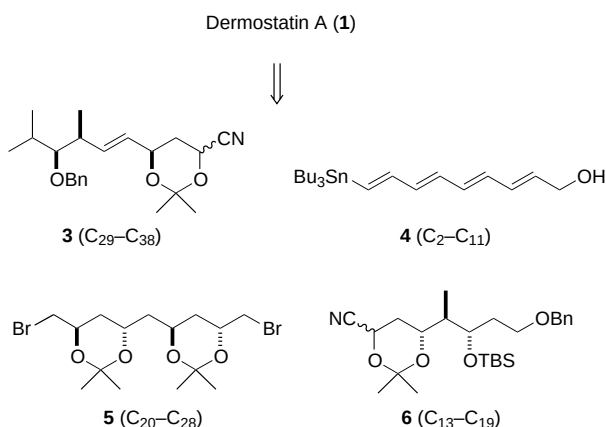
Dermostatin A (**1**) and B (**2**) are 36-membered macrolides that were isolated from the mycelium of *Streptomyces viridigriseus* Thirum.^[3] Their flat structures were determined by Rinehart and Pandey.^[4] The dermostatins show potent antifungal activity (comparable to amphotericin B) against a large number of human pathogens,^[5] and have been used clinically as a treatment for deep vein mycoses.^[6] Additionally, in an evaluation of a variety of polyene macrolides as potential HIV treatments, dermostatin A (**1**) and B (**2**) showed the highest antiproliferative activity against HIV in H9 cells.^[7] Although the dermostatins have demonstrated a broad range of biological activities, details of their mode of action remain unknown.

We set out to develop a highly convergent synthetic approach that would be sufficiently flexible to allow the facile generation of analogues for studies of the mode of action (Scheme 1). The central synthetic challenges posed by dermostatin A (**1**) are the complex polyol region and the conjugated hexaene. The acid- and light-sensitivity of the polyene necessitates delaying its introduction until a late stage. We intended to employ a palladium-mediated cross-coupling with vinyl stannane **4** as the penultimate carbon–carbon bond construction. Previous studies from our group have demonstrated the utility of cyanohydrin acetamide

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.



Scheme 1. Retrosynthesis of dermostatin A (1).

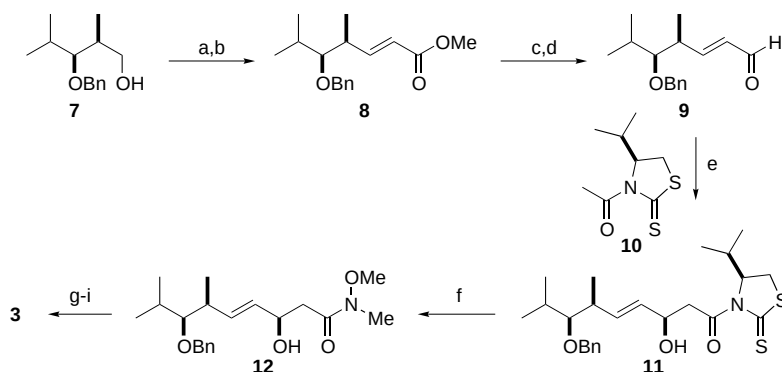
alkylations in the construction of complex 1,3-polyols.^[8] In the context of dermostatin A, the sequential connection of cyanohydrin acetonide fragments **3** and **6** with the C₂-symmetric dibromide **5** would provide the C₁₃–C₃₈ polyol region. Based on precedent from previous synthetic efforts, we anticipated that an intramolecular Horner–Wadsworth–Emmons macrolactonization would generate the macrolide under sufficiently mild conditions.^[9]

The synthesis of cyanohydrin acetonide **3** commenced with the oxidation and Wittig homologation of alcohol **7**,^[10] to provide enoate **8** (82%) (Scheme 2). Reduction with DIBAL-H (88%) and oxidation with *o*-iodoxybenzoic acid^[11] (99%) furnished enal **9**. Nagao's acetate aldol method^[12] has recently seen a resurgence in natural product synthesis.^[13] For our purposes, the addition of the tin enolate derived from *N*-acetyl thiazolidine-thione **10** to aldehyde **9** proceeded in excellent yield with 28:1 diastereoselectivity. The resulting compound **11** was converted into Weinreb amide **12** under mild conditions (90%). A three-step sequence (with no purification of intermediates required) provided cyanohydrin acetonide **3** in 80% yield.

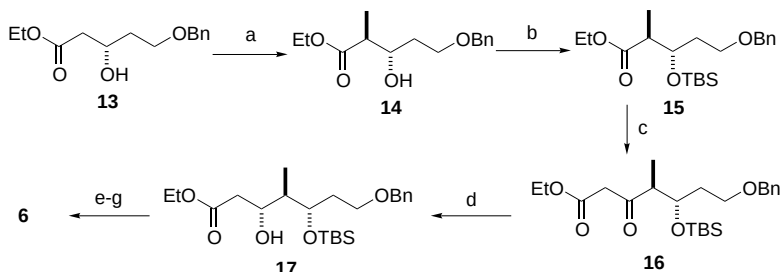
The Noyori hydrogenation product **13**^[10, 14] provided a convenient starting material for the synthesis of cyanohydrin acetonide **6** (Scheme 3). The required C₁₄–C₁₅ *anti* stereoarray was secured by a Frater–Seebach alkylation,^[15] to provide **14** in 78% yield with 14:1 diastereoselectivity. Silylation (TBSOTf, 2,6-lutidine) of **14** gave **15** in 94% yield. β -Keto ester **16** was procured by DIBAL-H reduction and Roskamp homologation^[16] of the intermediate aldehyde. Reduction with NaBH₄ in MeOH at –50 °C provided β -hydroxy ester **17** in 84% yield with 6.8:1 diastereoselectivity. Generation of the cyanohydrin acetonide proceeded without incident, providing **6** in 75% yield.

We recognized that carrying the C₂₀ and C₂₈ bromide substituents from the start of the syn-

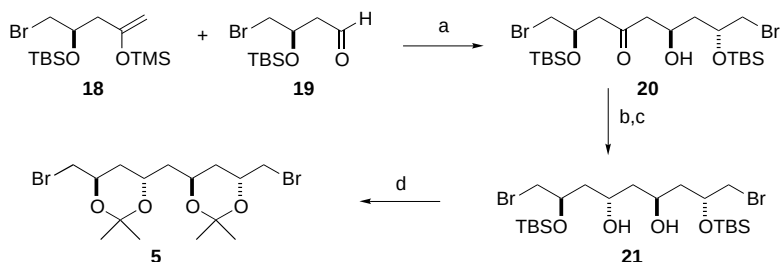
thetic sequence would constitute the most efficient approach to compound **5** (Scheme 4). To this end, a Mukaiyama aldol coupling of enol silane **18** and aldehyde **19**^[17] proceeded in 74% yield, with a modest preference^[18] for the desired 1,3-*anti* adduct **20** (3.3:1).^[19] We investigated a variety of protecting groups (e.g. the use of β -OBn- or β -OPMB-protected aldehydes), the use of cationic Lewis acids (Me₂AlCl) for chelation control,^[20] and reagent control with chiral Lewis acids.^[21] These modifications were not significantly more diastereoselective, and were much less practical than the original coupling. The conversion of **20** into *anti* 1,3-diol **21** was best accomplished by Evans–Tischenko reduction^[22]



Scheme 2. Reagents and conditions: a) DMSO, (COCl)₂, Et₃N, CH₂Cl₂; b) methyl(tri-phenylphosphoranylidene) acetate, CH₃CN, 90 °C, 82% over two steps; c) DIBAL-H, 88%; d) IBX, DMSO, 99%; e) **10**, Sn(OTf)₂, *N*-ethylpiperidine, CH₂Cl₂, –50 °C, 88%, 28:1 dr; f) MeNHOMe · HCl, imidazole, 90%; g) TMSCl, Et₃N; h) DIBAL-H; i) TMSCN, KCN/[18]crown-6; acetone, 2,2-dimethoxypropane, cat. $\pm$ -CSA, 80% over three steps. DMSO = dimethyl sulfoxide, DIBAL-H = diisobutylaluminum hydride, IBX = *o*-iodoxybenzoic acid, CSA = camphorsulfonic acid.



Scheme 3. Reagents and conditions: a) LDA, MeI, DMPU, THF, 78%, 14:1 dr; b) TBSOTf, 2,6-lutidine, 94%; c) DIBAL-H; ethyl diazoacetate, SnCl₂, 74%; d) NaBH₄, MeOH, –50 °C, 84%, 6.8:1 dr; e) TMSCl, Et₃N; f) DIBAL-H; g) TMSCN, KCN/[18]crown-6; acetone, 2,2-dimethoxypropane, cat. $\pm$ -CSA, 75% over three steps. LDA = lithium diisopropylamide, DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone, TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate.



Scheme 4. Reagents and conditions: a) BF₃ · OEt₂, CH₂Cl₂, –78 °C, 74%, 3.3:1 dr; b) cat. SmI₂, *i*PrCHO, THF, –20 °C; c) DIBAL-H, 75% over two steps, >95% de; d) Dowex-H⁺, MeOH, 75 °C; acetone, 2,2-dimethoxypropane, cat. $\pm$ -CSA, 50%.

followed by the reductive cleavage of the resultant ester (75 %, >95 % de). Desilylation under acidic conditions followed by protection of the crude tetraol gave bis-acetonide **5** in 50 % yield.

With the required fragments in hand, our attention turned to the coupling procedure (Scheme 5). Addition of LDA to a mixture of **5** (2.2 equivalents) and **6** in the presence of DMPU (5 equivalents) gave the monoalkylated adduct **22** in 65 % yield. Unreacted **5** could be readily recovered. Alkylation with the C₂₉–C₃₈ fragment **3** under the same conditions provided the protected polyol **23** in 70 % yield. Submission of **23** to a large excess of LiDBB (30 equivalents) at –78 °C effected reductive decyanation at C₁₃ and C₂₉ with concomitant deprotection of the C₁₃ and C₃₅ benzyl ethers, providing **24** in 75 % yield as a single diastereomer.^[23] Chemoselective oxidation with TEMPO under the conditions of Einhorn et al. gave aldehyde **25**, with no detectable oxidation of the C₃₅ secondary hydroxy group.^[24] Takai iodo-olefination of **25** gave vinyl iodide **26** in 88 % yield, with 11:1 *E/Z* selectivity.^[25] At

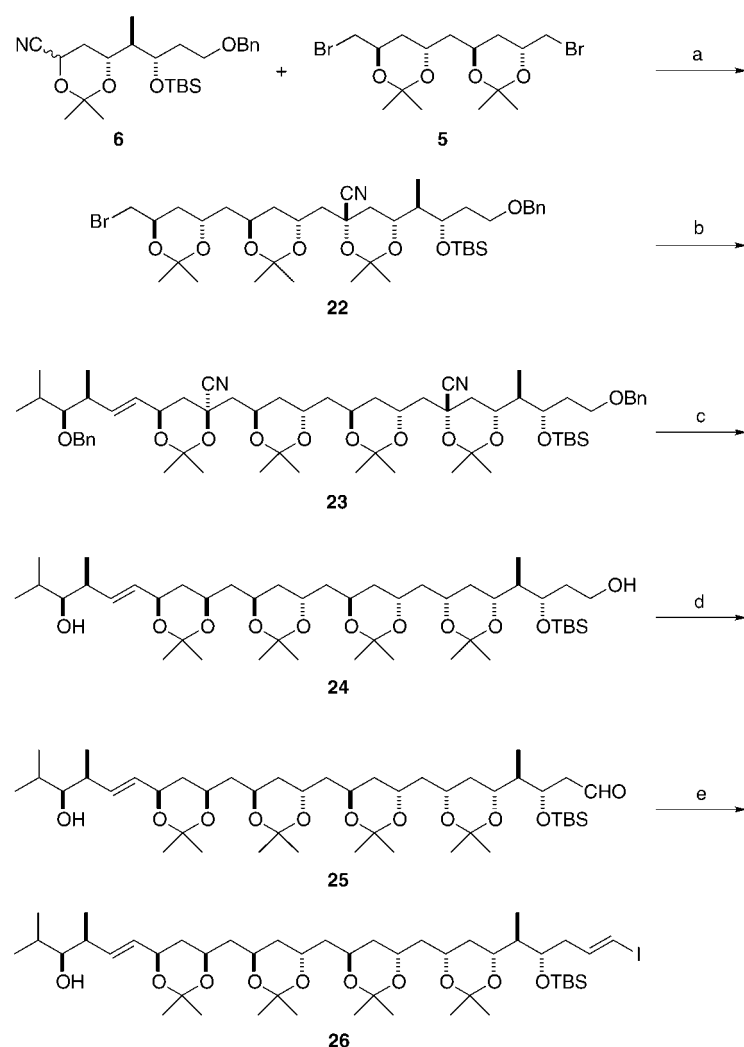
this point, the stage was set for installation of the polyene and subsequent macrocyclization.

In anticipation of the Horner–Wadsworth–Emmons macrocyclization, BOP/DMAP-promoted phosphonoesterification of **26** at C₃₅ gave **27** in quantitative yield (Scheme 6). The choice of a Stille coupling for the convergent introduction of the polyene^[26] was predicated on the belief that this transformation could be accomplished with highly functionalized substrates containing polar functional groups.^[27] In the event, palladium-mediated coupling of **27** with vinyl stannane **4**^[28] gave polyene **28** in 77 % yield, with no evidence of dimerization. Oxidation with the Dess–Martin periodinane to aldehyde **29** and submission to Masamune–Roush conditions^[9] provided macrolide **30** in 50 % yield over two steps. Deprotection proved to be surprisingly facile. Treatment of **30** with Dowex acidic resin in MeOH at ambient temperature effected the removal of the acetonide protecting groups and the partial deprotection of the C₁₅–TBS ether which could be driven to completion by gentle heating. Purification by HPLC

(high-pressure liquid chromatography) provided **1**, which was indistinguishable from natural dermostatin A by a variety of analytical methods (¹H NMR spectroscopic analysis in two solvents, high-resolution mass spectrometry, circular dichroism, HPLC).

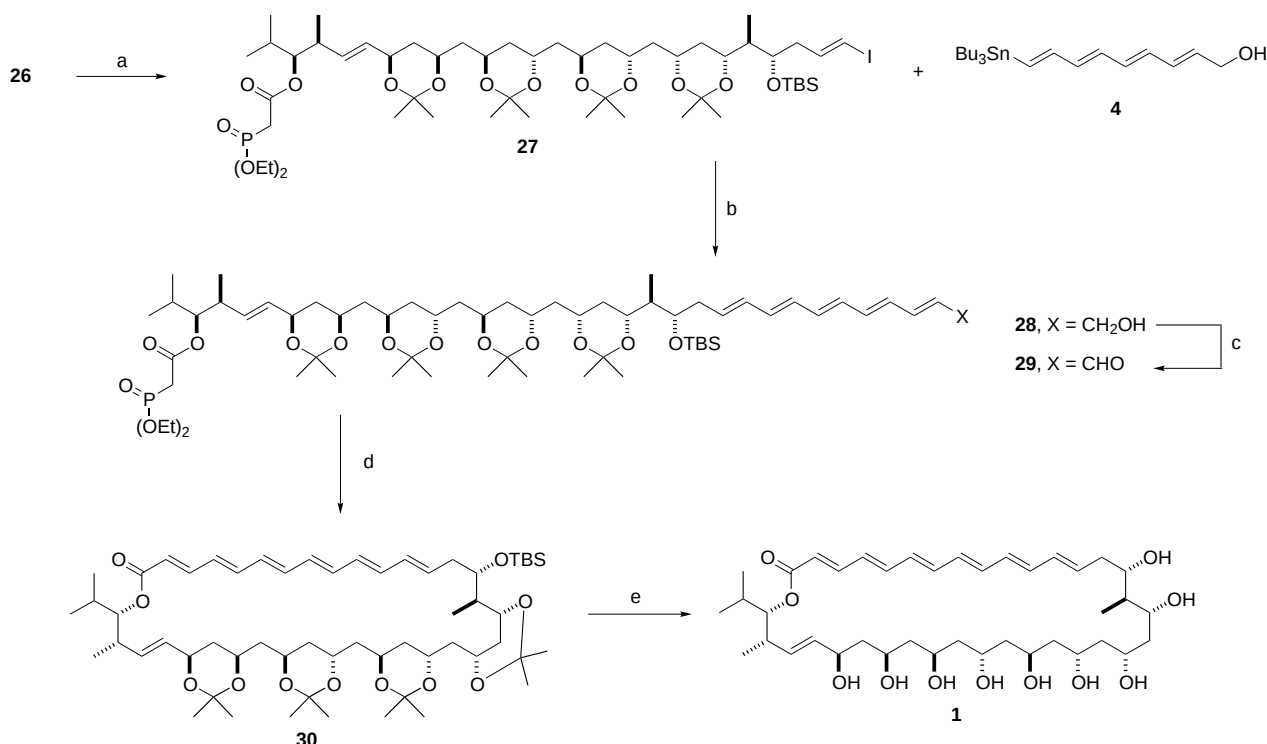
An efficient, convergent total synthesis of dermostatin A (**1**) has been achieved, thus confirming the reported stereostructure. The 1,3-polyol region was rapidly assembled by using the cyanohydrin acetonide method. The Stille cross coupling has been applied for the first time to the total synthesis of an oxo-polyene macrolide. The strategy and tactics described above should prove useful for the synthesis of analogues of dermostatin A as well as other polyene macrolides.

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Scheme 5. Reagents and conditions: a) LDA, DMPU, THF, –50 °C, 65 %; b) **3**, LDA, DMPU, THF, –50 °C, 70 %; c) LiDBB (30 equiv), THF, –78 °C, 75 %; d) TEMPO, Bu₄NCl, *N*-chlorosuccinimide, CH₂Cl₂, pH 8.6 buffer, 25 °C, 88 %; e) CrCl₂, CHI₃, dioxane/THF, 25 °C, 88 %, 11:1 *E/Z*. LiDBB = lithium di-*tert*-butylbiphenylide, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical.

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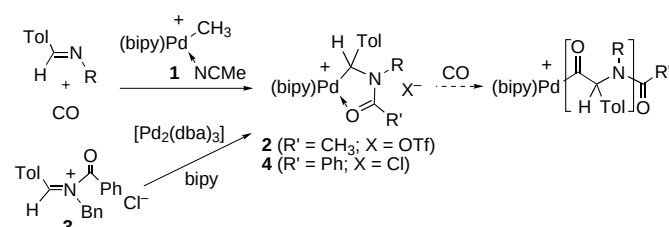
Scheme 6. Reagents and conditions: a) (diethylphosphono)acetic acid, BOP, DMAP, CH_2Cl_2 , 99 %; b) **4** (4 equiv), $\text{Pd}_2(\text{dba})_3 \cdot \text{CHCl}_3$, $i\text{Pr}_2\text{NEt}$, AsPh_3 , THF, 77 %; c) Dess – Martin periodinane, NaHCO_3 , CH_2Cl_2 ; d) LiCl , DBU, CH_3CN (**29**) = 0.002 M, 50 % over two steps; e) Dowex- H^+ , MeOH, 25 °C to 50 °C, 77 %. BOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate, dba = (*E,E*)-dibenzylideneacetone, DMAP = 4-dimethylaminopyridine.

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The Use of Carbon Monoxide and Imines as Peptide Derivative Synthons: A Facile Palladium-Catalyzed Synthesis of α -Amino Acid Derived Imidazolines**

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Of the many basic building blocks used in pharmaceutical development, the peptide unit is perhaps the most ubiquitous. In addition to linear polypeptides, this unit is found in a range of biologically relevant heterocyclic compounds.^[1] Traditional routes to prepare peptide-containing compounds have focused on the use of presynthesized or isolated α -amino acid derivatives as synthons.^[2] However, examination of the peptide structure suggests that it might also be conceived to arise from the coupling of an imine and carbon monoxide, and its synthesis approached by metal-mediated alternating insertion (Scheme 1).^[3] Considering the ready availability and



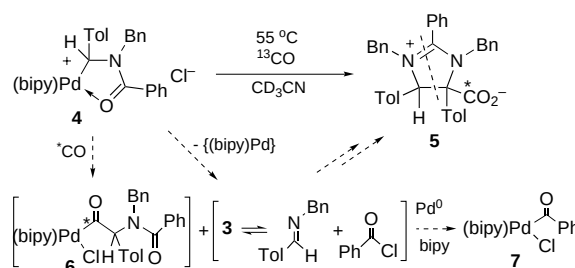
Scheme 1. Palladium-mediated synthesis of peptides. dba = dibenzylidene acetone.

flexibility of imines as substrates, CO and imine coupling would provide an attractive general route to both natural and nonnatural amino acid containing products. We have recently reported that the sequential insertion of CO and an imine can be achieved with $[(bipy)Pd(CH_3)(NCCH_3)]^+OTf^-$ (**1**) (*bipy* = 2,2'-bipyridine), which demonstrates the feasibility of this process.^[3–5] Here we report the development of a novel catalytic method to construct α -amino acid containing heterocycles, by using an imine, CO, and an acid chloride as the sole building blocks.

The addition of $Tol(H)C=NR$ ($Tol = p\text{-}C_6H_4CH_3$) and 1 atm CO to complex **1** has been found to lead to the formation of the Pd-chelated amide complex **2**.^[3] In order to generate an amino acid derivative from **2**, the further insertion of CO into the Pd–C bond is necessary. However, the attempted reaction of **2** with CO has been unsuccessful, even at elevated temperature and pressure. A plausible rationale for this behavior is that strong chelation of the amide ligand in **2** effectively blocks the coordination site required for CO

insertion.^[3] To address this issue, we examined the use of the more coordinating halide counteranion, which might weaken amide chelation through coordination, or stabilize the product of CO insertion.^[6]

Anion exchange of **2** with NaCl allows the in situ incorporation of the chloride anion into the metallacycle; however, a more facile route to this class of compounds is described in Scheme 1. This involves the oxidative addition of acyliminium salt **3** to $[Pd_2(dba)_3] \cdot CHCl_3$ in the presence of 2,2'-bipyridine to form **4** in 92% yield.^[7,8] In contrast to the behavior of **2**, the reaction of **4** with 1 atm CO in CD_3CN leads to the slow disappearance of starting materials over the course of 5 days at 55 °C. Surprisingly, however, product isolation yields the carboxylate-substituted imidazoline **5** in 35% yield (Scheme 2).



Scheme 2. Formation of the carboxylate-substituted imidazoline **5**.

Carboxylate-substituted imidazolines such as **5** are biologically relevant heterocycles, formally incorporating an α -amino acid residue into the heterocyclic core.^[10–12] Thus, the generation of **5** from palladium complex **4** suggests that the imine and CO have been coupled into a peptide unit, followed by subsequent reactions. To determine the origin of **5**, and to optimize its synthesis, the mechanism of the transformation was examined. Performing the reaction of **4** with ^{13}C leads to the incorporation of ^{13}C label in the carboxylate group ($\delta = 166.4$). This presumably arises from insertion of ^{13}CO into **4** to form the palladium-bound amino acid derivative **6** (Scheme 2). Examination of the structure of **5** suggests that it is formed by the coupling of the amino acid ligand in **6** with the imine. However, no imine was added to the reaction. The source of additional imine was determined by monitoring the reaction by ^1H NMR, which revealed the concurrent formation of $[(bipy)Pd(COPh)Cl]$ (**7**) (40% yield) along with imidazoline **5**. In the light of the synthesis of **4** by the oxidative addition of acyliminium salt **3**, a plausible explanation for the formation of **7** involves the reductive fragmentation of **4** to regenerate **3** and Pd^0 .^[9] The ^1H NMR of **3** in CD_3CN at 55 °C reveals that it is in rapid equilibrium with $PhCOCl$ and $Tol(H)C=NBn$. This would both provide a source of free imine, and allow for the formation of **7** by oxidative addition of $PhCOCl$ to Pd^0 .

The mechanism described in Scheme 2 shows that palladium complex **4** is in rapid equilibrium with imine and acid chloride under the reaction conditions. This suggests that the synthesis of the imidazoline core might be more easily achieved by starting directly from imine, CO, and acid chloride. Indeed, the one-pot reaction of $Tol(H)C=NBn$,

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PhCOCl, and 1 atm CO with $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$ and 2,2'-bipyridine results in the direct coupling of these fragments into imidazoline **5** in 70 % yield of isolated product.^[7] This reaction occurs with remarkable selectivity, and the four separate units are cleanly combined to form **5** as the only observable reaction product.

One limitation of this imine/acid chloride/CO coupling is that it is stoichiometric in palladium. However, monitoring this reaction by ^1H NMR reveals the formation of HCl as well as the imidazoline product. Thus, all fragments of the reagents added to Pd^0 (imine, acid chloride, CO) are liberated after the coupling, which suggests that Pd^0 is regenerated and may be able to mediate further coupling cycles. This does turn out to be the case. The reaction of $\text{ToI}(\text{H})\text{C}=\text{NBn}$, PhCOCl, and 1 atm CO with 5 mol % $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$ and 2,2'-bipyridine leads to the clean coupling of these fragments into a single imidazoline product (Table 1). This imidazoline formation proceeds in high yield with a number of imines of

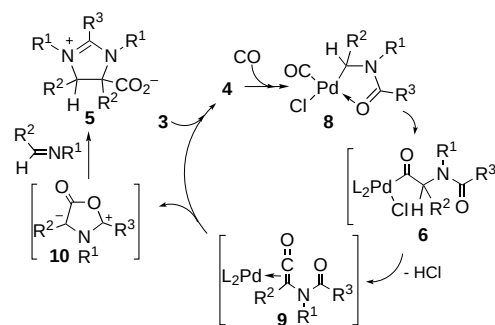
Table 1. Palladium-catalyzed synthesis of imidazolines.^[a]

$\text{R}^2\text{C}=\text{NR}^1 + \text{R}^3\text{C}(=\text{O})\text{Cl} + \text{CO} \xrightarrow[\text{CH}_3\text{CN}]{55^\circ\text{C}, [\text{Pd}_2(\text{dba})_3] (5 \text{ mol}\%), \text{ligand} (10 \text{ mol}\%)} \text{R}^1\text{N}^+\text{C}(\text{R}^2)\text{C}(\text{R}^3)\text{N}^-\text{R}^1$					
Entry	Ligand	R ¹	R ²	R ³	Yield [%] ^[b]
1	bipy	PhCH ₂	<i>p</i> -CH ₃ C ₆ H ₄	Ph	82
2	bipy	CH ₃	<i>p</i> -CH ₃ C ₆ H ₄	Ph	92
3	bipy	PhCH ₂	<i>p</i> -CH ₃ SC ₆ H ₄	Ph	73
4 ^[c]	bipy	PhCH ₂	<i>p</i> -ClC ₆ H ₄	Ph	62
5	bipy	CH ₂ CH ₂ OCH ₃	<i>p</i> -CH ₃ C ₆ H ₄	Ph	78
6	bipy	PhCH ₂	<i>p</i> -CH ₃ C ₆ H ₄	CH ₃	70
7	bipy	Ph	<i>p</i> -CH ₃ C ₆ H ₄	Ph	—
8	bipy	PhCH ₂	<i>p</i> -NO ₂ C ₆ H ₄	CH ₃	—
9	pyridine	PhCH ₂	<i>p</i> -CH ₃ C ₆ H ₄	Ph	87
10	diphos	PhCH ₂	<i>p</i> -CH ₃ C ₆ H ₄	Ph	—
11 ^[d]	—	PhCH ₂	<i>p</i> -CH ₃ C ₆ H ₄	Ph	83

[a] 0.57 mmol imine, 0.57 mmol acid chloride, 1 atm CO with 5 mol % $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$ and 10 mol % ligand for 4 days at 55 °C. [b] Yields of

aromatic aldehydes. Notably, the incorporation of functionality into the imine substrate (entries 3–5) does not retard the reaction. In addition, both aryl and alkyl acid chlorides can be employed (entry 6), as well as bidentate and monodentate nitrogen ligands (entry 9). However, phosphane ligands completely block the formation of imidazolines under these conditions (entry 10), as does the use of less nucleophilic imines (entries 7 and 8). The present methodology represents what is to our knowledge the first catalytic generation of a peptide-containing heterocycle (**5**) from an imine and CO. Considering that these heterocycles are typically prepared by the cyclization of presynthesized 1,2-diamino acids,^[13, 14] this four-component coupling provides an exceedingly simple and general method to both construct the α -amino acid unit, and incorporate it directly into the imidazoline core.

While the complete mechanistic details of this process are still under investigation, the above findings are consistent with the process shown in Scheme 3. The insertion of CO into the Pd–C bond of **4** would generate **6**. The role of chloride in



Scheme 3. Proposed mechanism for the formation of **5** involving Münchnone **10**.

allowing CO insertion with **4**, while the triflate salt (**2**) is inert, is postulated to arise from labilization of L_2 rather than dechelation of the amide ligand. Evidence for this is found by monitoring the catalytic reaction by ^1H NMR, which reveals the presence of the intermediate **8** in a 1:3 ratio to complex **4**.^[15] The generation of **8** implies that the added ligand L_2 may be slowing the catalytic process by hindering the complete formation of this intermediate. Consistent with this hypothesis, the strongly coordinating diphos ligand blocks imidazoline formation. More importantly, the reaction in the absence of any ligand results in greatly accelerated catalysis, with the reaction complete within 24 h (entry 11). Thus, the optimized catalysis conditions by using imine, acid chloride, and CO with $[\text{Pd}_2(\text{dba})_3] \cdot \text{CHCl}_3$ and no ligand provides a rapid and high yield route to synthesize imidazolines. Following CO insertion, the elimination of HCl would generate metallo–ketene complex **9**. Amido-substituted ketenes have been demonstrated to be in dynamic equilibrium with the mesoionic Münchnone structure **10**.^[16a] The latter can undergo a 1,3-dipolar cycloaddition with free imine, followed by C–O bond scission to generate imidazoline **5**.^[16, 17] This mechanism suggests, therefore, that the role of palladium in this process is to mediate the catalytic formation of an amino acid based Münchnone intermediate,^[18, 19] which is subsequently converted to the observed product with additional imine.^[20]

In conclusion, these studies have demonstrated that the palladium-catalyzed coupling of an imine and CO can be utilized for the construction of a peptide unit and its direct incorporation into heterocyclic substrates (i.e. imidazoline **5**). Considering the ready availability of these building blocks and the mild conditions employed (1 atm CO, 55 °C, palladium catalysis), this represents one of the most facile routes to prepare this class of compounds. The diverse reactivity of the putative Münchnone intermediates^[16, 19] generated in this process suggests this coupling may prove useful for the synthesis of range of amino acid derived and/or heterocyclic products. The further development of this chemistry towards these synthetic targets and the full elucidation of the reaction mechanism are currently the subject of research in our laboratories.

Experimental Section

All reactions were carried out under an N_2 atmosphere with a Vacuum Atmospheres 553-2 drybox or by standard Schlenk techniques.

Catalytic synthesis of **5**: Imine (0.57 mmol) and acid chloride (0.57 mmol) were combined in 10 mL of CH₃CN and stirred for 15 min. To this solution was added [Pd₂(dba)₃] · CHCl₃ (5 mol %) in 10 mL of CH₃CN. The reaction mixture was transferred to a 100 mL reaction bomb and left to stir at room temperature for 30 min. 790 Torr of CO was then added to the reaction mixture, and it was allowed to stir at 55 °C for 24 h. The resulting solution was filtered through celite, redissolved in CHCl₃, then washed with dilute HCl, saturated aqueous NaHCO₃, water, and saturated aqueous NaCl, followed by drying over Na₂SO₄. After filtration, the solvent was removed in vacuo, and the resultant material dissolved in diethyl ether and cooled to –40 °C. The imidazoline **5** was then collected as a white precipitate.

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Synthesis of a Trisaccharide Library by Using a Phenylsulfonate Traceless Linker on Synphase Crowns**

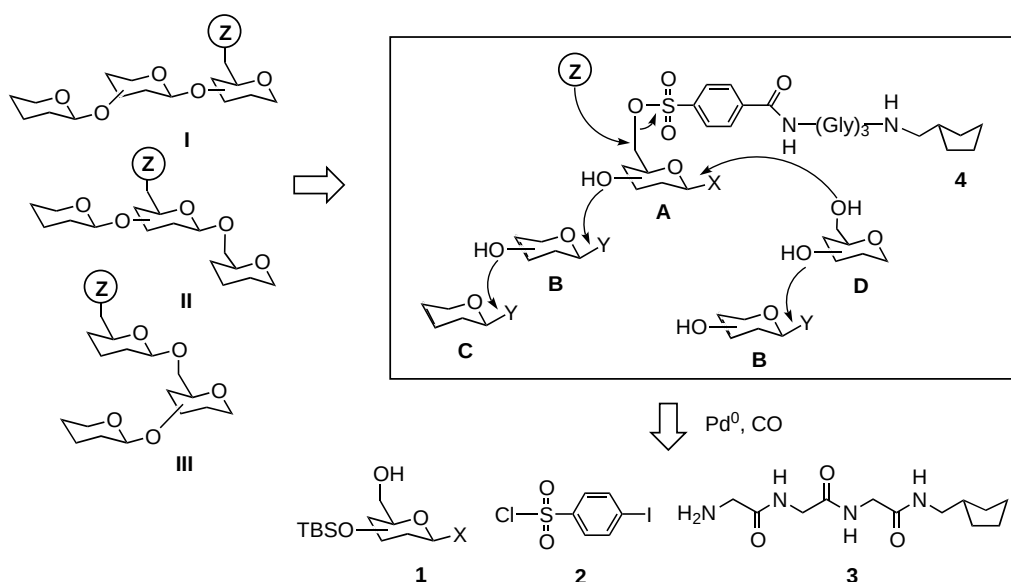
Takashi Takahashi,* Hitoshi Inoue, Yuichi Yamamura, and Takayuki Doi

The development of novel linkers and linkage strategies has become essential in solid-phase synthesis for the discovery of new drugs and materials. In recent years, many efficient linkers were developed.^[1] Traceless linkers are advantageous in that the original functional group of the linker does not remain in the product.^[2] We have reported a phenylsulfonate traceless linker,^[3] which acts as a leaving group under nucleophilic-displacement reaction conditions.^[4,5] With this linker a diversity of products can be obtained, because various functional groups can be introduced at the final stage in a solid-phase synthesis. Herein, we report a high-speed synthesis of a functionalized trisaccharide library utilizing the phenylsulfonate linker on Synphase Crowns.^[6,7]

The synthetic strategy is illustrated in Scheme 1. The trisaccharide derivatives **I**, **II**, and **III** which have various functional groups **Z** at the 6 position of their glucose unit could be synthesized from solid support **4**, which consists of a

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Scheme 1. Synthesis of an oligosaccharide library by using a phenylsulfonate traceless linker. TBS = *tert*-butyldimethylsilyl.

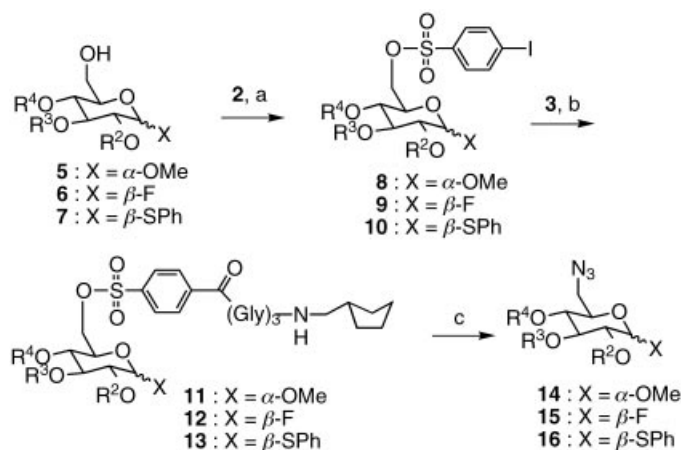
glucose unit **A** linked by a sulfonate linker at the 6 position. 1) Glycosylation of the supported glycosyl acceptor **4** (unit **A**) with glycosyl donor **B** followed by glycosylation of the **B** unit of the produced disaccharide with glycosyl donor **C** and displacement of the sulfonate linker of the **A** unit with a nucleophile **Z** lead to the trisaccharide **I**. 2) Glycosylation of the supported glycosyl donor **4** (unit **A**) with glycosyl acceptor **D** followed by glycosylation of either the **A** or the **D** unit with donor **B** (two-directional glycosylation)^[8] and displacement with **Z** lead to the trisaccharide **II** or **III**. Sulfonation of monosaccharide **1** at the 6 position with 4-iodobenzenesulfonyl chloride (**2**), which can be regarded as a precursor for an activated ester, was followed by Pd⁰-catalyzed carbonylative amidation with support **3**.^[9] This support, which can be easily handled, consists of aminomethyl crown residues that are distinguishable by the tagging stems clipped to them and a tripeptide spacer (Gly–Gly–Gly) to maintain a suitable distance between the reaction site and the solid support. In this strategy, it is feasible that the diversity of the oligosaccharide library could increase in a combinatorial fashion through variation of the position of the glycosylation and the number of nucleophiles. To synthesize each compound, in the pure form, in the library of trisaccharides, the crown residues were clipped with Trans-tems and were utilized in a split-and-mix method because the crown residues can be identified as and when necessary by “reading” the radiofrequency tags.^[6]

We optimized the reaction conditions of the Pd⁰-catalyzed carbonylative amidation with support **3**.^[10] Methyl glycosides **5**, glycosyl fluorides **6**, and thioglycosides **7** were converted into the precursors **8–10**, respectively, by

sulfonation with **2** (Scheme 2). The products were carbonylated and amidated with **3**,^[9] the yields were determined by HPLC with integration of the peak area of the corresponding 6-azido-6-deoxymonosaccharide. For methylglycoside **8a** coupling at 40 °C gave, after cleavage, **14a** in 46% yield (Scheme 2, entry 1).^[3, 11] When this carbonylation was carried out at room temperature or at 80 °C (entries 2 and 3, respectively), the yield decreased. The reactions with **8b** and glycosyl fluoride **9e**, both of which have a TBS group at the 3 position, were successful (entries 4 and 5, respectively). The attachment of thio-

glycoside **10e** onto **3** resulted in decomposition, whereas the attachment of triply benzoyl-substituted thioglycoside **10h** was successful. In the light of these results, we chose to use the glycosyl fluorides **9e–g** as solid-supported glycosyl donors.

A 44-member trisaccharide library was synthesized as follows: carbonylative amidation of methyl glycosides **8b–d** and glycosyl fluorides **9e–g** was carried out in parallel under the conditions given above to afford six different solid-supported monosaccharides, **11b–d** and **12e–g**. The three



Entry	Substrate	T [°C]	Product, yield [%]
1	8a	40	14a , 46
2	8a	RT	14a , 8
3	8a	80	14a , 35
4	8b	40	14b , 48
5	9e	40	15e , 38
6	10e	40	16e , 0
7	10h	40	16h , 35

	R ²	R ³	R ⁴
a	Bn	Bn	Bn
b	Bn	TBS	Bn
c	Bn	Bn	TBS
d	TBS	Bn	Bn
e	Bz	TBS	Bn
f	Bz	Bn	TBS
g	Bz	Bn	Bn
h	Bz	Bz	Bz

Scheme 2. Attachment of glycosides by Pd⁰-catalyzed carbonylative amidation. a) py, CH₂Cl₂; b) ArI (0.5 M), [Pd(PPh₃)₄] (0.01 M), NEt₃ (0.5 M), CO (10 atm), DMF, 40 °C, 24 h; c) NaN₃, DMF, 60 °C, 12 h; Bn = benzyl, Bz = benzoyl, py = pyridine.

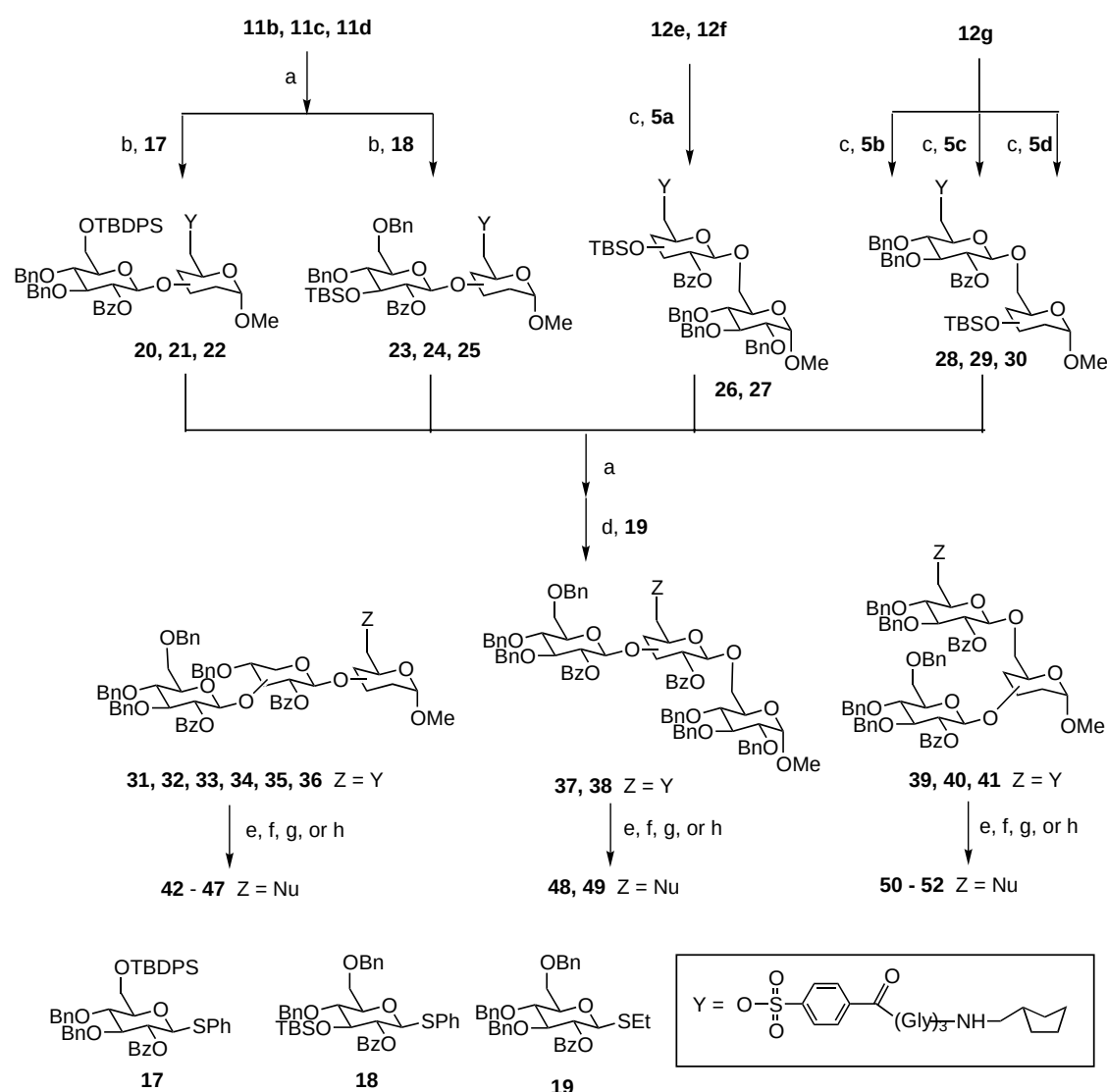
solid-supported methylglycosides **11b–d**, which differ in the position of the TBS group, were mixed, selectively deprotected and divided into two equal fractions (Scheme 3). Using DMTST^[12] as an activator, one fraction was treated with the glycosyl donor **17**, which has a TBDPS group at the 6 position, and the other was treated with the glycosyl donor **18**, which has a TBS group at the 3 position, to provide the six disaccharides **20–25**.^[13] Subsequently, the two solid-supported glycosyl fluorides **12e** and **12f** were placed in a single vial and treated with glycosyl acceptor **5a** and [Cp₂Zr(OTf)₂]^[14] to afford the two disaccharides **26** and **27** (Scheme 3). Next, the solid-supported glycosyl fluoride **12g** was divided into three portions, which were treated with glycosyl acceptor **5b**, **5c**, and **5d**, respectively, in parallel to provide the disaccharides **28–30** (Scheme 3).

The eleven prepared silyl-protected disaccharides **20–30** were combined. Deprotection of the silyl group and glyco-

sylation of all the solid-supported disaccharides with glycosyl donor **19** in a single vial^[15] afforded the solid-supported trisaccharides **31–41**^[16] (Scheme 3).

Finally, the crown compounds were sorted by means of their radiofrequency tags,^[6] and nucleophilic displacement—cleavage with sodium azide, sodium iodide, cesium acetate, and sodium borohydride—furnished the desired 44 functionalized trisaccharides **42–52**.^[3, 17] These reactions were performed in parallel to give each trisaccharide as a pure product, not as a mixture of trisaccharides (Table 1). The purities of the desired trisaccharides were determined by high-performance liquid chromatography (HPLC) and the structures of the respective major products in all the reactions were characterized by mass spectrometry and NMR spectroscopy after preparative HPLC.^[18, 19]

In summary, we have demonstrated a solid-phase synthesis of a functionalized trisaccharide library by using a phenyl-



Scheme 3. Synthesis of a 44-member trisaccharide library by glycosylation of a sulfonate-linked solid support followed by displacement with nucleophiles. a) 2 M HCl in MeOH/THF (1:1), RT; b) glycosyl donor **17** (TBDPS = *tert*-butyldiphenylsilyl) or **18** (0.2 M), DMTST (0.2 M), MS-4Å, CH₂Cl₂, RT, 24 h, repeated twice for improving the yield; c) glycosyl acceptor **5** (0.2 M), [Cp₂Zr(OTf)₂] (0.2 M), MS-4Å, CH₂Cl₂, RT, 24 h; d) glycosyl donor **19** (0.2 M), NIS (0.2 M), TfOH (0.05 M), MS-4Å, CH₂Cl₂, RT, 24 h;^[16] e) NaN₃ (0.1 M), DMF, 60 °C, 12 h; f) NaI (0.1 M), DMF, 60 °C, 12 h; g) CsOAc (0.1 M), [18]crown-6 (0.02 M), DMF, 60 °C, 12 h; h) NaBH₄ (0.1 M), DMSO, 60 °C, 12 h. DMTST = dimethyl(methylsulfanyl)sulfonium triflate, NIS = *N*-iodosuccinimide, Tf = trifluoromethanesulfonate.

Table 1. Nucleophilic displacement and cleavage to afford a 44-member trisaccharide library.^[a]

Components			Trisaccharides	Nu = N ₃	Nu = I	Nu = OAc	Nu = H
11b	17	19	Glcβ1 → 6Glcβ1 → 3(6-Nu)Glcα-OMe 42	55	52	49	50
11c	17	19	Glcβ1 → 6Glcβ1 → 4(6-Nu)Glcα-OMe 43	66	70	65	65
11d	17	19	Glcβ1 → 6Glcβ1 → 2(6-Nu)Glcα-OMe 44	51	48	48	46
11b	18	19	Glcβ1 → 3Glcβ1 → 3(6-Nu)Glcα-OMe 45	66	61	60	62
11c	18	19	Glcβ1 → 3Glcβ1 → 4(6-Nu)Glcα-OMe 46	54	52	49	46
11d	18	19	Glcβ1 → 3Glcβ1 → 2(6-Nu)Glcα-OMe 47	71	68	70	65
12e	5a	19	Glcβ1 → 3(6-Nu)Glcβ1 → 6Glcα-OMe 48	83	81	81	75
12f	5a	19	Glcβ1 → 4(6-Nu)Glcβ1 → 6Glcα-OMe 49	50	51	44	43
12g	5c	19	(6-Nu)Gluβ1 → 6Gluα(Gluβ1 → 4)-OMe 50	65	66	63	66
12g	5b	19	(6-Nu)Gluβ1 → 6Gluα(Gluβ1 → 3)-OMe 51	84	79	81	78
12g	5d	19	(6-Nu)Gluβ1 → 6Gluα(Gluβ1 → 2)-OMe 52	92	90	88	90

[a] Purities [%] of the trisaccharides **42**–**52** in the crude products. Glc = D-glucoside.

sulfonate traceless linker. In this study, an efficient strategy for diversification, Pd⁰-catalyzed carbonylative amidation to immobilize monosaccharides, glycosylation at various positions of the solid-supported glycosyl acceptors, and cleavage from the sulfonate linker with four nucleophiles has been developed.

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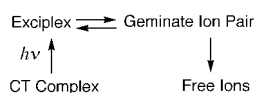
cossylation under the latter conditions completely consumed the disaccharide on the solid support.

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- [19] Removal of the protecting groups (benzoyl and benzyl) of two samples **45** (Nu = OAc) and **48** (Nu = N₃) (10 mg cleaved from the three crown compounds; NaOMe, THF/MeOH; Pd(OH)₂/C, MeOH/H₂O) afforded the corresponding free trisaccharides detected by ¹H NMR spectroscopy (400 MHz) and mass spectrometry (ESI-TOF) in quantitative yields. None of the problems mentioned by one of the referees (loss of the product or incomplete hydrogenolysis) was observed.

Direct Observation of a Distant Ion Pair Generated in a Photoconductive Poly(*N*-vinylcarbazole) Film with Dopant**

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Poly(*N*-vinylcarbazole) (PVCz) has received much attention as an organic photoconductor, since the PVCz film is one of the best functional materials for photodetection systems, electrophotographic imaging, etc.^[1] Over the past few decades, a considerable number of studies on the PVCz film have been made to clarify the photoconduction mechanism in the amorphous organic solid state.^[2] A simplified mechanism for the carrier generation prior to the carrier transport is proposed for the photoexcitation of charge-transfer (CT) complexes in acceptor-doped films in which the CT complexation takes place efficiently. The geminate ion pair is a key intermediate in the generation of the free ions (Scheme 1).



Scheme 1. Carrier generation process.

The interionic distance (r_0) of the ion pair was reported to be around 20–30 Å on the basis of the external electric field effects on the steady-state fluorescence from the exciplex^[3] and on the generation yield of the carriers (free ions).^[4] On the other hand, the dichroism relaxation measurements using picosecond optical spectroscopy gave a negative response to the presence of the hypothetical distant ion pair developed during a cooling process of the exciplex.^[5] In the present study, we detected a spin-correlated radical pair (SCRPs) and determined the distance between the transient pair radicals in 1,2,4,5-tetracyanobenzene (TCNB)-doped PVCz film^[6] by using time-resolved electron paramagnetic resonance (EPR) spectroscopy.^[7]

The spin-polarized EPR spectrum was clearly observed, as shown in Figure 1a, when the CT complex in the PVCz film was selectively excited by the laser-pulse irradiation at 532 nm. The spectral phase is enhanced absorption (A) in the low-field half and emission (E) in the high-field half. It is noteworthy that the signals are detected over a relatively wide region of more than 8 mT. The spectrum intensity decreased exponentially with the delay time after the laser flash, in

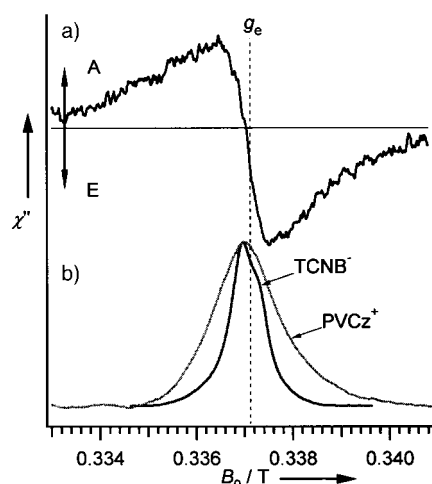


Figure 1. a) Time-resolved EPR spectrum of TCNB-doped PVCz film observed at 500 ns after the pulse excitation at the CT band at room temperature. b) Continuous wave (Cw)-EPR spectra obtained by numerical integration of the first-derivative signals of PVCz⁺ in concentrated H₂SO₄ and TCNB⁻ in MTHF at 8 K recorded using a lock-in detection.

keeping the same broad spectral shape with the A/E pattern during the decay. To assign the observed transient paramagnetic species, we measured separately the conventional steady-state EPR spectra for the TCNB anion^[8] and the PVCz cation^[9] in rigid glassy solvents (Figure 1b). Both free ions showed EPR signals at the magnetic field corresponding to $g = 2.0028$, but the spectral widths were quite different from each other, mainly because of the difference of the hyperfine interactions. The central field of the time-resolved EPR spectrum for the TCNB-doped PVCz film is also in agreement with $g = 2.0028$, which indicates that the transient species are associated with the TCNB⁻ and PVCz⁺ radicals. However, the spectral width of the transient signal in the film is much broader than that of the component free ions, the signals of which are broadened only by intramolecular interactions such as the anisotropic Zeeman and the hyperfine interactions. The broadness of the time-resolved EPR spectrum implies that the transient signal is attributed to the ion pair (TCNB⁻...PVCz⁺) rather than to the free ions, because the intermolecular magnetic interactions of the exchange and dipolar interactions between TCNB⁻ and PVCz⁺ can make the EPR spectrum broader.

The intensity of the time-resolved EPR signal increases with the incident power of the laser excitation. A plot of the EPR intensity versus the laser power is given in Figure 2. The linear laser-power dependence with a unit of slope indicates that the observed ion pair is created through a one-photon process.

The time-profiles of the EPR signal always showed an exponential decay with a time constant of about 1.0 μs, even when the laser power was varied. The exponential behavior, that is, first-order kinetics, supports the fact that the observed decay of the ion pair under zero external electric field represents recombination within a geminate pair rather than a free pair, because the free-pair recombination should obey second-order kinetics.^[10] Furthermore, the decay constant of the ion pair is comparable to the apparent time constant estimated from the exciplex fluorescence decay in the micro-

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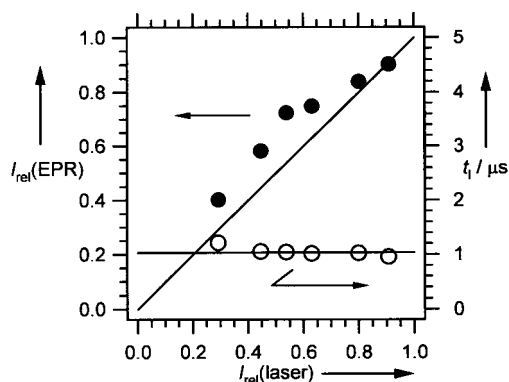


Figure 2. Laser power dependencies of the intensity and the decay constant of the transient EPR signal from the ion pair of PVCz⁺ and TCNB⁻; ● = EPR intensity ($I_{\text{rel}}(\text{EPR})$), ○ = lifetime (t_1).

second region. The agreement of the decay kinetic constants strongly suggests that the transient EPR signal arises from the geminate ion pair.

The irradiation with a linearly polarized light preferentially excites the CT complexes such that their transition moment in the CT band is along the polarized direction of light. Hence, the linearly polarized light enables an angle-selection experiment in the randomly oriented film sample to be performed. Since the EPR spectrum is broadened by the large anisotropy because of the spin dipolar interaction (see below), the shape of the EPR spectrum may be distorted by the angle-selection experiment,^[11] as long as the ion pair holds the initial orientation of the CT complex. However, excitation with the polarized light did not have any influences on the spectral shape. No effect of the angle-selection experiments could be interpreted in terms of the loss of memory of the initial orientation in the ion pair because of a charge migration. Therefore, it is suggested that the ion pair giving the spin-polarized EPR spectrum is a distant ion pair generated after the successive hole transfers from the exciplex, which occur in random directions and on the nanosecond time scale.^[5]

The multiplet spin polarization in the ion pair can be understood by the polarization mechanism of SCRP in which a coherent spin singlet–triplet interconversion takes place within the ion pair.^[12] The spin Hamiltonian for a pair consisting of radicals A and B ($\mathcal{H}_{\text{RP}}$) can be written in angular frequency units [Eq. (1)].

$$\mathcal{H}_{\text{RP}} = \omega_A S_{Az} + \omega_B S_{Bz} - J_{\text{SS}}(\mathbf{S}_A \mathbf{S}_B - 1/2) + D_{\text{SS}}(\cos^2 \theta - 1/3)(3S_{Az} S_{Bz} - \mathbf{S}_A \mathbf{S}_B) \quad (1)$$

ω_A and ω_B are determined by the Zeeman and hyperfine interactions within the individual radicals. J_{SS} and D_{SS} are the coupling constants for the exchange and dipolar interactions between the radicals, respectively. θ is the angle between the vector connecting the two radicals and the magnetic field. The coherent spin interconversion is induced by the difference between ω_A and ω_B . The SCRP born from the singlet exciplex under a magnetic field has the four sublevels of the electron spins shown in Figure 3a. The transitions from |2⟩ and |3⟩ to |1⟩ offer the absorptive EPR signals. The emissive signals arise from the transitions from |2⟩ and |3⟩ to |4⟩. The typical

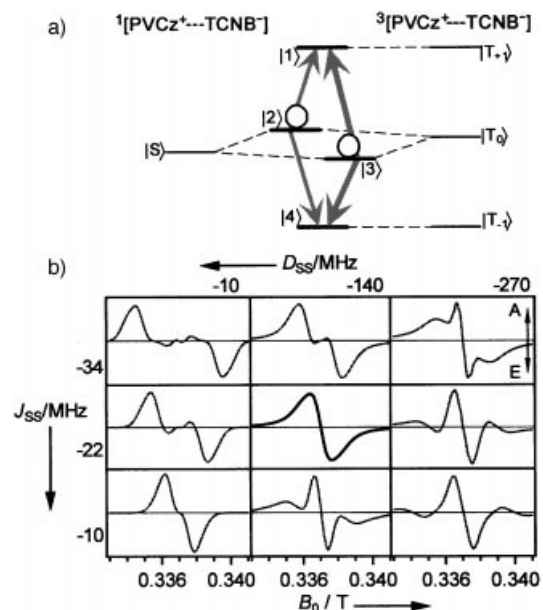


Figure 3. a) Energies of the four spin sublevels in a singlet-born SCRP model having a negative J_{SS} . Circles on the spin sublevels and arrows among the sublevels indicate the populations and the EPR transitions, respectively. b) Simulation spectra calculated by using various values as the intermolecular coupling constants of J_{SS} and D_{SS} on the SCRP model for the distant ion pair consisting of PVCz⁺ and TCNB⁻.

spectra calculated on the SCRP model^[13] are shown in Figure 3b. The spectral shape depends very much on both J_{SS} and D_{SS} . The simulation using the constants around $J_{\text{SS}} = -22 \pm 2$ MHz and $D_{\text{SS}} = -140 \pm 20$ MHz reproduced well the A/E-polarized broad spectrum observed for the distant ion pair between PVCz⁺ and TCNB⁻.

The magnitude of the dipolar interaction is proportional to r^{-3} , where r is the separation between the electron spins. The obtained D_{SS} value of the distant ion pair is reduced to $r_0 = 8.3 \pm 0.4$ Å within a point dipole approximation,^[14] which is much shorter than the values acquired in the static measurements of the electric field quenching.^[4] The analysis based on the Onsager model,^[15] in which the polymer matrix is regarded as an isotropic dielectric medium, has been adopted for the electric field quenching experiments. This model has offered understandable physical interpretation of the carrier generation. The assumption for the isotropic continuum, however, seems to be not sensitive on the local scale in the organic solid state. To ignore the molecular structure of the polymer may lead to an overestimate of r_0 . Taking into account the dichroism memory of up to several nanoseconds,^[5] the distant ion pair may be generated by the stepwise hole hopping rather than by an electron jump of around 8 Å during thermalization of a hot exciplex. The decay rate of the distant ion pair seems to be mainly determined by an endothermic activation step in the process back to the exciplex rather than by the hole hopping, since the EPR signal decayed without any change in the spectral shape.

In conclusion, the present study demonstrated that the distant geminate ion pair separated by ~ 8 Å with the lifetime of 1 μs exists at the carrier generation in the photoconductive TCNB-doped PVCz film. The presence of the distant ion pair

suggests stable trap site for the hole, the position of which is not far from the acceptor. The slow recombination of the geminate ion pair with the relatively short distance would be related to the trap-site structure or some sorts of reorganizations because of the charge shift in the PVCz film.

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C–S Bond Cleavage and C–C Coupling in Cyclopentadienylchromium Complexes To Give the First Dithiooxamide-Bridged and Doubly Dithiocarbamate-Bridged Double Cubanes: [Cp₆Cr₈S₈{(C(S)NEt₂)₂}] and [Cp₆Cr₈S₈(S₂CNEt₂)₂]**

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There have been extensive studies on the use of the dithiocarbamate ligand in a variety of bonding modes for stabilizing a wide range of oxidation states in coordination compounds of main-group and transition metals.^[1] Although generally unreactive in the majority of its complexes, this ligand can undergo C–S bond cleavage, a phenomenon first observed in a Mo complex in 1972 and becoming increasingly common in the last two decades.^[2] In most cases, the cleaved S atom takes on a distinctly different coordination role in the complex, except where it is abstracted by an S acceptor such as PEt₃.^[2c] For example, the cleaved S atom can assume a bridging mode (M–S–M),^[2a,b,f,3] form an M=S bond,^[2d,g] or interact with a neighboring ligand (e.g., the M≡C bond of a coordinated alkyne).^[2e] Cleavage of both C–S bonds of the ligand was reported for reactions with [Ru₃(CO)₁₂]^[2h] and [Mo₂(μ-O₂CMe)₄].^[3] Other reactions of the dithiocarbamate ligand include oxidative addition of a C–S bond to alkynes^[4] and coupling reactions with coordinated alkyne,^[2c,5] alkylidene,^[6] vinylidene/allenylidene,^[7] and cyanide^[8] ligands. Most of these studies involved Mo and W complexes, except for a few examples containing Cu,^[4d] Nb and Ta,^[2g] and Ru.^[2h,7] In the course of our investigations on the reactivity of [(CpCr(CO)₃)₂] (Cp = η⁵-C₅H₅) towards the E–E bonds of organic substrates such as diphenyl dichalcogenides Ph₂E₂ (E = S, Se, Te),^[9] and bis(diphenylthiophosphinyl)disulfane [Ph₂P(S)S]₂,^[10] we studied reactions with tetraalkylthiuram disulfides [R₂NC(S)S]₂, and here we report the first organochromium complexes containing dithiocarbamate and dithiooxamide ligands.

The instantaneous reaction of [(CpCr(CO)₃)₂] with one molar equivalent of tetraethylthiuram disulfide [Et₄NC(S)S]₂ in toluene at ambient temperature gave a dark red solution, which upon concentration yielded air-stable deep red crystals of [CpCr(CO)₂(S₂CNEt₂)] (**1**) in 87% yield. A similar reaction at 90 °C for 2 h gave a dark brown mixture, from which were obtained, in order of elution from a silica gel column, the following compounds: [(CpCr(CO)₂)₂S] (**7**) as a dark green solid (ca. 1% yield), (**1**) (22% yield), [CpCr(CO)₂(SCNEt₂)] (**2**) as a dark red crystalline solid (11% yield), [Cp₄Cr₄S₄] (**3**) as a dark solid (22% yield), [Cp₆Cr₈S₈{(C(S)NEt₂)₂}] (**4**) as a brown solid (6.8% yield),

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[Cr(S₂CNEt₂)₃] (**5**) as a dark blue crystalline solid (19% yield), and [Cp₆Cr₈(S₂CNEt₂)₂] (**6**) as a dark brown solid (11% yield). Heating **1** at 90 °C for 2 h gave **1–6** in similar yields (Scheme 1). All compounds were spectroscopically characterized, and satisfactory elemental analyses were obtained for **1**, **2**, and **4–6**, but only data for **4** and **6** are presented here.^[11] Dark brown rhombus-shaped crystals of **4** and **6** were obtained at –29 °C by diffusion of hexane into a toluene solution after 7 d, and by layering a toluene solution with hexane after 5 d, respectively. They were structurally characterized by X-ray diffraction analysis.^[12]

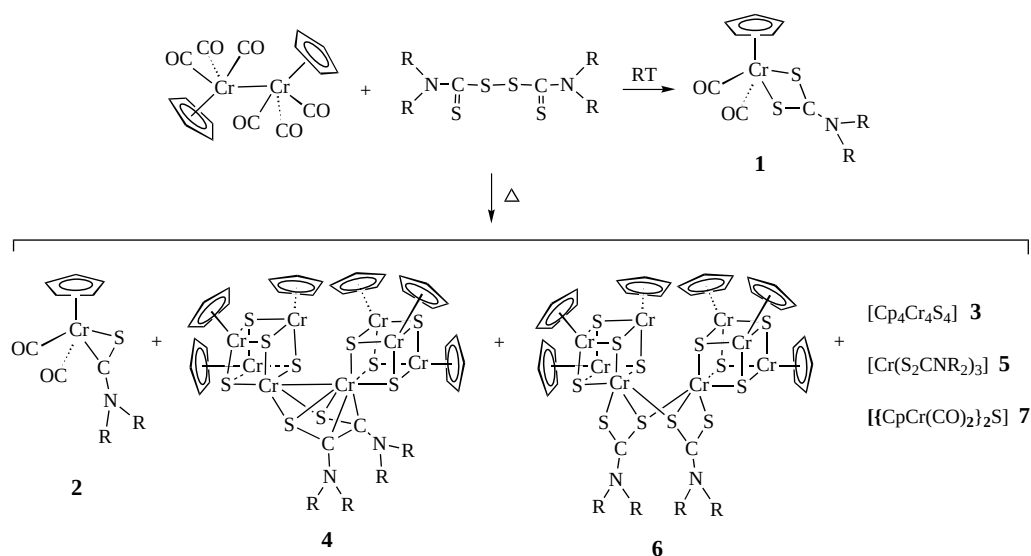
The structures of **4** and **6**, illustrated in Figure 1, consist of two [(η^5 -CpCr)₃CrS₄] cubes, linked at the Cr atoms Cr(1) and Cr(5), from which the η^5 -Cp ligands have been cleaved. In the molecule of **6**, the double cubane is doubly bridged by two dithiocarbamate ligands, each of which is coordinated in a μ - η^1 -S, η^2 -S,S' mode. Whilst such a bonding mode has been found in some dinuclear coordination compounds of Mo,^[13a] Re,^[13b] Fe,^[13c] Ru,^[13d–f] Rh,^[13g] Pt,^[13h] and other metals, this is unprecedented for double-cubane structures. However, numerous examples of bridging S ligands are found in the [Fe₄S₄] and FeMoS clusters, studied extensively by Holm et al. and Coucouvanis et al., including sulfide or disulfide,^[14a–c] alkyl thiolates,^[14d,e] and 1,2-ethane-dithiolates.^[14f,g]

If the μ - η^1 , η^2 -dithiocarbamate ligand is regarded as a five-electron donor, each of the [Cr₄S₄] units in **6** has 60 valence electrons. The average Cr–Cr distances are (2.86 ± 0.07) and (2.87 ± 0.06) Å for the cubes containing Cr(1) and Cr(5), respectively. The (C₂N)C(S)₂ units of both bridging dithiocarbamate ligands in **6** have a planar conformation. Planarity at C(1), C(2), N(1), and N(2) indicates sp² character of the C and N atoms. This is consistent with a double-bond nature of the C–N bond, which is reflected in the bond lengths of 1.37(3) and 1.40(3) Å for the two dithiocarbamate ligands; as expected, these bonds are shorter than the N–C(alkyl) single bonds in **6** (1.46(4)–1.51(3) Å); the values are within the reported range of 1.288(8)–1.52(9) Å for dithiocarbamate complexes.^[13d,g] The C–S distances (1.62(3) and 1.78(3) Å) are also within the reported range (1.700(7)–1.79(2) Å).^[13d,g,h]

The unit cell of **4** contains two independent molecules (A and B). Significant features are a dithiooxamide ligand (DTO = Et₂NC(S)–C(S)NEt₂) that links the two cubes in a μ - η^2 , η^4 bonding mode and a weak M–M bond between the two cubes. The Cr(1)–Cr(5) bond lengths of 3.101 and 3.118 Å in molecules A and B, respectively, are within the observed range of 2.676–3.310 Å for Cr–Cr bonds;^[9a] the longest bond is the one found in [(Cp*Cr(CO)₃)₂] (Cp* = η^5 -C₅Me₅).^[15] Interestingly, the η^4 -chromadithiacyclopentenyl ring [Cr(1)S(9)C(1)C(2)S(11)] is isoelectronic with the 26-electron C₅H₅[–] ligand that it displaced at Cr(5).

The bonding of C(1) and C(2) of the thioamidic ligand to Cr(5), but not to Cr(1), renders Cr(5) and Cr(1) formally eight- and six-coordinate, respectively. This unsymmetrical linking mode of the bridging ligand is reflected in the presence of three Cr–S bridging bonds (2.331(7)–2.364(7) Å) which are much shorter than the Cr(5)–S(9) bond (2.469(7) Å), which lies in the range of the six Cr–S bridging bonds (av (2.50 ± 0.07) Å) in the dithiocarbamate complex **6**. With the thioamidic bridge as an eight-electron donor and the presence of a bridging Cr–Cr bond, each of the cubane skeletons in **4** has 60 valence electrons, consistent with the observation of diamagnetic behavior in the complex. The Cr–Cr distances in the two cubes [av (2.83 ± 0.02) Å in molecule A for the cube containing Cr(1) and (2.82 ± 0.06) Å for the cube containing Cr(5)] are similar to those in **6**. The C(1)–C(2) distances in molecules A and B of **4** are 1.41(3) and 1.35(3) Å, respectively, which are intermediate between those of a C–C single (1.532(2) Å) and C=C double bond (1.335(3) Å) and agree well with those in complexes in which an alkene ligand exhibits the metallacyclopentane mode of coordination, for example, 1.43 Å in [Pt(C₂H₄)(PPh₃)₂],^[16a] 1.46(6) Å in [Fe(C₂H₄)(CO)₄],^[16b] 1.44 Å in [Pt(C₂F₄)(C₂H₄)₂],^[16c] and 1.45 Å in the metallacyclopentene structure of [Cp₂Zr(dime-thylbutadiene)].^[16d]

The C(1)–C(2) distances are shorter than those in bidentate (η^2 -S,S) DTO ligands, for which C–C bond lengths have been reported in the range 1.496(17)–1.526(13) Å.^[17] The C–S distances (1.72(3) and 1.81(2) Å in molecule A) are compa-



Scheme 1. Synthesis of **1–6**. R = Et.

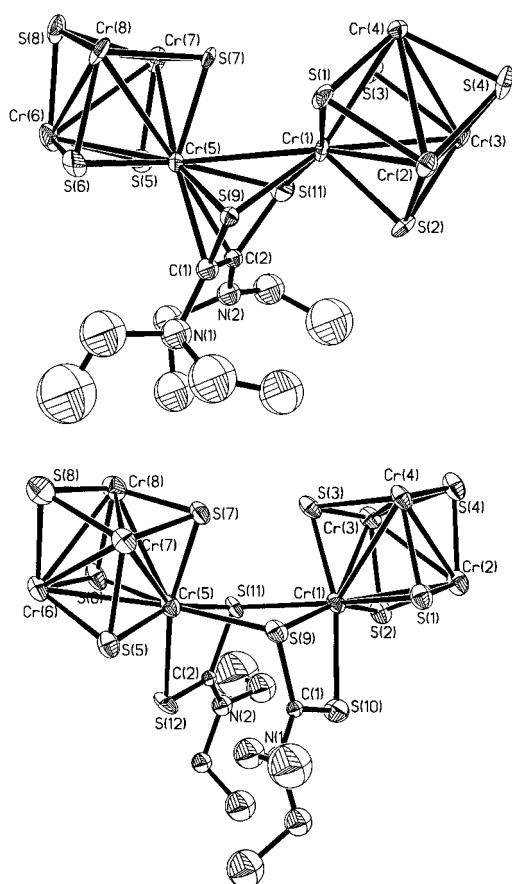
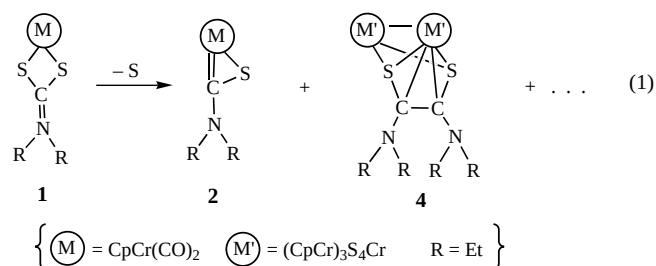


Figure 1. Ortep diagrams of **4** (top) and **6** (bottom). Cp rings on Cr(2)–Cr(4) and Cr(6)–Cr(8) are omitted for clarity. Selected distances [Å] and angles [°] in molecule A of **4** for the bridging dithiooxamide ligand: Cr(1)–Cr(5) 3.101(6), Cr(1)–S(9) 2.331(7), Cr(1)–S(11) 2.340(8), Cr(5)–S(9) 2.364(7), Cr(5)–S(11) 2.469(7), Cr(5)–C(1) 2.32(3), Cr(5)–C(2) 2.45(2), C(1)–S(9) 1.72(3), C(2)–S(11) 1.81(2), C(1)–C(2) 1.41(3), C(1)–N(1) 1.44(3), C(2)–N(2) 1.38(3); S(9)–Cr(1)–S(11) 81.4(3), S(9)–Cr(5)–S(11) 78.1(2), S(9)–Cr(5)–C(1) 43.1(6), S(11)–Cr(5)–C(2) 43.2(6), C(1)–Cr(5)–C(2) 34.4(8), Cr(1)–S(11)–Cr(5) 80.3(2), Cr(1)–S(9)–Cr(5) 82.7(2), S(9)–C(1)–Cr(5) 69.8(9), C(1)–S(9)–Cr(5) 67.1(9), C(2)–S(11)–Cr(1) 109.1(8), C(2)–C(1)–Cr(5) 77.8(15), C(1)–C(2)–Cr(5) 67.8(14), C(2)–S(11)–Cr(5) 67.8(8), S(11)–C(2)–Cr(5) 69.0(8), N(1)–C(1)–S(9) 122.8(19), N(2)–C(2)–Cr(5) 135.0(17), C(1)–C(2)–S(11) 114.2(18), C(2)–C(1)–S(9) 120.9(19), N(1)–C(1)–C(2) 114(2), N(2)–C(2)–C(1) 130(2). For the Cr₄S₄ cubes: Cr–S 2.208(7)–2.264(7), Cr–Cr 2.764(6)–2.887(6); S–Cr–S 98.6(2)–104.1(3), Cr–S–Cr 75.4(2)–79.2(3), Cr–Cr–Cr 57.70(13)–61.95(15). Selected distances [Å] and angles [°] for the bridging dithiooxamide ligand in **6**: Cr(1)–Cr(5) 3.853(7), Cr(1)–S(9) 2.495(9), Cr(1)–S(10) 2.443(10), Cr(1)–S(11) 2.552(10), Cr(5)–S(9) 2.581(10), Cr(5)–S(11) 2.505(9), Cr(5)–S(12) 2.441(9), C(1)–S(9) 1.77(3), C(1)–S(10) 1.71(3), C(2)–S(11) 1.78(3), C(2)–S(12) 1.62(3), C(1)–N(1) 1.37(3), C(2)–N(2) 1.40(3); S(9)–Cr(1)–S(10) 71.5(3), S(11)–Cr(5)–S(12) 71.1(3), Cr(1)–S(9)–Cr(5) 98.7(3), Cr(1)–S(11)–Cr(5) 99.2(3), Cr(1)–S(11)–C(2) 112.0(10), Cr(5)–S(9)–C(1) 109.2(10), C(1)–S(9)–Cr(1) 86.7(9), C(1)–S(10)–Cr(1) 89.8(9), C(2)–S(11)–Cr(5) 83.8(9), C(2)–S(12)–Cr(5) 89.3(10), S(9)–C(1)–S(10) 112.1(15), S(9)–C(1)–N(1) 121(2), S(10)–C(1)–N(1) 127(2), S(11)–C(2)–S(12) 115.7(15), S(11)–C(2)–N(2) 119(2), S(12)–C(2)–N(2) 125(2). For the Cr₄S₄ cubes: Cr–S 2.218(11)–2.290(11), Cr–Cr 2.783(8)–2.926(7); S–Cr–S 97.5(4)–103.2(4), Cr–S–Cr 75.8(3)–80.4(3), Cr–Cr–Cr 57.34(18)–61.88(18).

able to those previously observed (1.666–1.738 Å), but the C–N distances (1.44(3) and 1.38(3) Å in molecule A) are longer than earlier reported ones (1.279–1.318 Å),^[17] and are close to those of the N–C(alkyl) single bonds (1.37(4)–1.49(4) Å in molecule A). The conformation of the

[[C₂N)C(S)]₂ moiety of the dithiooxamide ligand is almost planar, whereby N(1) and N(2) lie slightly out of the plane defined by the three carbon atoms (0.42 and 0.15 Å in molecule A, and 0.14 and 0.39 Å in molecule B), and only slight torsion is present in the S–C–C–S chain (–4.8 and –8.8° in molecules A and B, respectively). The deviation from planarity is possibly a result of steric interactions between the NET₂ groups, which are brought close together by the C(1)–C(2) bond. The Cr–C distances (Cr(5)–C(1) 2.32(3) and Cr(5)–C(2) 2.45(2) Å) fall outside the range observed so far (1.93–2.19 Å) for a number of Cr “carbenoid” complexes.^[18]

The thermal transformation of **1** into the double cubanes **4** and **6** is unprecedented. Such behavior has not been reported for the Mo^[19] or W^[20] analogues of **1**, although the W analogues were obtained by UV irradiation and thermolysis of the monodentate complex [CpW(CO)₃(S₂CNMe₂)]. The various reactions of related cyclopentadienyl metal complexes such as [(η⁵-C₅R₅)Fe(CO)₂(η¹-S₂CNR'₂)] (R = H, Me; R' = alkyl), which have been extensively investigated by Astruc et al., involve chelation of the monodentate ligand, initiated chemically, photolytically or by redox processes; ligand exchange; substitution; redox reactions; and electrophilic attack at the uncoordinated S atom of the ligand.^[21] In the cyclopentadienylchromium complexes investigated here, the thermolysis of **1** gives rise to complexes containing the intact dithiocarbamate ligand (**5** and **6**), and to complexes in which the ligand has undergone C–S bond cleavage (**2** and **4**). It was experimentally demonstrated that **4** did not derive from the thiocarbenoid complex **2** or the dithiocarbamate-bridged double cubane **6**. It is conceivable that **6** may derive directly from **1** without C–S bond cleavage. Alternatively, the occurrence of a C–S bond cleavage in **1** would lead to **2**, and when accompanied by a concurrent intermolecular C(1)–C(2) coupling across two thiocarboxamido ligands, would produce the bridging unit in the cubane **4** [Eq. (1)]. Such dimerization



processes have been well-established to be one of the principal reaction pathways of carbenes.^[17] A subsequent or concomitant coordination of the ethene ligand so formed to a Cr center, would then produce the metallacyclopropane [Cr(5)C(1)C(2)] moiety in complex **4**.

This first study of the reaction of an organochromium complex with tetraalkylthiuram disulfide showed that the resulting mononuclear dithiocarbamate complex has unusual reactivity, which results in the formation of cubane structures that are doubly bridged by dithiocarbamate or dithiooxamide

ligands. We are extending our investigations by varying the transition metal complex and the ligand.

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- [11] Spectroscopic data for **4**: ^1H NMR (300 MHz, C_6D_6 , 27°C): $\delta = 5.17$ (1 $\times$ Cp), 5.14 (2 $\times$ Cp), 5.08 (2 $\times$ Cp), 5.06 (1 $\times$ Cp), 3.44 and 3.22 (each dq, $J = 14$, 7 Hz; 2 $\times$ CH_2), 1.21 (t, $J = 6$ Hz; 4 $\times$ CH_3); ^{13}C NMR (75.47 MHz, C_6D_6 , 27°C): $\delta = 92.2$, 91.9, 91.5, 91.4 (Cp), 48.6 (CH_2), 14.0 (CH_3). IR (KBr): $\tilde{\nu} = 1434$ (m, C=N), 1006 (w, NC₂), 802 cm^{-1} (vs, C=S). MS (FAB⁺): m/z : 1295 [$\text{Cp}_6\text{Cr}_8\text{S}_8\{(\text{C}(\text{S})\text{NEt}_2)_2\}$], 1063 [$\text{Cp}_6\text{Cr}_8\text{S}_8$], 531 [$\text{Cp}_3\text{Cr}_4\text{S}_4$], 233 [Cp_2Cr_2]. Spectroscopic data for **6**: ^1H NMR (300 MHz, $\text{C}_6\text{D}_5\text{CD}_3$, 27°C): $\delta = 5.09$ ($\nu_{12} = 22$ Hz, 3 $\times$ Cp; at -50°C : 5.40, 5.28, 5.06 (Cp)), 3.57 (unres. d, $J = 7$ Hz, 2 $\times$ CH_2), 0.99 (t, $J = 7$ Hz, 2 $\times$ CH_3). ^{13}C NMR (75.47 MHz, C_6D_6 , 27°C): $\delta = 91.7$ (Cp), 45.5 (CH_2), 13.5 (CH_3), 353.8 (S_2CNEt_2). IR (KBr): $\tilde{\nu} = 1495$ (s, C=N), 1145 (m, NC₂), 801 cm^{-1} (s, C=S). MS (FAB⁺): m/z : 679 [$\text{Cp}_3\text{Cr}_3\text{S}_4(\text{S}_2\text{CN}(\text{Et})_2)_2$], 614 [$\text{Cp}_2\text{Cr}_2\text{S}_4(\text{S}_2\text{CN}(\text{Et})_2)_2$], 563 [$\text{Cp}_2\text{Cr}_3\text{S}_4(\text{S}_2\text{CN}(\text{Et})_2)_2$], 530 [$\text{Cp}_2\text{Cr}_3\text{S}_3(\text{S}_2\text{CN}(\text{Et})_2)_2$], 348 [$\text{Cr}(\text{S}_2\text{CN}(\text{Et})_2)_2$], 200 [$\text{Cr}(\text{S}_2\text{CN}(\text{Et})_2)_2$], 116 $\text{SCN}(\text{Et})_2$.
- [12] Crystal data for **24**: $\text{C}_4\text{H}_4\text{O}$: $M_r = 2658.91$, monoclinic, space group $P2_1/n$, $a = 9.7539(3)$, $b = 50.869(2)$, $c = 21.1727(9)$ Å, $\beta = 91.659(2)^\circ$, $V = 10500.9(7)$ Å³, $Z = 4$, $\rho = 1.682$ Mg m⁻³, $F(000) = 5392$, $\lambda(\text{MoK}\alpha) = 0.71073$ Å, $\mu = 2.021$ mm⁻¹, $T = -50^\circ\text{C}$, crystal dimensions $0.32 \times 0.14 \times 0.04$ mm. Siemens SMART diffractometer, equipped with a CCD detector. Of 69642 reflections measured, 15082 unique reflections were used in refinement. Final $R = 0.1678$, ($R_w = 0.3400$). Crystal data for **6**: C_6H_{14} : $M_r = 1445.71$, monoclinic, space group $P2_1/c$, $a = 9.6283(9)$, $b = 26.798(2)$, $c = 21.0638(19)$ Å, $\beta = 99.133(4)^\circ$, $V = 5365.9(8)$ Å³, $Z = 4$, $\rho = 1.790$ Mg m⁻³, $F(000) = 2952$, $\lambda(\text{MoK}\alpha) = 0.71073$ Å, $\mu = 2.060$ mm⁻¹, $T = -50^\circ\text{C}$, crystal dimensions $0.03 \times 0.08 \times 0.30$ mm. Siemens SMART diffractometer, equipped with a CCD detector. Of 13987 reflections measured, 5828 unique reflections were used in refinement. Final $R = 0.1319$, ($R_w = 0.3047$). 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-159452 (**4**) and CCDC-159453 (**6**). 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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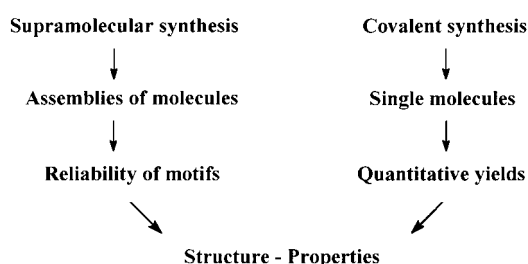
“Total Synthesis” Supramolecular Style: Design and Hydrogen-Bond-Directed Assembly of Ternary Supermolecules**

Christer B. Aakeröy,* Alicia M. Beatty, and Brian A. Helfrich

“Molecular chemistry, thus, has established its power over the covalent bond. The time has come to do the same for non-covalent intermolecular forces.”^[1]

Chemical synthesis, the making and breaking of covalent bonds, is a fundamental scientific discipline with consequences for all aspects of human existence. A vast number of reactions have been devised, discovered, and refined allowing more and more complicated molecules to be made using extraordinary multistep synthetic processes.^[2] In contrast, crystal engineering, a new but rapidly expanding area of science concerned with the construction and properties of assemblies and extended networks of molecules, has yet to reach anywhere near the same level of sophistication.^[3] Nevertheless, covalent *and* supramolecular (noncovalent) synthesis can be seen as having considerable overlap in terms of both methods and focus (Scheme 1).

“The consequences of directed and selective hydrogen-bond interactions on sets of molecules... are to a solid-state chemist what a new synthesis is to a solution chemist.”^[4]



Scheme 1. Comparison between covalent and supramolecular synthesis.

In recent years many systematic and elegant studies have been witnessed that demonstrate how molecular assemblies of increasing complexity and dimensionality can be put together with complementary intermolecular interactions, for example, hydrogen bonds and π - π interactions. Typically, these are homomeric architectures: “most crystals are built from identical (or enantiomeric) copies of the same molecule”.^[5]

However, in order to bring crystal engineering to a higher level of refinement and versatility it is necessary to identify reliable supramolecular reactions and synthetic strategies that will allow us to build multicomponent heteromeric structures, such as binary and ternary supermolecules.^[6]

Much of the important ground work regarding the preparation and utility of cocrystals was carried out by Etter and Frankenbach:^[7] binary cocrystals were synthetic goals, but these cocrystallization reactions were also used as probes of the competition between different hydrogen-bonding interactions. An important rule of thumb resulted from this work: in a system with a multitude of hydrogen-bonding functionalities “the best hydrogen-bond donor and the best hydrogen-bond acceptor will preferentially form hydrogen bonds to one another”.^[8] From this it also follows that the second-best donor will form a hydrogen bond to the second-best acceptor, etc. It is still important to remember, however, that solid-state assembly is an enormously complex process governed by a subtle balance between relatively weak and often ill-defined intermolecular forces (in addition to possible complications posed by competition between kinetic and thermodynamic factors). This certainly helps to explain the fact that multistep intermolecular synthesis is not commonplace. In fact, to the best of our knowledge,^[9–11] there is not a single example in the Cambridge Crystallographic Database^[12] (amongst over 224 000 entries) of a 1:1:1 ternary cocrystal containing supermolecules with well-defined and predictable connectivity that was synthesized using a deliberate supramolecular design strategy.^[13]

Our approach to the design of ternary structures is based upon two simple principles: 1) Hydrogen bonds often form in a hierarchical fashion (best donor to best acceptor, second-best donor to second-best acceptor, etc.)^[8] and 2) a small number of specific intermolecular interactions can provide a large part of the stabilization energy of molecular crystals.^[14]

The centerpiece in our supramolecular systems is isonicotinamide. This molecule readily reacts with aromatic carboxylic acids to form 1:1 binary cocrystals that contain a robust and reproducible hydrogen-bonded motif (shown in Figure 1 for benzoic acid as the carboxylic acid (**1**)).^[15]

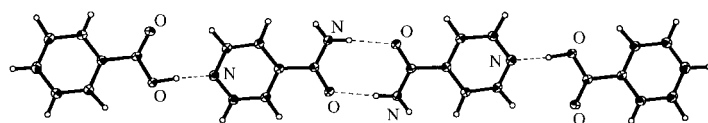


Figure 1. The supermolecule in the 1:1 cocrystal **1** of isonicotinamide and benzoic acid held together by acid...pyridine and amide...amide hydrogen-bonding interactions.

The best donor (carboxylic acid) and the best acceptor (pyridine nitrogen atom) form a short intermolecular N...O hydrogen bond, and the supermolecule is completed through a self-complementary amide...amide interaction. Isonicotinamide is a good example of a suitable building block for ternary cocrystals: it has two distinctly different, yet relatively strong, hydrogen-bonding moieties, and it shows good “structural flexibility” (it can form well-defined crystals with different components).^[16] The primary hydrogen bond in this

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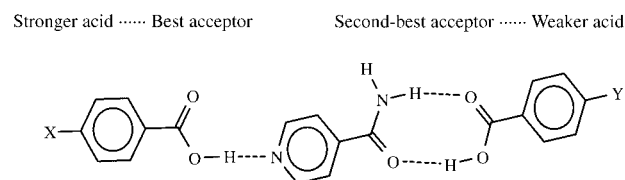
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system is the acid...pyridine interaction and, since hydrogen bonds have large electrostatic components, the strength of this interaction is governed by the acidity of the carboxylic acid donor. Of course, if the acid is too strong, the proton will be transferred to the nitrogen atom; this will result in an ionic compound and not in a cocrystal.^[17]

The “weaker” link in the tetrameric motif in **1** is the amide...amide interaction. Since many reported structures contain heteromeric amide...acid hydrogen bonds (in preference to the corresponding homomeric options),^[12, 18] our plan was to replace the amide...amide interaction with a more favorable heteromeric acid...amide interaction, thereby allowing the introduction of a third component in a specific manner. By offering two different carboxylic acids to the isonicotinamide molecule, we expected the stronger acid to interact preferentially with the best acceptor (the pyridine nitrogen atom) and the weaker acid to form a heteromeric motif with the remaining amide moiety (Scheme 2).



Scheme 2. Proposed scheme for the construction of a three-component supermolecule.

This supramolecular design strategy was put to the test by allowing equimolar amounts of a weaker acid, a stronger acid, and isonicotinamide to react in an aqueous solution. Herein we present the crystal structures of three different cocrystals obtained using this approach.^[15, 19]

Compound **2** is a 1:1:1 ternary cocrystal of 3,5-dinitrobenzoic acid, isonicotinamide, and 3-methylbenzoic acid that contains the desired three-component supermolecule with the expected connectivity. The stronger acid ($pK_a = 2.8$)^[20] interacts with the pyridine nitrogen atom, and the weaker acid ($pK_a = 4.3$)^[20] competes successfully for the amide moiety and forms a heteromeric hydrogen-bonded motif (Figure 2). Compound **3** is a ternary cocrystal of 3,5-dinitrobenzoic acid, isonicotinamide, and 4-(dimethylamino)benzoic acid with the intended three-component supermolecule. Again, the strong acid...pyridine nitrogen atom interaction persists, and the weaker acid ($pK_a = 6.5$)^[20] generates a heteromeric motif with the amide moiety (Figure 2). Finally, compound **4** is a 1:1:1 ternary cocrystal of 3,5-dinitrobenzoic acid, isonicotinamide, and 4-hydroxy-3-methoxycinnamic acid with the same primary supramolecular connectivity as in **2** and **3** (Figure 2). It is important to note that the desired trimer persists even in the presence of the potentially disrupting OH moiety on the weaker acid ($pK_a = 4.4$).^[21]

Two important intermolecular synthons,^[22] carboxylic acid...pyridine and carboxylic acid...carboxamide, have been utilized in the assembly of a family of 1:1:1 ternary cocrystals. All three structures contain a heteromeric, three-component supermolecule synthesized through the use of hierarchical (best donor/best acceptor, second-best donor/

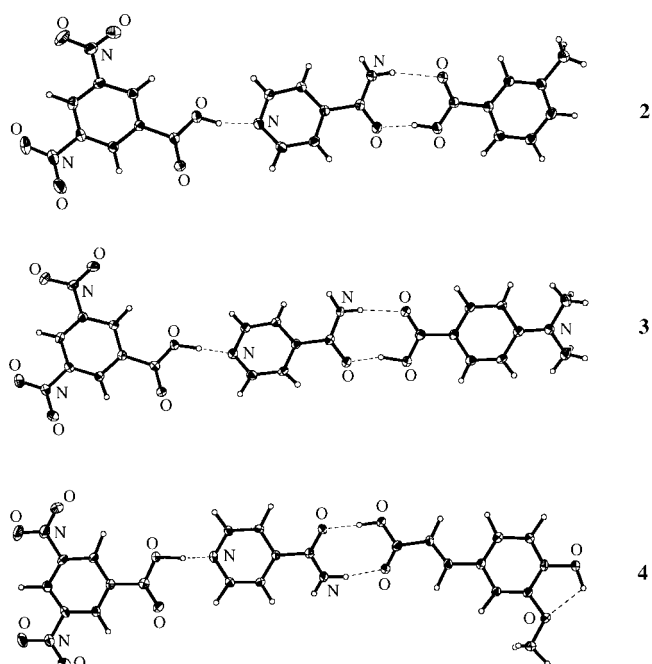


Figure 2. Structures of the ternary supermolecules in **2** (top), **3** (middle), and **4** (bottom) with the stronger acid...pyridine interaction being the “best” hydrogen bond and the weaker acid...amide interaction being the “second-best” hydrogen bond.

second-best acceptor) hydrogen-bonding interactions. None of the crystals incorporate solvent molecules in the lattice. There is no ambiguity about the classification of these compounds: they are molecular cocrystals, not ionic compounds. All the hydrogen atoms were located from difference density maps, and the C–O distances on each carboxylic acid moiety are distinctly different, and indicate the presence of one C–O and one C=O bond. In addition, the C–N–C angles on the isonicotinamide molecules are in the range of 117.7–118.5°, which is consistent with those exhibited by other nonprotonated pyridine derivatives.^[23] All three structures (**2–4**) are also stabilized by additional $\pi(\text{weak acid})-\pi^*(\text{strong acid})$ charge-transfer interactions (see Figure 3).

The ternary compounds are all colored whereas their constituent parts range from off-white to pale yellow in color. The colors of **2–4** reflect the changes in the resulting $\pi-\pi^*$ separation. The weakest acid, 4-(dimethylamino)benzoic acid, has the highest energy HOMO, and therefore the smallest $\pi-\pi^*$ separation, which results in the deep red color of **3**. Compound **4** is orange and **2** is yellow; these colors correspond to an expected increase in the energy of the $\pi-\pi^*$ charge-transfer interaction in these compounds.^[24] It is unlikely that this charge transfer takes place through the isonicotinamide molecule from the weak acid (π) to the strong acid (π^*). Instead, as the supermolecules are stacked in an antiparallel fashion (Figure 3) with considerable donor–acceptor overlap between neighboring weak and strong acids, it is reasonable to assume that the charge transfer is through space. The intrastack phenyl–phenyl separations are about 3.35 Å. Charge-transfer interactions are also known to be important for stabilizing 1:1 binary cocrystals containing weak and strong aromatic carboxylic acids.^[25, 26] Of course it must be

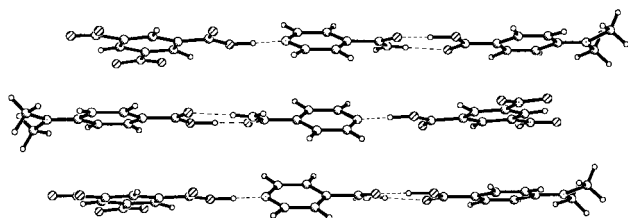


Figure 3. Side-on view of a stack of supermolecules arranged in an antiparallel fashion in **3** to illustrate the π (weak acid)– π^* (strong acid) charge-transfer interactions.

noted that we have presented a strategy for the construction of ternary supermolecules: real crystals are created through the three-dimensional assembly of such building blocks. Neighboring trimeric supermolecules are linked laterally through a variety of weaker N–H...O and C–H...O interactions, which lead to lamellar architectures in all three cases.

This work has demonstrated that three-component supermolecules can be constructed through the use of known intermolecular synthetic operations. Although these forces are weaker than covalent interactions, it is clearly possible to assemble more than two different building blocks in a preconceived manner. We have every reason to believe that more complex supermolecules can be readily synthesized through an improved awareness of the balance and competition between intermolecular interactions. In addition, supramolecular reactions of this type may have important ramifications for the pharmaceutical industry (circumventing patents, modifying crystal habit, and facilitating processing), and may also lead to new strategies for chiral separation.

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Application of Chemical Shift Imaging for Simultaneous and Fast Acquisition of NMR Spectra on Multiple Samples**

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NMR spectroscopy has become an indispensable part of molecular structure determination and verification processes in all fields of chemical and biostructural research. Only recently have the emerging field of combinatorial chemistry, in which large compound libraries need to be characterized, and the demand for quality control of compound depositories in pharmaceutical industry shown that the sample throughput achievable with setups used in modern NMR laboratories is too low and prevents efficient application of NMR spectroscopy in the above-mentioned contexts. The two automated setups nowadays in use comprise a) automated sample changers that position one NMR sample tube after the other in the magnet and b) the pumping of single samples in a capillary system to a properly designed flow-through probe.^[1] Both approaches are designed for sequential sample input and lack any aspect of parallelization.

A setup with four detection volumes mounted into a home-built flow-through probe was described in the literature.^[2] The application of an inhomogeneous magnetic z -field together with a tailored data processing was used to measure and decompose the spectra. This method, however, is restricted to measurements of only four samples in parallel and necessitates a coil around each sample tube. Therefore, a specially adapted NMR probe is needed.

To increase the parallelization and the sample throughput, as well as circumventing the need for additional costly hardware, we propose^[3] the combination of a technique widely applied in the field of medical NMR spectroscopy known under the acronym CSI^[4] (chemical shift imaging) with a compartmented detection volume realized by a bundle of 1-mm NMR-suited glass capillaries (Figure 1).^[5] Each capillary is filled with a different solute. The acquisition of regular NMR data on such a compartmented detection volume results in a spectrum that is identical to that obtained from a mixture of all the compounds in a single-volume detection cell. The decomposition of the data to single spectra is a prerequisite for getting useful information. We adopted the CSI technique that is in use in magnetic resonance imaging (MRI) to perform this task.^[6] A typical question to be answered in medical applications of this technique is what is the spatial distribution of substances in certain organs within a human (animal) body. CSI combines spatial information (organs, for example, liver, or arbitrary coordinates) with chemical information (for example, the NMR spectrum of glucose).

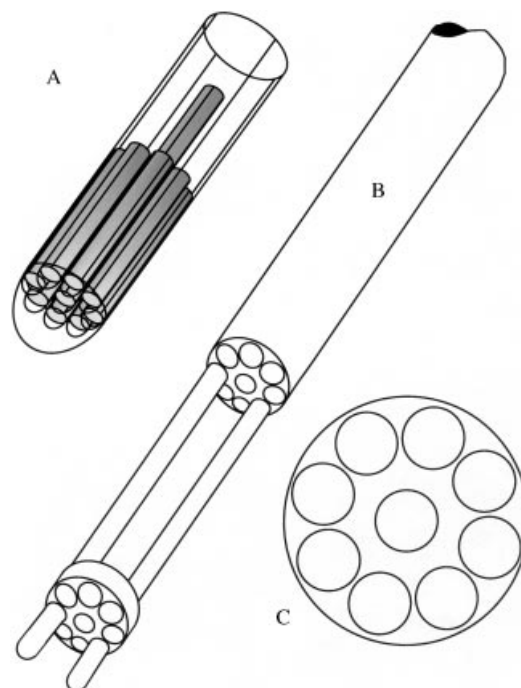


Figure 1. Schematic drawing (A and B) and cross section (C) of a nine-capillary bundle (A) to fit into a standard 5.0 mm NMR tube. The bundle is fixed at the bottom and the top in carriers made of plexiglas (B) in our in-house workshop. The upper carrier is integrated in a cylindrical module made of the same material to enable proper positioning in the NMR tube. To circumvent the need for cleaning the containers, disposable low-price capillaries can be used if a large number of samples have to be measured without significant loss of spectral quality.

The principle of the method rests on the application of controlled inhomogeneous magnetic field pulses (gradient pulses) to obtain information on the spatial distribution of spins within a sample volume.

Only recently were x - and y -direction gradient coils integrated in NMR spectrometers used in analytical chemistry for the purpose of coherence pathway selection and solvent suppression.^[7] This hardware feature is commercially available for NMR spectrometers and meets exactly the requirements necessary to obtain spatially resolved chemical shift information. An exemplary pulse sequence suited for the acquisition of simple x,y -resolved one-dimensional (1D) ^1H NMR spectra is shown in Figure 2. The three-dimensional (3D) data set obtained comprises two spatial (x,y) dimensions and a spectral one. The initial phases of the free induction decays (FIDs) that were acquired as a function of the

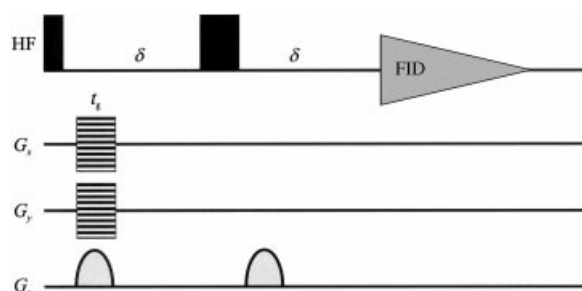


Figure 2. CSI pulse sequence for spatially x - and y -resolved 1D ^1H spectra.

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independently incremented gradient pulse strengths contains the information on the x and y position of the spins within the capillary bundle. The number of increments N_i and the strength G_i of the gradient pulses applied along a certain dimension $i \in \{x, y, z\}$ determine the so-called field-of-view (FOV_i), which is given by $2\pi(\gamma_H G_i t_g N_i)^{-1}$. The spatial resolution is given by FOV_i/N_i . All the FOV_i values must be set large enough to comprise the diameter of the object along the corresponding direction (for example, the diameter of the capillary bundle) to avoid folding. The spatial resolution must be set high enough to resolve the individual capillaries in the image. The parameters of the spectral dimension can be optimized as usual to enable extraction of chemical information as needed (chemical shift, J couplings, integrals). The spectral information is obtained as usual by Fourier transformation (FT) of the all FIDs with respect to the acquisition time. The image of the sample is obtained by independent FT of the data with respect to both incremented gradient strengths. It is clear from the theory of NMR imaging^[8] that small “cross-talk” between extracted traces cannot be circumvented completely. Numerical simulation based on theory shows that the expected transfer of amplitudes between spectra in our experimental setup is below 6 % of the intensity of the spectrum of interest. This unwanted “contaminant” can be further reduced by use of optimized spatial sampling schemes.^[9]

In Figure 3 we show the skyline projection of the data acquired as described in the Experimental Section after FT only with respect to the spatial dimensions. The expected

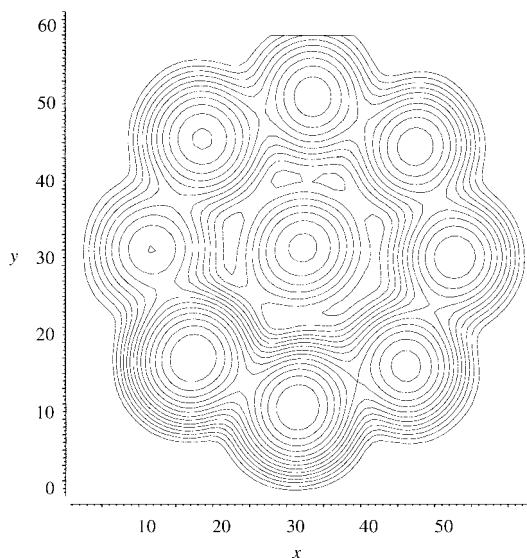


Figure 3. Cross-sectional image of the bundle of 1-mm capillaries obtained by FT along the spatial dimensions only. The contours code for spin density.

image of our object is clearly visible. The expanded regions of the spectra shown in Figure 4 were extracted after an additional FT with respect to the acquisition time, which was obtained from orthogonal traces taken out of the 3D data set at the x, y coordinates of the maxima located in each capillary. This task can be automated using known peak-picking algorithms. No cross-talk between the traces is seen at the given signal-to-noise level.

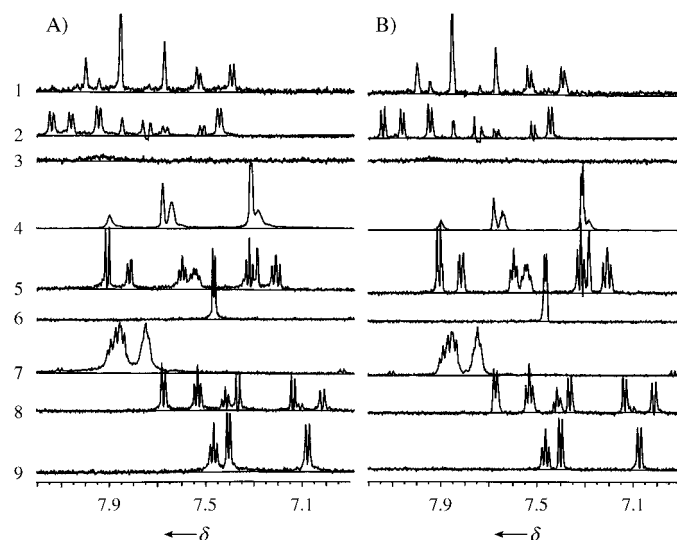


Figure 4. Expanded regions of the extracted ^1H 1D NMR spectra obtained with (A) and without (B) filling the interstice between the capillaries with $[\text{D}_6]\text{DMSO}$. The spectra allow for the extraction of chemical shifts and J couplings larger than 5 Hz. The reduced spectral resolution relative to those obtained from a standard setup can be explained by contributions to the B_0 -field inhomogeneity from susceptibility jumps in our sample geometry. Nevertheless the quality of the spectra is high enough for structure validation of known structures needed for quality control of compound libraries. The signal-to-noise ratio measured fits (with the exception of the spectrum in trace 4) nicely with the expected value when taking into account the small detection volume per capillary. The result in trace 4 can be explained by a higher initial sample concentration. Trace 3 is blank as this substance has no aromatic moiety—signals are visible in other regions of this spectrum.

CSI is not restricted to the 1D homonuclear pulse sequence used here to extract chemical information. Multidimensional homo- and heteronuclear pulse sequences play a substantial role in modern NMR spectroscopy. The modification of most pulse sequences in use to include the spatial encoding part shown in Figure 2 prior to the detection of the signal is straightforward. As will be discussed below, a substantial decrease in the spectrometer time needed per sample by using the CSI technique is only achieved if the time needed for sample replacement dominates the total cycle time per sample in the conventional setup. This prerequisite can not be fulfilled by any multidimensional NMR experiment. The x, y -encoding scheme of an arbitrarily compartmented arrangement can easily be expanded by an additional incrementation of the strength of the z -gradient pulse to obtain spatial information along the z dimension of the compartment arrangement to obtain 3D spatial information.^[10] This enables the application of the method to further optimized geometries of compartmentation.

The experimental time that allows a single scan per gradient setting is 5 min for our geometry of 9 samples. For comparison, an automated standard setup allows the acquisition of a simple 1D proton spectrum within 5.5 min per sample (typical cycle time in our service NMR department including automated sample exchange, shimming, locking). Thus, we have shown that an increase in the throughput of samples by a factor of roughly ten is readily achieved by use of CSI-based methods, and this can be performed on any commercially available state of the art NMR spectrometer. The only

additional hardware needed is a miniaturized compartmented sample container suitable for NMR spectroscopy. For a typical small molecular weight substance, 30 μg dissolved in a capillary is enough to provide a spectrum of sufficient quality to deduce the structural integrity of the substance and impurities in the 10% range.

As the achievable signal-to-noise ratio is determined only by the sensitivity of the NMR probe and the number of spins in the detection volume, the method presented here by no means generates an improvement in the sensitivity of the NMR equipment. The gain in throughput is based solely on the reduction of the lengthy time required for frequent change of samples. This is only possible at the relatively high concentration of samples available in all fields mentioned in the introduction. Automation of the manually difficult filling of the capillary bundles is currently in progress in our workshop.^[11] Further miniaturization of the diameters of the capillaries is due to limitations of available technology for automated liquid handling is not advisable.

The application of large diameter (7–10 mm) probes to increase the number of capillaries included in the detection volume is hampered by the lower intrinsic sensitivity of these probes, which reduces the quality of the spectra. This limitation might change in the near future if cryogenic technology^[12] becomes available for larger diameter probes. A simple geometry for positioning 19 capillaries in a standard detection volume is described in the technical section. A bundle of flow-through capillaries measured by use of the CSI technique might also help to further increase the throughput of samples.

Experimental Section

For the experiments presented here each capillary tube was filled with 10 μL by using a syringe. The nine substances dissolved at concentrations below 10 mM in deuterated dimethyl sulfoxide ($[\text{D}_6]\text{DMSO}$) were arbitrarily obtained from our routine NMR laboratory. The solvent served as an internal lock. All data presented were taken on a DMX 600 Bruker spectrometer equipped with a standard triple-axis gradient TXI probe housing all the gradient coils. The temperature was set to 300 K. The NMR experimental parameters were set as follows: Proton pulse 8 μs ; interscan delay 1 s; eddy current compensation δ 2 ms. FIDs of 4 K complex time points were acquired in the digital filtering and quadrature detection mode of DMX spectrometers. The x - and y -gradient pulses ($t_g = 1$ ms) were ramped in 16 steps from -5 to $+5$ G cm^{-1} to obtain the needed spatial resolution of 300 μm . The 3 G cm^{-1} G_z gradient was used to remove 180° pulse imperfections.

This acquisition gave a data matrix of $(16 \times 16)_{\text{spatial}} \times 4K_{\text{complex,time}}$ data points. Eight dummy scans were allowed for thermal equilibration of the spin system prior to acquisition of the data. The data were processed using a matched exponential filtering along the chemical shift dimension and time-centered sine functions along both spatial dimensions together with first order phase correction (as needed) in all dimensions. Small truncation artifacts seen for multiplets are due to evolution of J -coupling during the spin-echo delays δ of the pulse sequence. A final data matrix of $(64 \times 64)_{\text{spatial}} \times 8K_{\text{chemical shift}}$ data points was obtained after zero filling. A bundle of capillaries (and interstice) filled with a 90% $\text{H}_2\text{O}/\text{D}_2\text{O}$ mixture was used for a standard two-step z -gradient shimming protocol for the determination of initial shim settings. Low order off-axis shims were adjusted manually. The intercapillary space was originally filled with the same solvent as used in the capillaries to improve the homogeneity of the magnetic field over the bundle. Our highly symmetric arrangement comprises one central capillary surrounded by a second layer with eight capillary tubes. In the course of the experiments it turned out that the distortion of the magnetic field

homogeneity introduced by the removal of the intercapillary liquid is negligible (Figure 4). If the bundle is included in a standard NMR tube with a glass wall of 0.4 mm the active detection volume of the spectrometer cannot be filled completely with capillaries. The outer NMR tube is not necessary as the intercapillary liquid can be removed. Therefore, a bundle of 19 capillaries consisting of a central capillary surrounded by a second layer of 6 and a third layer of 12 capillaries will perfectly fill the available detection volume. Currently work is in progress in our laboratory to build such an improved geometry.

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Landmark Crystal Structure of an Experimentally Utilized Tetralithium–Tetrapotassium Amide–Alkoxide Superbase**

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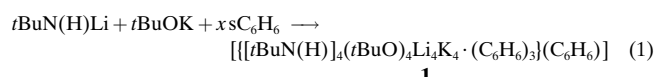
It has long been known that mixtures of organolithium derivatives (e.g., alkyls, amides, or enolates) and heavier alkali metal alkoxides (e.g. *tert*-butoxides or *tert*-pentoxides) can exhibit special proton-abstracting powers well beyond the capability of the lithium compounds on their own.^[1] A forest of literature has grown around this superbasicity focussing mainly on its exploitation in organic synthesis^[2] and, to a lesser extent in polymerization.^[3] Yet for all this accumulated knowledge, there is still much uncertainty about the precise

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identities and structures of these superbasic reagents, which seems remarkable given that in general their two separate components (e.g. *n*BuLi^[4] and *t*BuOK^[5]) have themselves been fully characterized both in solution and in the solid state. But the situation is complicated by the vast number of structural permutations possible when the two components are mixed together in solution. To summarize, each separate component can autoaggregate and may exist in several oligomeric forms, or combine with the other component to generate mixed oligomers, and in theory all of these distinct species could form adducts with the substrate to be metallated or with the solvent employed in the reaction, or even with both.^[6] Hence the reason why structural studies^[7] are deemed to be so important if the secrets of superbasicity are to be finally revealed. Several research groups have already made significant progress along this path by reporting the structures of “model superbases”: these compounds contain a combination of anions, and in some cases a combination of metals, representative of those found in superbasic mixtures though they differ in one or more ways from the actual combinations used in practice (thus the need for the qualification “model”). Model structures of this type are known for a variety of classes of superbase.^[8] Our approach to this problem has been more direct in that we have reinvestigated authentic, experimentally utilized superbasic mixtures and endeavored to grow crystals from them. Most attempts have predictably ended in failure for reasons all too familiar to researchers in the field: solids isolated from such mixtures tend to be microcrystalline or single-component (homometallic) species. However, a breakthrough came in revisiting a reaction first studied by Lochmann and Trekoval in 1979 in their pioneering work on the reactions of substituted *N*-lithium amides with heavier alkali metal alkoxides; specifically they reported^[9] that mixtures of lithium *tert*-butylamide and potassium *tert*-butoxide in solution in benzene exhibited increased metalation properties toward toluene, yielding mainly benzylpotassium. We have now been successful in isolating and crystallographically characterizing a mixed-metal mixed-anion complex from this mixture and confirmed that it readily metalates toluene. We therefore report herein the first crystal structure of a bona fide superbase.

Lochmann and Trekoval highlighted the said reaction as atypical because unlike the other amide systems studied by them, no precipitate separated from it upon mixing the two components together. As this implied the superbase remained in solution, we decided to use a much more concentrated benzene solution of the alkoxide/amide mixture (2M) than that employed originally (0.2–0.4M). Furthermore we amended the alkoxide:amide stoichiometry from that used by Lochmann and Trekoval (1.3:1) to 1:1. This adapted reaction [Eq. (1)] afforded colorless crystals of **1**, a benzene solvate.



Four (metal–heteroatom)₂ dimeric rings arranged in two pairs provide the building blocks of the centrosymmetric molecular structure of **1** (Figure 1).^[10] Symmetry dictates that

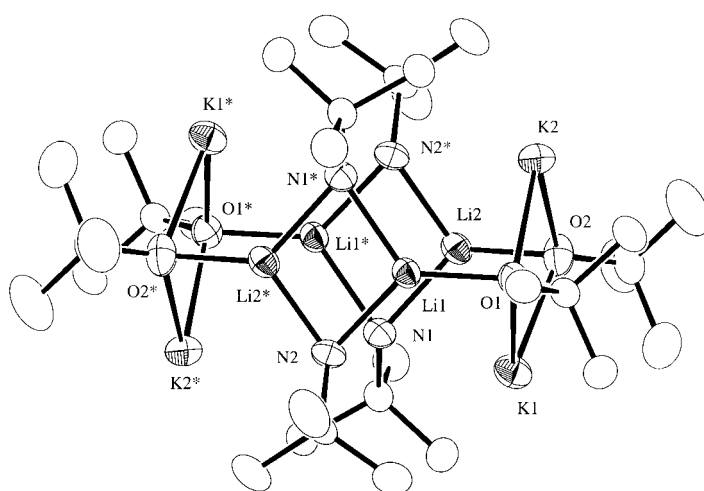


Figure 1. Core structure of **1** without hydrogen atoms and benzene ligands. Selected bond lengths [Å]: K1–O1 2.6106(17), K1–O2 2.658(2), K1–N1 3.192(2), K2–O1 2.6312(17), K2–O2 2.6334, K2*–N2 3.325(2), O1–Li1 1.879(4), O2–Li2 1.865(4), N1–Li2 2.007(4), N1*–Li1 2.040(4), N2*–Li2 2.006(5), N2–Li1 2.046(4). * denotes the symmetry operation 1 – *x*, –*y*, –1 – *z*.

the two equivalent rings within each pair lie strictly parallel to each other, while the distinct sets (e.g., K1–O1–K2–O2 and Li1–N2–Li2*–N1*) lie essentially perpendicular (e.g., angle between least-squares planes of rings, 82.92°) to each other. The rings join together in a carousel arrangement through strong Li–O bonds, which collectively make up a Li₄O₄ plane (“seat”) running through the center of the structure. Bent away from the (LiN)₂ or (KO)₂ ring planes to which they are attached, the two distinct types of *tert*-butyl substituent adopt exocyclic positions with respect to the hexadecanuclear inorganic core, while the symmetry-equivalent types eclipse each other. Furthest from the core lie benzene solvent ligands which complete the coordination spheres of the K atoms. Those attached to K2/K2* bind terminally and symmetrically (η^6) with six K–C distances under 4 Å in length (range, 3.71–3.83 Å). In contrast, those attached to K1/K1* adopt a “slipped” asymmetrical (η^3) arrangement which enables them to bridge between neighboring Li₄K₄N₄O₄ units using either side of the arene π system (range of K–C distances, 3.67–4.00 Å) to generate an extended structure of one-dimensional zigzag chains (Figure 2). An additional benzene molecule of crystallization (one per Li₄K₄N₄O₄ unit) occupies a gap between the chains.

What information can be gleaned about the structures of superbases using the architecture of **1** as an exemplar? One point to note is that the structure organizes around Li–O

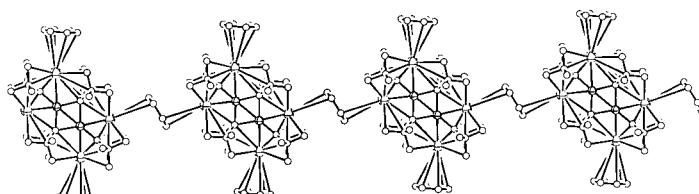


Figure 2. Extended structure of **1** showing ligating benzene molecules. Benzene molecules of crystallization are omitted.

bonding. Thus the $(\text{LiN})_2$ rings align themselves almost perfectly with the $(\text{KO})_2$ rings to create the Li_4O_4 plane (root mean square (RMS) deviation from planarity, 0.016 Å) which maximizes the overlap between the small cation and small anion centers. Short Li–O bond lengths (mean, 1.872 Å) are formed as a result (cf. 1.916 Å in $(t\text{BuOLi})_6$).^[11] That said, it is perhaps surprising that the number of Li–O bonds is not maximized (i.e., there is only one per Li). This contrasts with the two such bonds per Li center in $[\{\text{PhN(H)}\}_2(t\text{BuO})\text{-LiNaK}\cdot 2\text{TMEDA}]_2$ (TMEDA = tetramethylethylenediamine^[8] (mean length, 1.948 Å) reflecting the different bridging role of its heteronuclear (N–Na–N–Li) ring towards the $(\text{KO})_2$ ring. A further surprise is the absence of any significant K–N bonding in **1** as the mean contact distance is 3.259 Å (some 0.34 Å longer than the mean K–N(amido) bond length in the aforementioned trimetallic complex) and the $\text{K1}\cdots\text{K2}^*$ and $\text{N1}\cdots\text{N2}$ vectors are poorly aligned (RMS deviation from planarity, 0.159 Å). Therefore the amido ligands are held in place mainly by two Li–N bonds (mean length, 2.025 Å). Contrast this with the situation in the known homometallic analogue $[\{t\text{BuN(H)Li}\}_8]$,^[12] a cyclic ladder molecule, in which three such bonds (mean length, 2.050 Å) hold the amido anions much more tightly within the aggregate. It would appear that this weakening of the Li–N bonding is an important factor in the enhanced basicity of **1** versus its parent lithium amide, which significantly cannot deprotonate toluene under the conditions studied here. The presence of the heavier alkali metal gives **1** another possible advantage, a potential binding site for the arene substrate (here demonstrated by the K– π interactions with benzene). It is well established in enolate chemistry that carbonyl substrates can take the place of conventional donor solvent ligands by coordinating to a metal atom as a prerequisite to undergoing deprotonation.^[13] In the case of **1** the benzene ligands could similarly be replaced by toluene molecules prior to the deprotonation step. The absence of a heavier alkali metal coupled with the steric crowding (comprising three bulky amido ligands) about the smaller lithium cations, negate this possibility in $[\{t\text{BuN(H)Li}\}_8]$. This prebinding of the toluene molecules would lower the entropy of the subsequent deprotonation reaction (see Schlosser's article in reference [1] for an illuminating discussion of such proximity effects).

To verify that **1** is indeed a superbases, we isolated it, redissolved it in benzene, then added toluene. The presence of benzyliumpotassium was subsequently confirmed by ^1H NMR spectroscopic studies.^[14] However, note that **1** is only superbasic in the sense that it has superior proton-abstracting powers to its parent lithium amide. It should not be measured against classical alkyl (carbanion) superbases which are inherently more basic.

Experimental Section

1: Under a protective dry oxygen-free argon atmosphere in a Schlenk tube, $t\text{BuNH}_2$ (10 mmol) was added dropwise to a solution of $n\text{BuLi}$ (10 mmol) in hexane. Butane was evolved as the primary amide $t\text{BuN(H)Li}$ formed in solution. This was subsequently isolated and the dry solid and solid $t\text{BuOK}$ (10 mmol) were placed together in another Schlenk tube and covered with benzene (5 mL) to give a dark brown suspension which required gentle heating for complete dissolution. Cooling the solution on the bench

afforded a crop of colorless crystals of **1**. Yield (disregarding benzene molecules most of which are lost upon isolation), 75%. Elemental (C, H, N) analysis was hampered by the loss of solvent immediately upon isolation of the crystals. During the analysis itself the sample lost weight too rapidly for reliable results to be obtained. M.p. 102–103 °C; ^1H NMR (400 MHz, $[\text{D}_6]\text{benzene}$, 25 °C): δ = 3.87 (s, 1H, NH), 1.24 (s, 9H, $t\text{BuO}/t\text{BuN}$), 1.21 (s, 9H, $t\text{BuO}/t\text{BuN}$).

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- [10] Crystal structure data for **1**: $\text{C}_{56}\text{H}_{100}\text{K}_4\text{Li}_4\text{N}_4\text{O}_4$, $M_r = 1077.56$, colorless chip, approximately $0.35 \times 0.20 \times 0.20$ mm. MoK_α graphite-monochromated radiation ($\lambda = 0.71073$ Å), $T = 150$ K; triclinic, space group $P\bar{1}$; $a = 12.4570(5)$, $b = 12.6523(5)$, $c = 13.1390(5)$ Å; $\alpha = 93.621(2)$, $\beta = 116.846(2)$, $\gamma = 109.314(2)^\circ$; $V = 1687.21(11)$ Å³; $Z = 1$, $\mu = 0.304$ mm^{−1}, $\rho_{\text{calc}} = 1.061$ Mg m^{−3}, $2\theta_{\text{max}} = 54.96^\circ$, 12560 reflections collected, 7712 unique used ($R_{\text{int}} = 0.0270$). The structure was solved and refined to convergence on F^2 using published programs and techniques (G. M. Sheldrick, SHELXS and SHELXL-97, University of Göttingen, Germany **1997**). $R1 = 0.0595$ (for 5070 reflections with $I > 2\sigma(I)$), $wR2 = 0.1649$ and $S = 1.021$ for 343 parameters. Maximum residual electron density 0.701 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-162484. 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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- [14] Recorded in $[\text{D}_5]\text{pyridine}$ at 400 MHz, the ^1H NMR spectrum of the reaction mixture most characteristically reveals a CH_2 resonance at $\delta = 3.04$, identical to that found for an authentic sample of pure benzyliumpotassium. Free amine is also observed in the spectrum at $\delta = 1.13$ ($t\text{Bu}$), confirming that it is a by-product of the arene deprotonation process, that is, $t\text{BuN(H)}^-$ is the active base unit within the superbases molecule.

Large-Pore Mesoporous Materials with Semi-Crystalline Zeolitic Frameworks**

Do Trong On and Serge Kaliaguine*

Mesoporous molecular sieves with well-defined pore diameters of 20–500 Å^[1–3] overcome the pore diameter constraint of zeolites (<10 Å)^[4] and allow the diffusion of larger molecules. Furthermore, high surface area and tunable pore size are among the many desirable properties that have made such materials the focus of great interest. However, these materials do not show short-range order and, from this point of view, are closer to amorphous than zeolitic materials. As a consequence, the mesoporous silica–alumina have sites that are less acidic than those of zeolites, and do not exhibit the spectacular catalytic properties of acidic zeolites. Moreover, their hydrothermal stability is low and their industrial use as catalysts is rather limited to date.^[1–3, 5, 6] Therefore, to upgrade the performances of mesoporous molecular sieves and zeolites, attempts have been made to synthesize a new type of material, which would combine the advantages of these two kinds of molecular sieves. To date, only a few articles concerning this study have been published.^[7–12] For example, Kloetstra et al.^[7] prepared MCM-41/FAU composites which gave a clear improvement in catalytic activity in the cracking of vacuum gasoil. Huang et al.^[9] reported the synthesis of an MCM-41/ZSM-5 composite containing an interconnected mesopore and micropore structure. Attempts to crystallize the mesopore walls of mesostructured materials resulted in the formation of materials with increased thermal stability and catalytic activity.^[10–14] A synthesis of a partially crystalline bimodal pore system with combined micro- and mesopores, for example, ITQ, has been achieved by delaminating the layered zeolite MCM-22 and ferrierite (FER).^[10] Entrapped unit cells of ZSM5 contained in Al-MCM-41 and Al-HMS were prepared by ion exchange of the mesoporous aluminosilicates with tetrapropylammonium cation (TPA⁺) as an MFI-structure directing agent and then digestion of the mesoporous materials in glycerol at 120 °C.^[11] Pinnavaia and

co-workers^[12, 17] and Zhang et al.^[18] recently reported steam-stable aluminosilicate mesostructures assembled from zeolite-type seeds such as zeolite Y, ZSM-5, and zeolite beta. In the latter cases, the X-ray diffraction (XRD) patterns did not show crystalline features. Evidence for the presence of zeolite units cells may however be provided by the acid catalytic activity of such materials toward cumene cracking.^[11, 12]

Herein, we describe a new general method for the production of a new type of material with semi-crystalline zeolitic mesopore walls. This procedure involves a templated solid-state secondary crystallization of zeolites starting from the amorphous mesoporous materials of corresponding elemental composition. It is important that the walls of the amorphous mesoporous precursor material are as thick as possible. References [2, 3] provide methods for the preparation of mesoporous precursors that are useful in this context including the synthesis and characterization of the mesoporous materials with semi-crystalline ZSM-5 walls by the use of poly(alkylene oxide) triblock copolymer HO(CH₂CH₂O)₂₀(CH₂CH(CH₃)O)₇₀(CH₂CH₂O)₂₀H (designated as EO₂₀PO₇₀EO₂₀ or Pluronic P-123, BASF) and tetrapropylammonium hydroxide (TPAOH) as the surfactant and zeolite template, respectively.

Table 1 summarizes the physico-chemical properties of the series of samples obtained after different lengths of crystallization time and designated as [x]UL-ZSM-5, where *x* is crystallization time in days (*x*=0 is the sample before crystallization). The zeolite ZSM-5 was used as a reference. Reliable information about the effect of crystallization conditions on the mesopore structure as well as the crystalline phase has been obtained from nitrogen adsorption experiments, transmission electron micrograph (TEM) images, and from XRD patterns.

The nitrogen adsorption/desorption isotherm from the calcined [2]UL-ZSM-5 sample after 2 days of crystallization at 130 °C, exhibits typical type IV adsorption/desorption isotherm shape with a H₁ hysteresis loop and steep rises at low relative *P/P*₀ pressure indicating the presence of both mesopore and micropore structures (Figure 1). The micro-

Table 1. Physico-chemical properties of UL-ZSM-5^[a] and ZSM-5.^[b]

Material	<i>S</i> _{BET} [m ² g ^{−1}]	<i>S</i> _{BJH} [m ² g ^{−1}]	<i>S</i> _{micropore} [m ² g ^{−1}]	Micropore volume [cm ³ g ^{−1}]	Mesopore volume [cm ³ g ^{−1}]	Mesopore diameter [Å]	Crystallinity [%]
[0]UL-ZSM-5	865	745	120	0.020	1.25	56	–
[1]UL-ZSM-5	825	560	265	0.100	1.83	118	ca. 3
[2]UL-ZSM-5	645	275	370	0.145	1.86	205	14
[5]UL-ZSM-5	565	130	435	0.158	1.25	290	42
ZSM-5	490	35	465	0.195	–	–	100

[a] [x]UL-ZSM-5 where *x* = crystallization time in days (atomic Si/Al = 100/1). [b] ZSM-5 (atomic Si/Al = 57/1).

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pore and mesopore size distribution (MMSD) was calculated using Horváth–Kawazoe (HK) and Barrett–Joyner–Halenda (BJH) methods, respectively (Figure 1 insert) which show a narrow pore-size distribution of both micropores and mesopores with an average pore diameter of 5.7 and 205 Å, respectively. With increasing crystallization time, the samples exhibit nitrogen adsorption/desorption isotherms with similar inflection but a shift toward higher *P/P*₀ values over a larger

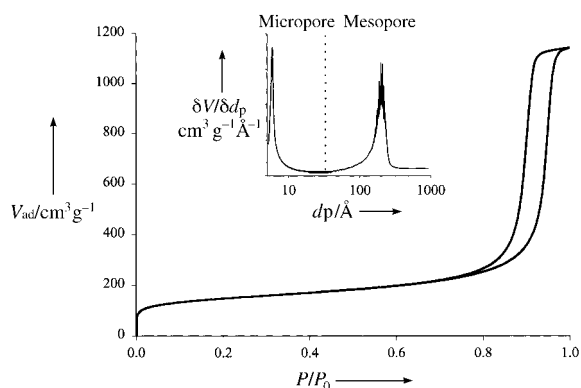


Figure 1. Adsorption/desorption isotherms of nitrogen at -196°C on the calcined [2]UL-ZSM-5 sample. Insert: HK micropore and BJH mesopore size distributions. d_p = pore diameter.

P/P_0 range. A significant increase in the pore diameter and a broader pore-size distribution were observed with increasing crystallization time, while the micropore volume increased from 0.020 to $0.158\text{ cm}^3\text{ g}^{-1}$ with no essential change in the micropore diameter (ca. $5.7\text{ \AA}$). The evolution of the MMSD is shown in Figure 2. The micropore diameter of ca. $5.7\text{ \AA}$ is comparable to the value known for the crystallographic aperture of the 10-membered-ring zeolite ZSM-5. Note that for highly crystalline ZSM-5, the micropore volume is approximately $0.20\text{ cm}^3\text{ g}^{-1}$ (see Figure 2e and Table 1). The increase in micropore volume suggests that nanoparticles of this zeolite have grown within the mesopore walls. Further-

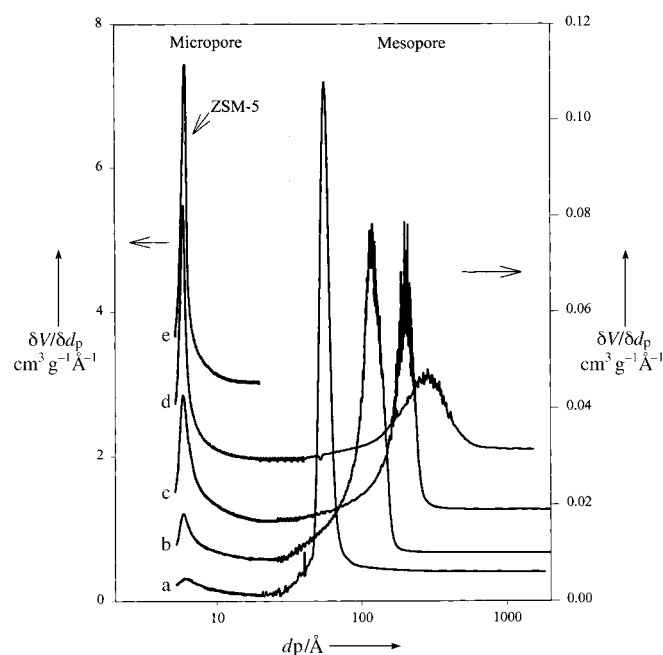


Figure 2. HK micropore and BJH mesopore size distributions for ZSM-5 and the [x]UL-ZSM-5 samples at different lengths of crystallization time: a) before crystallization, b) 1 day, c) 2 days, d) 5 days, and e) ZSM-5.

more, as the crystallization time is increased, the total surface area of the sample (S_{BET} ; BET = Brunauer–Emmet–Teller) varies from 865 to $565\text{ m}^2\text{ g}^{-1}$ and the mesopore surface area (S_{BJH}) varies from 745 to $130\text{ m}^2\text{ g}^{-1}$. Concomitantly, the

micropore surface area ($S_{\text{micropore}}$) increases from 120 to $435\text{ m}^2\text{ g}^{-1}$ for this series of samples (Table 1). This indicates some modification of the tubular channels of these materials during crystallization.

The crystalline phase in [x]UL-ZSM-5 upon crystallization was characterized by wide-angle XRD diffractograms, as shown in Figure 3. The mesoporous precursor with amorphous walls (Figure 3a) provides a starting material from

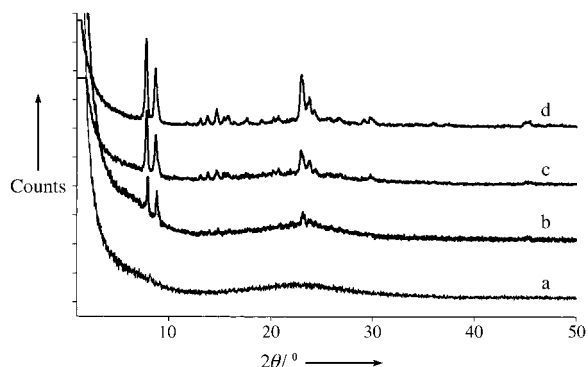


Figure 3. XRD patterns of the calcined [x]UL-ZSM-5 samples after different lengths of crystallization time: a) before crystallization, b) 1 day, c) 2 days, and d) 5 days.

which nano-crystalline domains can nucleate within the walls. The XRD diffractogram of the calcined sample in Figure 3b shows broad peaks, which match those of ZSM-5. These peaks grow in intensity as the crystallization time is increased (Figure 3c, d). By considering ZSM-5 as being 100% crystalline, the crystallinity of the samples was determined (Table 1). About 42% crystallinity was reached after 5 days of crystallization at 130°C . These data indicate that the initially amorphous walls of the mesoporous material are progressively transformed into crystalline nanoparticles. Furthermore, the [5]UL-ZSM-5 sample shows an FT-infrared (IR) absorption band at $561/547\text{ cm}^{-1}$ (doublet) which is not present in the mesoporous precursor (the FT-IR spectra are not shown). This band around 550 cm^{-1} has been assigned to the asymmetric stretching mode in five-membered ring blocks; the splitting of this lattice-sensitive band into a doublet at $561/547\text{ cm}^{-1}$ is characteristic of nanocrystals of the MFI structure (ZSM-5).^[13]

A TEM image of the precursor sample before crystallization is shown in Figure 4 and exhibits a uniform pore size with a highly disordered pore structure. This is reminiscent of MSU-1^[14] and KIT-1,^[15] which have wormhole-type pore structures. Figures 5A and 5B show bright- and dark-field TEM images recorded on the same area of [5]UL-ZSM-5, the bright spots in the image correspond to ZSM-5 nanocrystals. The nanocrystals are embedded in the continuous amorphous inorganic matrix to form semicrystalline wall structures while preserving the mesoporous structure. The wormhole pore lattice is however still present and micro-domains of the order of $10\text{--}30\text{ nm}$ are observed. This indicates that the pore walls consist of nanocrystals of this zeolite. However, some agglomerates of segregated zeolite crystals, as seen from the dark field images, indicate that the synthesis procedure could

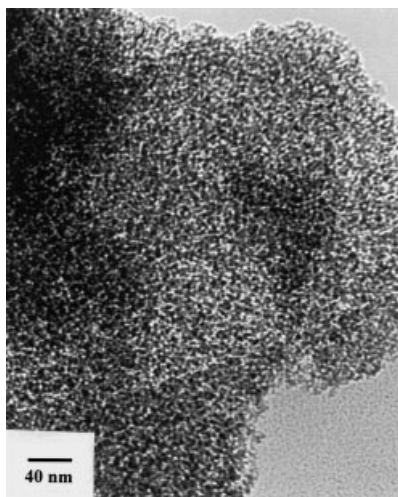


Figure 4. TEM image of the calcined [0]UL-ZSM-5 sample.

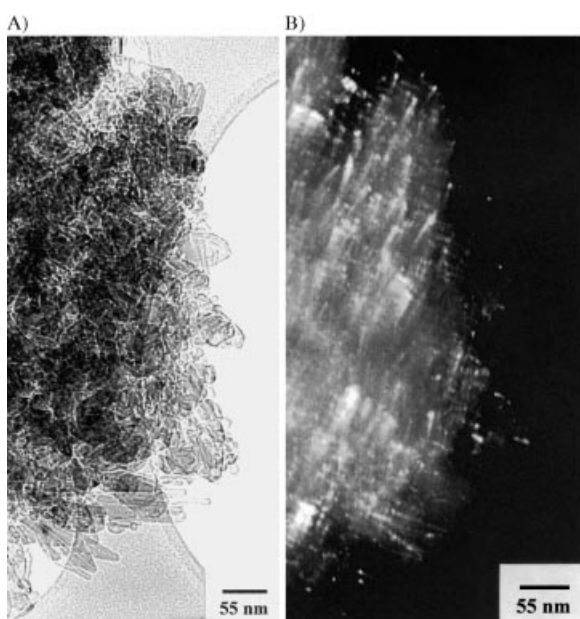


Figure 5. Bright-field (A) and dark-field (B) TEM images of the same area of the [5]UL-ZSM-5 sample.

still be optimized to generate products containing uniform nanocrystals within the mesopore walls. The ^{27}Al MAS NMR spectra of the samples exhibit a single resonance centered at $\delta = 54\text{--}58$, which is characteristic of tetrahedral aluminum. To determine the acidity, pyridine adsorption/desorption experiments were performed. The IR spectra of the pyridine adsorbed on the samples before and after crystallization for 5 days, and of ZSM-5 (atomic Si/Al = 57/1) as the reference, are shown in Figure 6. For the [0]UL-ZSM-5 sample after evacuation at 150°C , two very weak IR bands at 1547 cm^{-1} and 1455 cm^{-1} , which are characteristic of Brønsted and Lewis-acid sites respectively,^[16] are observed. However, the FT-IR spectra of the [5]UL-ZSM-5 and ZSM-5 samples show two intense bands at $1547, 1455\text{ cm}^{-1}$ which were retained even after evacuation at 200°C , while these bands disappeared in the [0]UL-ZSM-5 sample. This suggests a much stronger acidity in [5]UL-ZSM-5 and ZSM-5 than in [0]UL-

ZSM-5. The order of the acid strength was found to be $\text{ZSM-5} > [\text{5}] \text{UL-ZSM-5} \gg [\text{0}] \text{UL-ZSM-5}$ and it opens a new possibility of using this new type of material as acid catalysts.

The highly dispersed amorphous material of a mesoporous molecular sieve as a precursor phase was used to generate unusual mesostructured semizeolitic materials (UL-zeolites) by secondary solid-phase crystallization. The controlled size of the pore walls is taken advantage of, in controlling the crystal size of the zeolite product. For example, in the case of sample [5]UL-ZSM-5, Figure 5B indicates an average nanoparticle diameter on the order of 5 nm. This approach is not limited to the use of ZSM-5 type zeolites. In this process, zeolite nanoparticles are grown from the mesopore walls and at the same time the end product retains some of the mesopore surface of the precursor. As a consequence a rather high mesoporous surface is composed of the zeolite particles so that this zeolite surface is accessible to large molecules. It is thus expected that these materials will allow the typical acid reactions catalyzed by ZSM-5 but for large molecules. Actually, such effects are also expected with all UL-zeolites, which should allow catalysis to be extended to larger molecules, not converted in standard zeolites because of spatial restrictions. Moreover, the relatively short diffusion pathways through the thin walls are expected to improve mass transfer and catalytic reaction efficiency and minimize channel blocking. Detailed studies of the sorption behavior and catalytic properties are currently underway.

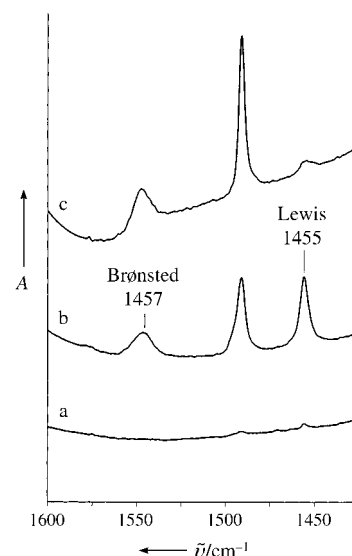


Figure 6. FT-IR spectra of the adsorbed pyridine in the $1350\text{--}1800\text{ cm}^{-1}$ range after pyridine desorption at 150°C : a) [0]UL-ZSM-5, b) [5]UL-ZSM-5, and c) ZSM-5.

Experimental Section

Synthesis: A series of materials, namely UL-ZSM-5, with atomic ratio Si/Al of 100/1 was prepared using poly(alkylene oxide) triblock copolymer $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_{20}(\text{CH}_2\text{CH}(\text{CH}_3)\text{O})_{70}(\text{CH}_2\text{CH}_2\text{O})_{20}\text{H}$ and tetrapropylammonium hydroxide as surfactant and zeolite template, respectively. The synthesis of the mesoporous zeolitic materials consists of two steps: 1) preparation of the amorphous mesoporous precursor followed by 2) transformation of the amorphous mesopore walls into crystalline walls.

A typical synthesis procedure was as follows: Step 1: An amorphous mesoporous aluminum containing silica (Si/Al = 100/1) was synthesized using SiCl_4 and AlCl_3 in ethanol, as in ref. [3]. The surfactant-containing mesoporous solid products were recovered and air-dried at room temperature. Step 2: the dried surfactant-containing mesoporous precursor was impregnated with a 10 wt% solution of TPAOH (free from inorganic alkali). The Si/TPAOH ratio was 8/1. After aging at room temperature, the solid was heated at 60 °C to eliminate water, and dried under vacuum for about 24 h at room temperature. Finally, the solid was transferred into a Teflon-lined autoclave and heated at 130 °C for different lengths of time. It is considered that the quantity of water adsorbed on the solid plays an important role in the crystallization process. Therefore, the partly crystalline solid was further crystallized at the same temperature for a given time after introducing a small amount of water. Because the solid-state crystallization continues in the presence of this small amount of water, the above process permits control of the crystallinity and the mesopore size of the solid materials. The products were washed with distilled water, dried in air at 80 °C, and finally calcined at 550 °C for 6 h to remove the organics.

Characterization: Powder XRD patterns of the materials were recorded on a Philips X-ray diffractometer (PW 1010 generator and PW 1050 computer-assisted goniometer) using nickel-filtered $\text{Cu}_{K\alpha}$ ($\lambda = 1.5406 \text{ \AA}$) radiation, 0.025° step size and a 1 s step time. Nitrogen adsorption and desorption isotherms at -196°C were established using an Omnisorp-100 apparatus. The specific surface area (S_{BET}) was determined from the linear part of the BET equation ($P/P_0 = 0.05-0.15$). The micropore size distribution was calculated from argon adsorption isotherms with the Horváth–Kawazoe method. The calculation of the mesopore-size distribution was performed using the desorption branch of the N_2 adsorption/desorption isotherms and the BJH formula. The mesopore surface area (S_{BJH}) and mesopore volume (V_{BJH}) were obtained from the pore-size distribution curves. The average mesopore diameter, d_p , was calculated as $4V_{\text{BJH}}/S_{\text{BJH}}$. Although its accuracy is limited, the BJH method, which is still universally utilized in the mesoporous molecular sieves (MMS) literature yields results that are comparable with the current literature values. High-resolution TEM images were obtained on a JEOL 200 CX transmission electron microscope operated at 120 kV. The samples for TEM were prepared by dispersing the fine powders of the products in a slurry in ethanol onto honeycomb carbon copper grids. Solid-state ^{27}Al and ^{29}Si MAS NMR spectra were recorded at room temperature on a Bruker ASX 300 spectrometer.

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A Redox Switch Based on Dihydro[5]helicene: Drastic Chiroptical Response Induced by Reversible C–C Bond Making/Breaking upon Electron Transfer**

Jun-ichi Nishida, Takanori Suzuki,* Masakazu Ohkita, and Takashi Tsuji

Helicenes are a special series of chiral molecules with huge optical activities, and thus attract considerable attention for use as nonlinear optical (NLO) materials and asymmetric catalysts.^[1] Chiroptical photoswitches that function by modulating the helical geometries of helicenes by light have also been constructed.^[2] However, the electron-transfer (ET) reaction of chiral helicenes has not been reported although they can serve as novel “electrochiroptical” transducers when redox-active groups are incorporated. We have found that dihydro[5]helicenes **1** which contain two electron-donating spiro rings undergo reversible C–C bond breaking to give

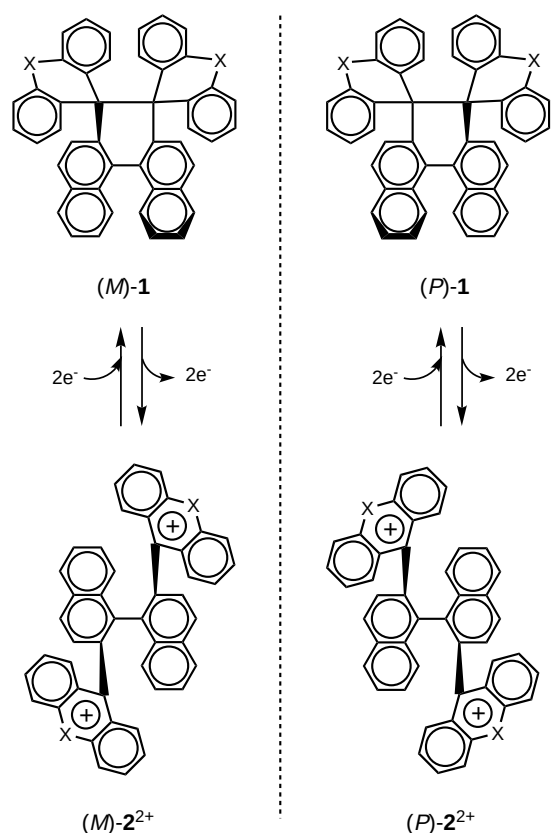
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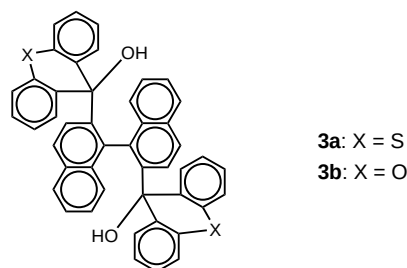
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binaphthyl dications 2^{2+} (Scheme 1). Furthermore, drastic changes of circular dichroism (CD) occurred during the interconversion between optically active **1** and 2^{2+} , thus demonstrating a new entry into the rare chiroptical redox switches.^[3]



Scheme 1. Interconversion between chiral dihydro[5]helicene **1** and the binaphthyl dications 2^{2+} ; **1a**, $2a^{2+}$ X = S, **1b**, $2b^{2+}$ X = O.

Diols **3a**^[4] and **3b**^[4] were prepared in 55 and 62% yield, respectively, by the reaction of 2,2'-dilithiobinaphthyl^[5] with thioxanthone or xanthone. Deeply colored salts $2a^{2+}(\text{BF}_4^-)_2$



and $2b^{2+}(\text{BF}_4^-)_2$ ^[4] were obtained in 96 and 97% yield, respectively, by treating these diols with HBF_4 in $(\text{EtCO})_2\text{O}$. The single-crystal X-ray analysis on $2a^{2+}(\text{BF}_4^-)_2$ shows that the distance between the two methylenium carbon atoms is 3.53 Å (Figure 1a);^[6] reduction with zinc led to formation a very long C–C bond between these methylenium carbon atoms (1.651(6) Å for **1a**; Figure 1b) to afford bridged 1,1'-

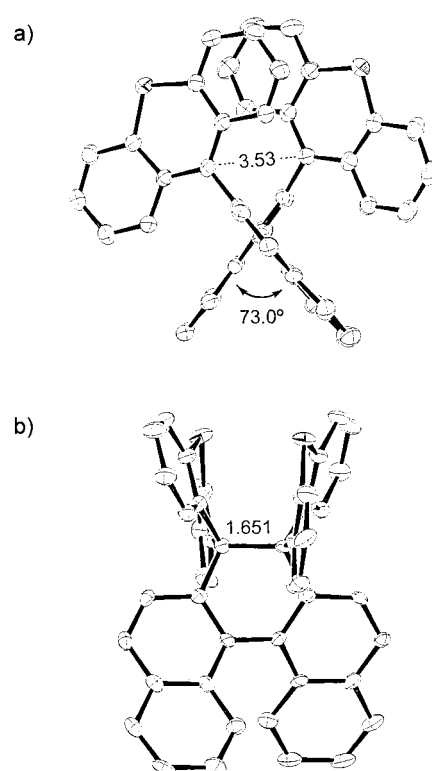


Figure 1. a) Molecular structure of $2a^{2+}$. The dication has a pseudo- C_2 symmetry and the molecular halves in $2a^{2+}$ are twisted by 73.0° around the binaphthyl axis. The two cationic planes overlap in a face-to-face manner (interplanar distance 3.36 Å; dihedral angle 4.5°). b) Molecular structure of **1a**. The dihedral angle between two naphthalene units is 37.1° .

binaphthyls **1a**^[4] and **1b**^[4] in 92 and 96% yield, respectively. Oxidation of **1a** and **1b** with 2 equivalents of $(p\text{-BrC}_6\text{H}_4)_3\text{N}^+\text{SbCl}_6^-$ led to fission of the weak C–C bond to regenerate the dications $2a^{2+}$ and $2b^{2+}$, which were isolated as SbCl_6^- salts in 89 and 81% yield, respectively. The high-yield interconversion between **1** and 2^{2+} indicates that they constitute “reversible” redox pairs, in which the bond making/breaking are induced upon ET. Furthermore, dynamic structural change upon ET causes a large separation of redox potentials of **1** and 2^{2+} (Figure 2, Table 1), thus endowing the present system with high electrochemical bistability.^[7]

Optical resolution was carried out by transforming $2a^{2+}$, $2b^{2+}$ into pairs of neutral, diastereomeric ethers (*P*)-**4a**, **4b**^[4, 8] and (*M*)-**5a**, **5b**^[4, 8] with (*R*)-1,3-butanediol in the presence of pyridine. The absolute configurations of the binaphthyl moieties were determined by X-ray analyses of (*P*)-**4a** and (*M*)-**5b**.^[6] By treating these ethers with HBF_4 then zinc, chiral dihydro[5]helicenes (*P*)- and (*M*)-**1** which have relatively large optical rotations because of their helicene-type structure were prepared (Table 2). Resolved **1a**, **b** and $2a^{2+}$, $2b^{2+}$ show no indication of racemization at room temperature.

The electrochemical response was examined by UV/Vis spectroscopy. As shown in Figure 3a, on interconversion, the redox pairs exhibit electrochromism with a vivid color change; the helicenes **1** show absorption only in the UV region, whereas for the dicationic dyes 2^{2+} the absorption bands occur in the visible region (Table 2). The isosbestic

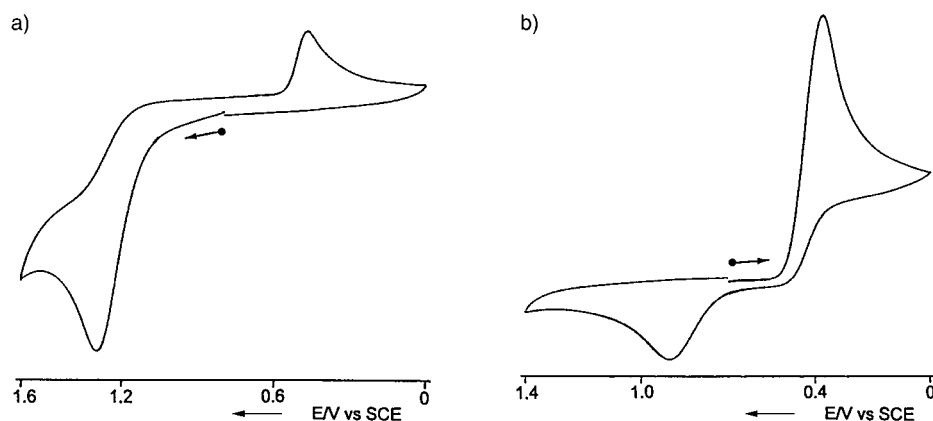


Figure 2. a) Cyclic voltammogram of **1b** in CH_2Cl_2 . The reduction peak at +0.46 V was absent when the voltammogram was first scanned in the cathodic direction. b) Cyclic voltammogram of $\text{2a}^{2+}(\text{BF}_4^-)_2$ in CH_2Cl_2 . The oxidation peak at +0.93 V was absent when the voltammogram was first scanned in the anodic direction. Voltammograms were measured under the conditions shown in Table 1; SCE = standard calomel electrode.

Table 1. Redox potentials of **1** and $\text{2}^{2+}(\text{BF}_4^-)_2$.^[a]

	E_p^{ox}	E_p^{red}
1a	+0.93 V	
1b	+1.30 V	
2a^{2+}		+0.37 V
2b^{2+}		+0.46 V

[a] Peak potentials in Volts versus SCE; solvent: CH_2Cl_2 ; electrolyte: $0.1 \text{ mol dm}^{-3} \text{ nBu}_4\text{NBF}_4$; Pt electrode, scan rate 100 mV s^{-1} . Voltammograms were unchanged on measurement at a scan rate of 500 mV s^{-1} .

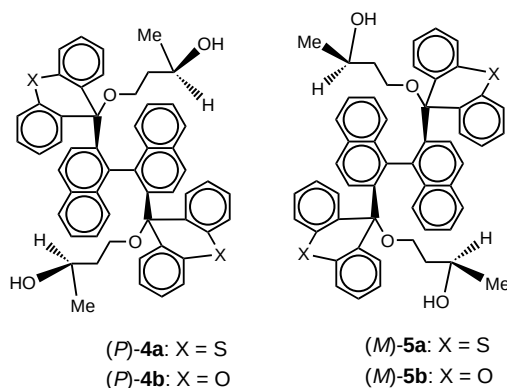


Table 2. Optical rotation for **1**, UV/Vis and CD data for **1** and $\text{2a}^{2+}(\text{BF}_4^-)_2$.

$[\alpha]_D^{27}$ (c) in CHCl_3 : (**P-1a**): +677°(0.50); (**M-1a**): −678°(0.58); (**P-1b**): +642°(0.16); (**M-1b**): −634°(0.62).

UV/Vis λ_{max} (lge) in MeCN: **1a**: 358 sh (4.01), 345 (4.08), 266 (4.65), 219 nm (4.97); **1b**: 358 (3.99), 344 (4.03), 291 (4.08), 255 (4.69), 219 nm (4.98); $\text{2a}^{2+}(\text{BF}_4^-)_2$: 551 sh (3.86), 516 (3.92), 388 (4.33), 288 (4.92), 227 nm (5.02); $\text{2b}^{2+}(\text{BF}_4^-)_2$: 502 sh (3.86), 382 (3.91), 382 (4.60), 263 (4.77), 225 nm (5.06).
CD λ ($\Delta\epsilon$) in MeCN: (**P-1a**): 381 (−0.76) 364 (+4.75), 336 (−11.4), 303 (+33.0), 270 (+82.0), 250 nm (+91.1); (**M-1a**): 381 (+0.83) 364 (−4.68), 336 (−11.5), 303 (−32.1), 270 (−79.4), 250 nm (−89.1); (**P-1b**): 338 (+14.3), 305 (−8.27), 264 (+96.8), 227 nm (+137); (**M-1b**): 338 (−13.4), 305 (+9.31), 264 (−93.1), 227 nm (−140); (**P-2a**) $^{2+}(\text{BF}_4^-)_2$: 503 (−19.0), 394 (−30.7), 362 (+11.4), 288 (−120), 261 (+49.3) 227 nm (+231); (**M-2a**) $^{2+}(\text{BF}_4^-)_2$: 503 (+19.3), 394 (+30.6), 362 (−10.9), 288 (+121), 261 (−47.2), 227 nm (−227); (**P-2b**) $^{2+}(\text{BF}_4^-)_2$: 466 (−17.1), 388 (−85.6), 355 (+43.3), 275 (+11.7), 255 (−44.6) 225 nm (+180); (**M-2b**) $^{2+}(\text{BF}_4^-)_2$: 466 (+17.1), 388 (+85.2), 355 (−42.4), 275 (−10.8), 255 (+46.9) 225 nm (−180).

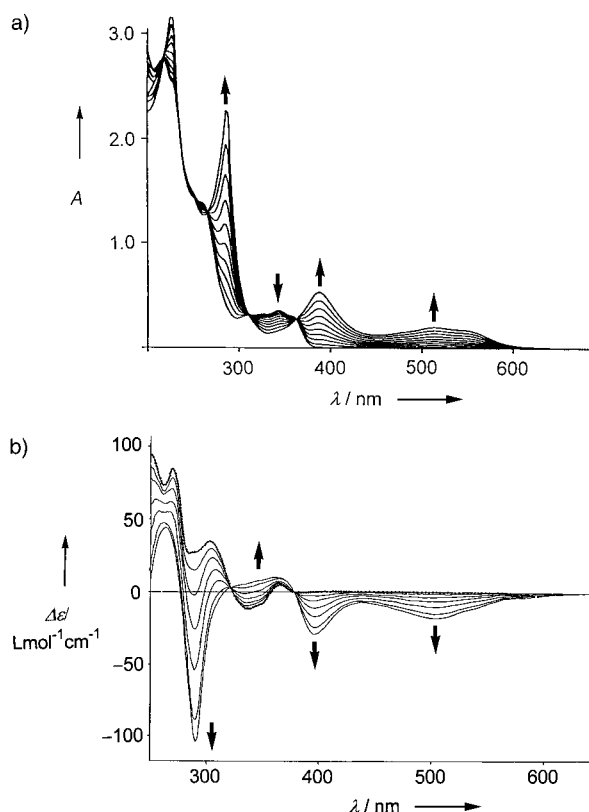


Figure 3. a) Continuous change in the UV/Vis spectrum of **1a** (3.0 mL; $2.96 \times 10^{-5} \text{ mol dm}^{-3}$ in MeCN containing $0.05 \text{ mol dm}^{-3} \text{ nBu}_4\text{NBF}_4$) upon constant-current electrochemical oxidation (26 μA) at 10 min intervals. b) Continuous change in the CD spectrum of (**P-1a**) (3.0 mL; $3.90 \times 10^{-5} \text{ mol dm}^{-3}$ in MeCN containing $0.05 \text{ mol dm}^{-3} \text{ nBu}_4\text{NBF}_4$) upon constant-current electrochemical oxidation (26 μA) at 10 min intervals. The dotted line indicates the spectrum before electrolysis.

$140 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$. Moreover, presence of isosbestic points (322, 378 nm) indicates the clean interconversion for the chiroptical response.

To conclude this is the first example of chiral helicenes where helicity and the axial chirality of biaryls are reversibly interconverted in a process controlled by external inputs.

Further studies of chiroptical redox switches based on this novel approach are now in progress.

Received: April 5, 2001 [Z16905]

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- [8] Although chirality of binaphthyl derivatives is commonly designated by (*R*)/(*S*), stereochemistry of **2**²⁺, **4**, and **5** is shown by (*P*) or (*M*), which indicates that the configuration corresponds to the binaphthyl framework of (*P*)-**1** or (*M*)-**1**, respectively.

COVER PICTURE

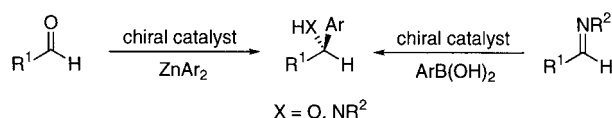
The cover picture shows that chemical and biological knowledge need to be linked more to ensure that highly effective and target-specific drugs can be found more quickly. Additionally, the main and side effects must be controlled in the best interests of the individual patient. Despite the intensive use of high-throughput technologies in drug research, despite new insight in genomic and proteomic research and in structural biology, and despite the progress made in bio- and chemoinformatics, there is still a worldwide shortage of new and innovative drugs. The bottleneck in lead-structure generation lies in the preparation of new biologically relevant substances and therefore to a major extent in chemistry. The way medicinal chemists perceive their subject and its role in the natural sciences as well as in the value chain of drug research is crucial to counteract this shortage, as explained by Wess et al. on page 3341–3350.



REVIEWS

Contents

The asymmetric catalytic aryl transfer to prochiral substrates expands the scope of enantioselective C–C bond-forming reactions. Previously, variants of palladium-catalyzed cross-coupling reactions mostly allowed for asymmetric aryl transfer. In recent times, however, this picture has changed significantly. This overview demonstrates that not only are asymmetric cross-couplings available to the synthetic organic chemist today, but that there are various other transformations for the enantioselective introduction of aryl groups (see scheme).



Angew. Chem. 2001, 113, 3382–3407

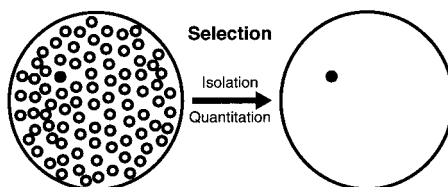
C. Bolm,* J. P. Hildebrand, K. Muñiz,
N. Hermanns 3284–3308

Catalyzed Asymmetric Arylation
Reactions

Keywords: arylation • asymmetric
synthesis • homogeneous catalysis •
transition metals

If you were to ask preparative chemists whether they would be interested in a method that rapidly and efficiently isolates a desired product from a reaction mixture, or analytical chemists whether they would use a technique for quantitative analysis that gives large, clear signals and practically no noise, the answers would likely be a resounding “Yes!”. Genetic selection (see schematic representation) is an approach that is useful for biological chemists and can provide both advantages. This review is an overview of the use of selection systems in enzymology, structural analysis, and protein (re)design.

Angew. Chem. **2001**, *113*, 3408–3436



S. V. Taylor, P. Kast,
D. Hilvert* 3310–3335

Investigating and Engineering Enzymes
by Genetic Selection

Keywords: directed evolution • enzyme catalysis • enzymes • genetic selection • protein design • protein structures

ESSAYS

The set of molecules assembled during a combinatorial experiment, is the antithesis of a library, says the author. Creating all combinations is a way of special significance for some, but is devoid of meaning for others—this debate has a venerable history. Playing with flowers and chocolates, his all too tardy gift to the talented concoctors of combinatorial pools, is a new, less pretentious, name.

Angew. Chem. **2001**, *113*, 3439–3443

R. Hoffmann* 3337–3340

Not a Library

Keywords: combinatorial chemistry

The sequencing of the human genome has led to a wealth of new pharmaceutical targets. However, medicinal chemistry has proved to be a bottleneck in the search for active drugs. This bottleneck can be overcome by chemical biology, which is crucial in the transition from a trial-and-error mentality to a predictive approach.

Angew. Chem. **2001**, *113*, 3443–3453

G. Wess,* M. Urmann,
B. Sickenberger 3341–3350

Medicinal Chemistry: Challenges and
Opportunities

Keywords: chemical biology • combinatorial chemistry • drug research • medicinal chemistry

VIPs

The following communications are “Very Important Papers” in the opinion of two referees. They will be published shortly (those marked with a diamond will be published in the next issue). Short summaries of these articles can be found on the *Angewandte Chemie* homepage at the address <http://www.angewandte.com>

High Turnover Numbers for Catalytic Selective Epoxidation of Alkenes with 1 atm Molecular Oxygen

Y. Nishiyama, Y. Nakagawa,
N. Mizuno* ◆

Experimental Detection of Tetraoxygen

F. Cacace,* G. de Petris,
A. Troiani

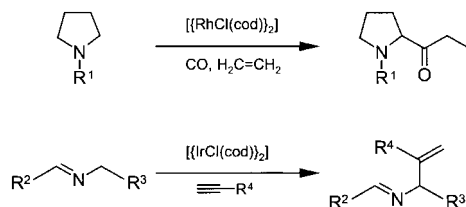
[12.12]Paracyclophanedodecaynes $C_{36}H_8$ and $C_{36}Cl_8$: The Smallest Paracyclophynes and Their Transformation into the Carbon Cluster Ion C_{36}^-

Y. Tobe,* R. Furukawa,
M. Sonoda, T. Wakabayashi

Fluorescence Quenching via Sequential Hydrogen, Electron, and Proton Transfer in the Proximity of a Conical Intersection

A. Sinicropi, R. Pogni*,
R. Basosi, M. A. Robb,
G. Gramlich, W. M. Nau,*
M. Olivucci*

A very promising approach for the efficient synthesis of complex amines is provided by the transition metal catalyzed activation of sp^3 C–H bonds in the position α to a nitrogen atom. Two recent publications show that this approach can be realized impressively by the use of rhodium and iridium catalysts (see scheme; cod = 1,5-cyclooctadiene).



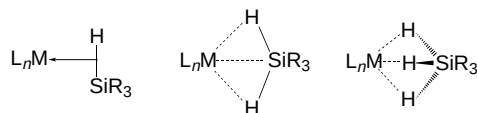
Angew. Chem. **2001**, *113*, 3455–3457

S. Doye * 3351–3353

Catalytic C–H Activation of sp^3 C–H Bonds in α -Position to a Nitrogen Atom—Two New Approaches

Keywords: carbonylation • C–H activation • homogeneous catalysis • iridium • rhodium

Dominated for two decades by agostic and σ complexes, the family of complexes with nonclassical interligand interactions is rapidly extending to include new types of compounds. Multicenter $(\text{H})_2 \cdots \text{Si}$ and $(\text{H})_3 \cdots \text{Si}$ interactions have recently been documented in transition metal silicon chemistry (see scheme).



Angew. Chem. **2001**, *113*, 3457–3459

G. I. Nikonov * 3353–3355

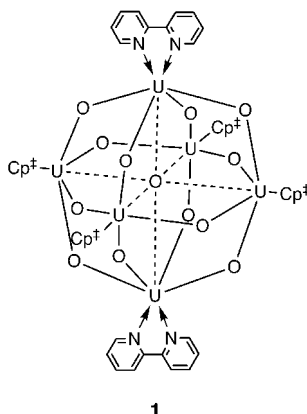
Going Beyond σ Complexation: Nonclassical Interligand Interactions of Silyl Groups with Two and More Hydrides

Keywords: bond theory • hydrido complexes • hypervalent compounds • nonclassical interactions • silicon

COMMUNICATIONS



Known for the Group 6 metals for over 100 years, the family of isopolyoxometalates has now been extended to include the first uranium derivative **1**, which contains a $[\text{U}_6\text{O}_{13}]$ core. In comparison to the well-studied transition metal analogues, the structural and electronic properties of **1** reflect the chemical attributes of the actinides. $\text{Cp}^+ = 1,2,4\text{-}t\text{Bu}_3\text{C}_5\text{H}_2$.



Angew. Chem. **2001**, *113*, 3462–3465

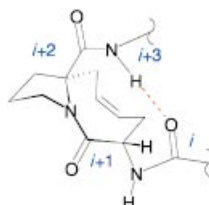
P. B. Duval, C. J. Burns,* D. L. Clark,*
D. E. Morris, B. L. Scott, J. D. Thompson,
E. L. Werkema, L. Jia,
R. A. Andersen 3358–3361

Synthesis and Structural Characterization of the First Uranium Cluster Containing an Isopolyoxometalate Core

Keywords: actinides • cluster compounds • polyoxometalates • uranium



A fruitful combination of molecular dynamics based design and modern synthetic reactions led to a conformationally rigidized *cis*-peptidyl proline surrogate (see picture). NMR spectroscopic experiments clearly show the existence of type VIa β -turn properties that are of interest in drug discovery.



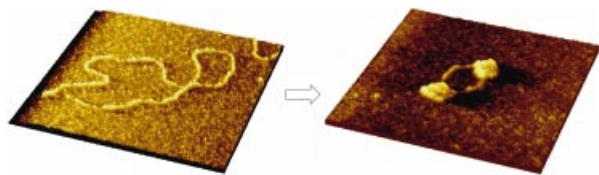
Angew. Chem. **2001**, *113*, 3465–3468

T. Hoffmann, H. Lanig,* R. Waibel,
P. Gmeiner * 3361–3364

Rational Molecular Design and EPC Synthesis of a Type VI β -Turn Inducing Peptide Mimetic

Keywords: β -turns • metathesis • molecular dynamics • peptidomimetics • semiempirical calculations

DNA condensation by a fullerene vector has been imaged at the molecular level by atomic force microscopy (AFM); analysis of a mixture of plasmid DNA and DNA-binding fullerene with the aid of this carbonaceous vector provided the first information on the mechanism of transfection. The picture shows AFM images of pBR322 DNA in the absence of (left) and partially condensed by the fullerene (right).



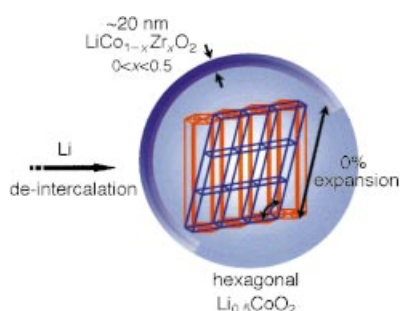
Angew. Chem. **2001**, *113*, 3468–3471

H. Isobe, S. Sugiyama, K.-i. Fukui,
Y. Iwasawa, E. Nakamura* 3364–3367

Atomic Force Microscope Studies on
Condensation of Plasmid DNA with
Functionalized Fullerenes

Keywords: DNA recognition •
fullerenes • polycations •
scanning probe microscopy • transfection

No strain, big gain: the possibility of producing more-efficient rechargeable lithium batteries is offered by a zero-strain LiCoO_2 cathode material produced by coating particles with thin films of high-fracture-toughness oxides. This method suppresses the changes in the lattice constant during electrochemical cycling and thereby suppresses phase transitions (see scheme). The order of capacity retention correlates with the fracture toughness of the coating thin-film oxides. The ZrO_2 -coated sample does not show any noticeable capacity fading over 70 electrochemical cycles.



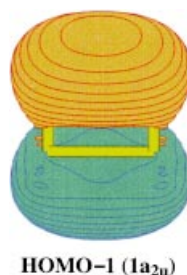
Angew. Chem. **2001**, *113*, 3471–3473

J. Cho, Y. J. Kim, T.-J. Kim,
B. Park* 3367–3369

Zero-Strain Intercalation Cathode for
Rechargeable Li-Ion Cell

Keywords: electrochemistry •
intercalation • lithium • thin films •
zirconium

Mercury forms a wide range of alloys (amalgams), some of which are known from ancient times. Square-planar Hg_4^{6-} units are known to be particularly favorable building blocks in many amalgams. It is reported here that the unusual Hg_4^{6-} square is aromatic (the picture shows the HOMO – 1 ($1a_{2u}$) molecular orbital), analogous to the recently discovered all-metal Al_4^{2-} ion.



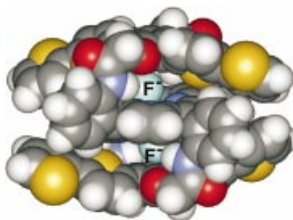
Angew. Chem. **2001**, *113*, 3473–3476

A. E. Kuznetsov, J. D. Corbett,*
L.-S. Wang,* A. I. Boldyrev* 3369–3372

Aromatic Mercury Clusters in Ancient
Amalgams

Keywords: ab initio calculations •
amalgams • aromaticity •
chemical bonding • cluster compounds

 **Fluoride fills cavities and insulates polymers:** A doubly strapped porphyrin has been designed that is a highly specific receptor for fluoride ions (see picture, yellow: sulfur, red: oxygen, blue: nitrogen, and light blue: fluoride). The strap defines a small cavity about the porphyrin core, and fluoride ion was observed to bind with cooperative allostereism. Larger anions did not bind in the cavity or interfere with fluoride binding. The straps are composed of bis(dithienyl)phenyl groups that can be electropolymerized to give conducting polymers. Exposure of these polymers to fluoride ions results in a dramatic irreversible reduction in conductivity.



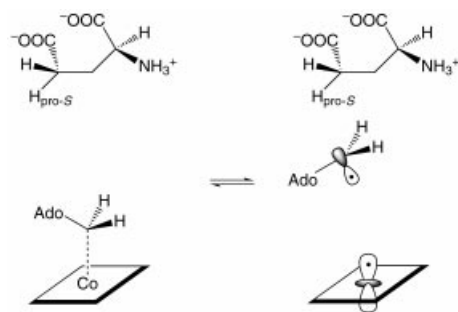
Angew. Chem. **2001**, *113*, 3476–3480

M. Takeuchi, T. Shioya,
T. M. Swager* 3372–3376

Allosteric Fluoride Anion Recognition by
a Doubly Strapped Porphyrin

Keywords: conducting materials •
fluoride ions • molecular recognition •
polymers • porphyrinoids

Two alternate conformations of the adenosyl ligand of the cofactor are observed in the active site of the coenzyme B₁₂ dependent enzyme glutamate mutase. This result shows ribose pseudorotation to be an elegant and safe mechanism for shuttling the “hot” methylene radical between the cofactor and substrate (see scheme).



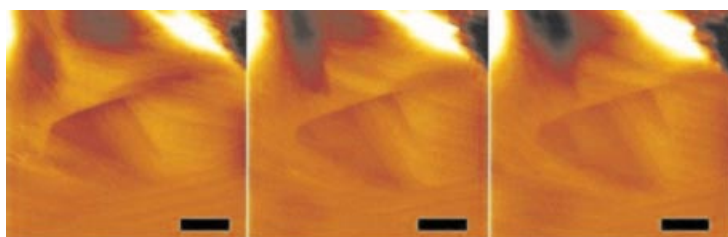
Angew. Chem. **2001**, *113*, 3481–3484

K. Gruber,* R. Reitzer,
C. Kratky 3377–3380

Radical Shuttling in a Protein: Ribose Pseudorotation Controls Alkyl-Radical Transfer in the Coenzyme B₁₂ Dependent Enzyme Glutamate Mutase

Keywords: enzyme catalysis • metalloenzymes • protein structures • radical reactions • reaction mechanisms

Etching edges: In situ measurement by atomic-force microscopy (AFM) is employed to investigate the dissolution of calcium phosphate crystals in the presence of different polymeric additives. The dissolution occurs in a way that is dependent on the pattern of functional groups on each polymer. The influence of poly(sodium)aspartate, polylysine, and serum albumins on the resulting crystal morphologies is discussed. The pictures show the dissolution of the (010) plane of brushite in the presence of polyaspartate (the bar = 1 μm).



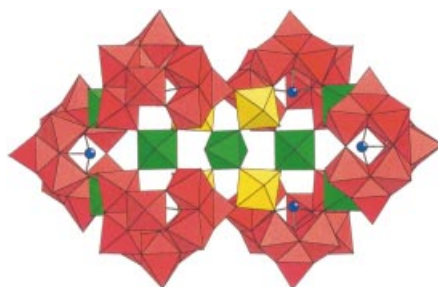
Angew. Chem. **2001**, *113*, 3484–3488

A. Peytcheva,
M. Antonietti* 3380–3383

“Carving on the Nanoscale”: Polymers for the Site-Specific Dissolution of Calcium Phosphate

Keywords: biomimetic synthesis • crystal engineering • molecular recognition • polymers

The largest tungstoarsenate(III) known to date is [As^{III}₆W₆₅O₂₁₇·(H₂O)₇]^{26−} (**1**). This novel polyoxoanion is isolated as its potassium salt in aqueous, acidic medium in good yield. Structural characterization is based on single-crystal X-ray diffraction, ¹⁸³W NMR, FT-IR spectroscopy, and elemental analysis. The compact structure of **1** with an idealized C_{2h} symmetry (see picture) consists of four inner (β-AsW₉O₃₃) and two outer (α-AsW₉O₃₃) fragments (red) that are linked together by a total of eleven corner-sharing WO₆ octahedra (green, yellow).



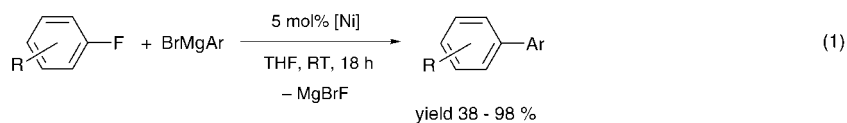
Angew. Chem. **2001**, *113*, 3488–3491

U. Kortz,* M. G. Savelieff, B. S. Bassil,
M. H. Dickman 3384–3386

A Large, Novel Polyoxotungstate:
[As^{III}₆W₆₅O₂₁₇(H₂O)₇]^{26−}

Keywords: arsenic • cluster compounds • polyoxometalates • self-assembly • tungsten

 **The activation of strong sp² C–F bonds** in catalytic C–C cross-coupling reactions with aryl Grignard reagents under mild conditions is possible: A nickel complex bearing an N-heterocyclic carbene ligand was identified as the most active catalyst for this reaction [Eq. (1)]. With the selective activation of aromatic C–F bonds over C–H bonds present, cross-coupling chemistry employing aryl halides has finally overcome its most challenging hurdle with regard to catalyst activity.



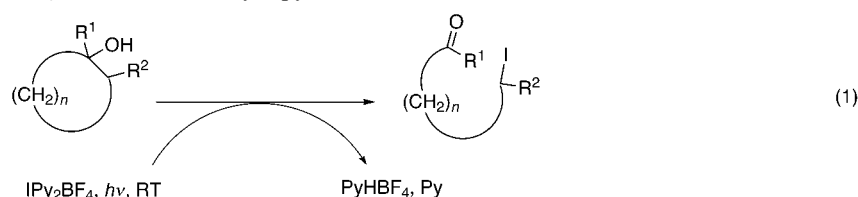
Angew. Chem. **2001**, *113*, 3500–3503

V. P. W. Böhm, C. W. K. Gstöttmayr,
T. Weskamp,
W. A. Herrmann* 3387–3389

Catalytic C–C Bond Formation through Selective Activation of C–F Bonds

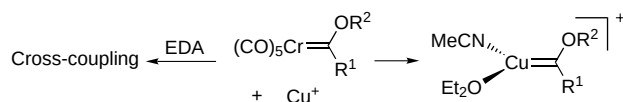
Keywords: biaryls • carbene ligands • C–C coupling • C–F activation • homogeneous catalysis • nickel

Simply mix and switch on the light: This is all that is required to obtain ω -functionalized aldehydes and ketones from readily available cyclic alcohols and IPy_2BF_4 . The unusual oxidation process is outlined in Equation (1). $\text{R}^1, \text{R}^2 = \text{H}, \text{CH}_3$; $n = 2, 3, 4, 10$; Py = pyridine.



Angew. Chem. **2001**, *113*, 3491–3494

The chromium–copper interaction is evidenced for chromium–carbene complexes and Cu^+ in the CuBr-catalyzed cross-coupling reaction of ethyl diazoacetate (EDA) and Fischer alkoxy carbene–chromium complexes (see scheme). In contrast, with $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$ (0.5 equiv) instead of CuBr a carbene ligand exchange between a chiral alkenylchromium carbene complex ($\text{R}^1 = (E)\text{-CH=CH-2-furyl}$, $\text{R}^2 = (1R, 2S, 5R)\text{-menthyl}$) and this Cu compound occurs at room temperature. The resulting novel trigonal-planar copper(I)–carbene complex, which contains MeCN and Et_2O as the remaining ligands, has been isolated and structurally characterized by X-ray diffraction.



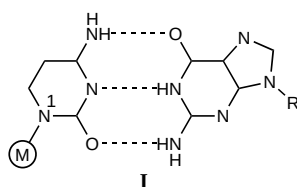
Angew. Chem. **2001**, *113*, 3495–3497

AlMe₃ and ethanethiol can be used to cleave peptides directly and efficiently from a variety of commercial resins to give C-terminal thioesters. Successful synthesis of the 37 amino acid long BPTI^{1–37}-SEt, an activated fragment used to prepare bovine pancreatic trypsin inhibitor (BPTI; see formula) by native chemical ligation, shows that even thioesters of long peptides of complex composition are accessible using standard resins and 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.

RPDFCLEPPYTGPCKARI IRYFYNAKAGLCQTFVYGG↓CRAKRN NFKSAEDCMRTCGGA

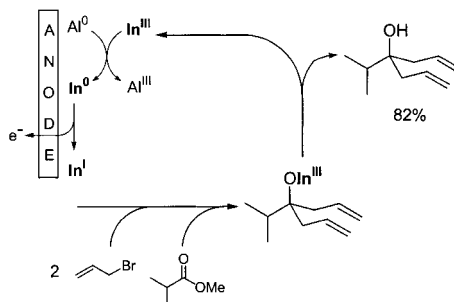
Angew. Chem. **2001**, *113*, 3503–3505

Stronger than the natural Watson–Crick base pairing is the association between N1-platinated cytosine and neutral, unplatinated guanine in DMSO. Nevertheless, in aqueous solution the Pt-containing associate **I** ($\text{M} = \text{Pt}$) is stable in a narrower pH range.



Angew. Chem. **2001**, *113*, 3497–3500

It seems almost paradoxical that reactive low-valent indium(I) species can be generated at the anode through reduction of indium(III) salts under electrochemical conditions (see scheme). The resulting indium(I) species are highly effective catalysts for the allylation of different aldehydes, ketones, and esters on a preparative scale.



Angew. Chem. **2001**, *113*, 3514–3516

J. Barluenga,* F. González-Bobes, S. R. Ananthoju, M. A. García-Martín, J. M. González 3389–3392

Oxidative Opening of Cycloalkanols:
An Efficient Entry to ω -Iodocarbonyl Compounds

Keywords: alcohols • cleavage reactions • iodine reagents • oxidation • radical reactions

J. Barluenga,* L. A. López, O. Löber, M. Tomás, S. García-Granda, C. Alvarez-Rúa, J. Borge ... 3392–3394

Chromium–Copper Exchange of Fischer Carbene Complexes: X-Ray Crystal Structure of a $[\text{Cu}\{\text{=CR}^1(\text{OR}^2)\}\text{-(MeCN)(Et}_2\text{O)}][\text{PF}_6]$ Complex

Keywords: carbene ligands • chromium • copper • cross-coupling • transmetalation

A. Sewing, D. Hilvert * 3395–3396

Fmoc-Compatible Solid-Phase Peptide Synthesis of Long C-Terminal Peptide Thioesters

Keywords: ligation • peptide fragment condensation • peptides • peptide thioester • solid-phase synthesis

W. Brüning, R. K. O. Sigel, E. Freisinger, B. Lippert * 3397–3399

Pt^{II} Binding to N1 of Cytosine:
Strengthening the Watson–Crick Pair with Guanine and yet Confining Its pH Existence Range

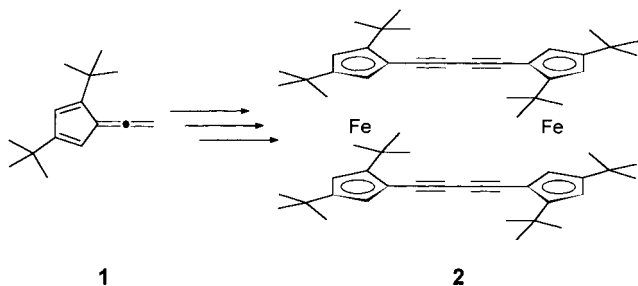
Keywords: hydrogen bonds • nucleobases • platinum

G. Hilt,* K. I. Smolko 3399–3402

Electrochemical Regeneration of Low-Valent Indium(I) Species as Catalysts for C–C Bond Formations

Keywords: allylation • aluminum • carbonyl compounds • electrochemistry • indium

Building butadiyne bridges: The kinetically stabilized 1,1'-diethynylferrocene, readily prepared from the pentafulvenoid allene **1**, can be transformed into the [4.4]ferrocenophane **2** by oxidative coupling. This compound exhibits a remarkably symmetrical structure in which the electrons are delocalized through the butadiyne bridges; in the crystalline state **2** exists in a helical-chiral conformation.



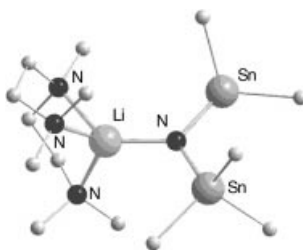
Angew. Chem. **2001**, *113*, 3517–3520

K. H. H. Fabian, H.-J. Lindner,
N. Nimmerfroth, K. Hafner* 3402–3405

Synthesis and Properties of the First
[4.4]Ferrocenophane-1,3,15,17-tetrayne

Keywords: alkyne complexes • alkynes •
C–C coupling • cumulenes • cyclic
voltammetry

Monomers as well as dimers have been established for the structures of *N*-lithiostannylamines in the solid state as well as in solution (the picture shows the crystal structure of the monomer [(Me₃Sn)₂NLi{(Me₂NCH₂CH₂)₂NMe}]). These compounds, which were synthesized for the first time from stannylamines with *n*-butyllithium, are remarkably reactive in solution despite having extremely short Li–N and Sn–N bonds. The selective and successive cleavage of the Li–N and Sn–N bonds under mild conditions holds great promise for their synthetic application.



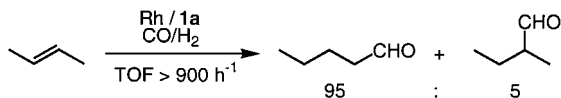
Angew. Chem. **2001**, *113*, 3511–3514

C. Neumann, T. Seifert, W. Storch,*
M. Vosteen,
B. Wrackmeyer* 3405–3407

New Building Blocks in Amide
Chemistry—*N*-Lithiobis(trimethylstannyl)-
amine and *N*-Lithiotrimethylstannyl-
(trimethylsilyl)amine

Keywords: amides • lithium • tin

Readily accessible and highly efficient: Substituted 2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl (NAPHOS) ligands **1** can be readily prepared from 2,2'-bis(dichlorophosphanyl)-1,1'-binaphthyl. Especially ligands with electron-withdrawing aryl substituents (for example, 3,4,5-F₃C₆H₂ (instead of Ph), **1a**) show a remarkable high activity and *n,i* selectivity in the rhodium-catalyzed hydroformylation of internal olefins (see scheme).



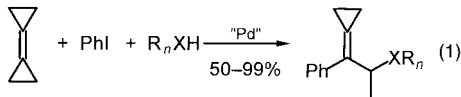
Angew. Chem. **2001**, *113*, 3505–3508

H. Klein, R. Jackstell, K.-D. Wiese,
C. Borgmann, M. Beller* .. 3408–3411

Highly Selective Catalyst Systems for the
Hydroformylation of Internal Olefins to
Linear Aldehydes

Keywords: homogeneous catalysis •
hydroformylation • P ligands • rhodium

The primary carbopalladation intermediate resulting under Heck conditions from bicyclopropylidene and phenyl iodide in the presence of tris(α -furyl)phosphane rearranges to give a π -allylpalladium complex which is efficiently trapped by various nucleophiles R_nXH. This rearrangement leads to substituted methylenecyclopropanes in good to excellent yields (42–99%) [Eq. (1); X = C (*n* = 3), N (*n* = 2), O (*n* = 1)]. The intermediate 1,1-dimethylenecyclopropylpalladium complex was isolated and fully characterized by an X-ray crystal-structure analysis.



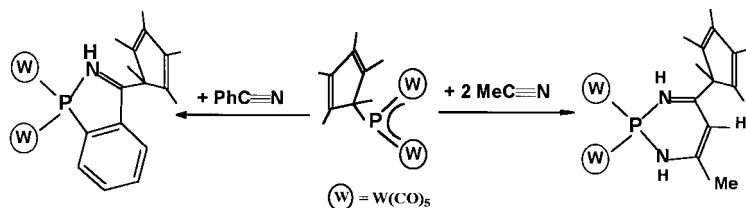
Angew. Chem. **2001**, *113*, 3509–3511

H. Nüske, M. Noltemeyer,
A. de Meijere* 3411–3413

Nucleophilic Trapping of π -
Allylpalladium Intermediates Generated
by Carbopalladation of
Bicyclopropylidene: A Novel Three-
Component Reaction

Keywords: domino reactions •
Heck reaction • multicomponent
reactions • palladium • small ring systems

Unexpectedly, nitriles are inserted at room temperature into the unstrained P–C bond of $[\text{Cp}^*\text{P}\{\text{W}(\text{CO})_5\}_2]$. Depending on the nitrile used, dihydro-1,3,2-diazaphosphinine and benzo-1,2-azaphosphole derivatives, which are stabilized by $[\text{W}(\text{CO})_5]$ moieties, are formed (see scheme). The synthesis of the azaphosphole is accompanied by an additional C–H activation at room temperature and offers a novel and straightforward synthetic approach to this class of compounds.



Angew. Chem. **2001**, *113*, 3520–3523

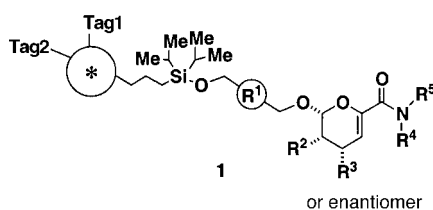
M. Schiffer, M. Scheer* 3413–3416

Insertion Reactions of Nitriles into the P–C Bond of $[(\eta^1\text{-C}_5\text{Me}_5)\text{P}\{\text{W}(\text{CO})_5\}_2]$ —A Novel Approach to Phosphorus-Containing Heterocycles

Keywords: insertion • phosphorus heterocycles • tungsten



Pathway development and library realization are reported for the generation of spatially segregated stock solutions of more than 4000 dihydropyrancarboxamides **1**. This is achieved by a diversity-oriented synthesis with use of enantioselective catalysis, high-capacity polystyrene macrobeads, encoding, and split-pool strategies.



Angew. Chem. **2001**, *113*, 3525–3529

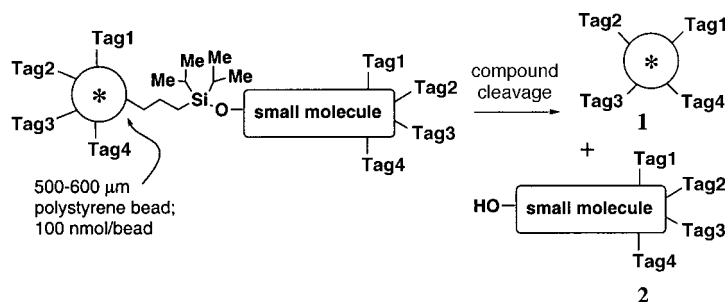
R. A. Stavenger, S. L. Schreiber* 3417–3421

Asymmetric Catalysis in Diversity-Oriented Organic Synthesis: Enantioselective Synthesis of 4320 Encoded and Spatially Segregated Dihydropyrancarboxamides

Keywords: asymmetric catalysis • combinatorial chemistry • cycloaddition • enantioselectivity • solid-phase synthesis



Binary encoding with chloroaromatic diazoketone tags allows for efficient decoding from either beads (**1**) or stock solutions (**2**), which were derived from a “one-bead, one-stock-solution” chemical technology platform. This process is demonstrated in the partial decoding of a library of 4,320 dihydropyrancarboxamides.



Angew. Chem. **2001**, *113*, 3529–3533

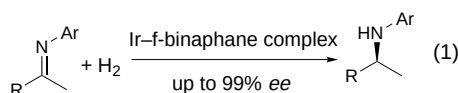
H. E. Blackwell, L. Pérez, S. L. Schreiber* 3421–3425

Decoding Products of Diversity Pathways from Stock Solutions Derived from Single Polymeric Macrobeads

Keywords: combinatorial chemistry • library encoding • polystyrene beads • solid-phase synthesis • structure elucidation



The key control factor in the hydrogenation of imines is the chiral ferrocene phosphane ligand f-binaaphane. High yields and enantioselectivities were obtained in the Ir-catalyzed conversion of imines into amines [Eq. (1)].



Angew. Chem. **2001**, *113*, 3533–3536

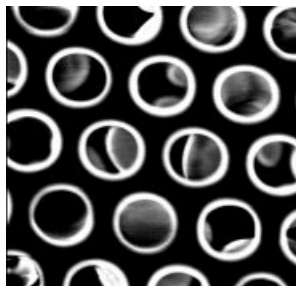
D. Xiao, X. Zhang* 3425–3428

Highly Enantioselective Hydrogenation of Acyclic Imines Catalyzed by Ir–f-Binaaphane Complexes

Keywords: asymmetric catalysis • hydrogenation • imines • iridium • P ligands

Star pores! Star and block polymer architectures are made using living radical polymerization. Isoporous honeycomb films (see scanning electron microscope image) are cast under humid conditions from solutions of these macromolecules. The pore diameter is shown to be dependent not only on the casting conditions but on the specific molecular architecture and molecular weight of the polymers.

Angew. Chem. **2001**, *113*, 3536–3540

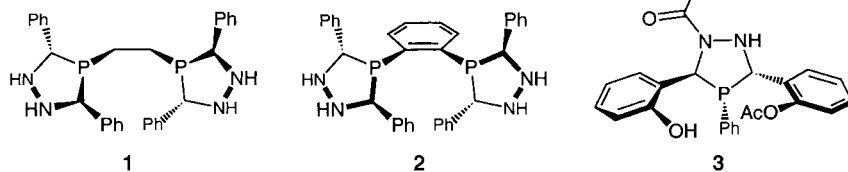


M. H. Stenzel-Rosenbaum, T. P. Davis,*
A. G. Fane, V. Chen 3428–3432

Porous Polymer Films and Honeycomb Structures Made by the Self-Organization of Well-Defined Macromolecular Structures Created by Living Radical Polymerization Techniques

Keywords: membranes • polymers • radical reactions • self-assembly • styrene

 **Effective ligands for transition metals:** a diverse array of chiral five-membered phosphacycles, for example, **1–3**, can be synthesized in two steps starting from simple primary phosphanes, hydrazine, and aldehydes.



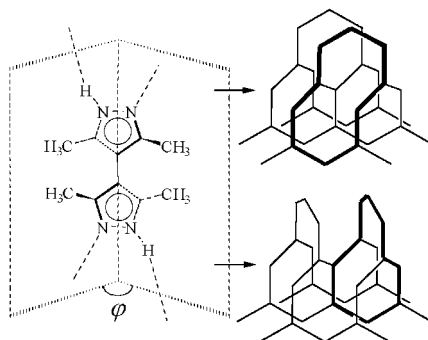
Angew. Chem. **2001**, *113*, 3540–3542

C. R. Landis,* W. Jin, J. S. Owen,
T. P. Clark 3432–3434

Rapid Access to Diverse Arrays of Chiral 3,4-Diazaphospholanes

Keywords: cyclization • homogeneous catalysis • P ligands • phosphorus heterocycles

 **The inter-ring angle φ** of 4,4'-bipyrazolyls, which can be controlled by varying the 3,3'- and 5,5'-substituents, determines their association into different three-dimensional arrays through interaction of their self-complementary hydrogen-bond donor and acceptor sites. Thus, for $\varphi \approx 90^\circ$, a (10,3)-*b* net is formed (see picture, top), while for $\varphi \approx 60^\circ$, the inherently chiral (10,3)-*c* net is obtained (see picture, bottom).



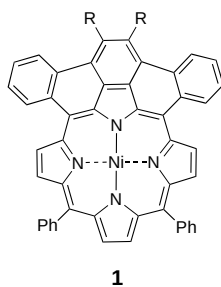
Angew. Chem. **2001**, *113*, 3543–3546

I. Boldog, E. B. Rusanov, A. N. Chernega,
J. Sieler, K. V. Domasevitch* 3435–3438

Acentric Extended Solids by Self-Assembly of 4,4'-Bipyrazolyls

Keywords: chirality • crystal engineering • hydrogen bonds • self-assembly • supramolecular chemistry

 **Bergman cyclization** of 2,3-dialkynyl-*meso*-tetraphenylporphyrins and carbon–carbon bond formation (mediated by 1,4-diradicals) with the adjacent *meso*-phenyl rings lead to formation of an unprecedented multicarbocycle fused onto the porphyrin periphery. The highly π -extended parent molecules (**1**; R = Bu, H, Ph) are described and are named picenoporphyrins after their [5]phenacene structure.



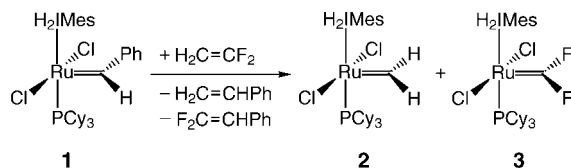
Angew. Chem. **2001**, *113*, 3547–3549

H. Aihara, L. Jaquinod, D. J. Nurco,
K. M. Smith* 3439–3441

Multicarbocycle Formation Mediated by Arenoporphyrin 1,4-Diradicals: Synthesis of Picenoporphyrins

Keywords: C–C coupling • cyclization • diradicals • nickel • porphyrinoids

Unsaturated halocarbene complexes can be synthesized in the first example of olefin metathesis with a directly fluorinated substrate. The ruthenium catalyst **1** reacts with 1,1-difluoroethylene to yield the corresponding methyldiene $[Ru] = CH_2$ (**2**) and difluorocarbene $[Ru] = CF_2$ complexes (**3**). H_2IMes = 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene; Cy = cyclohexyl.



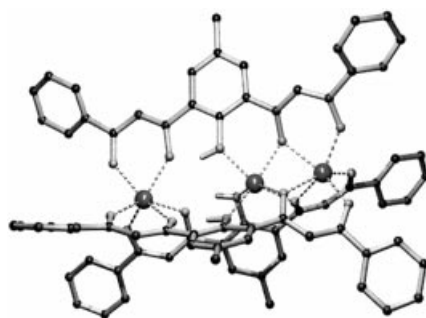
Angew. Chem. **2001**, *113*, 3549–3552

T. M. Trnka, M. W. Day,
R. H. Grubbs* 3441–3444

Olefin Metathesis with 1,1-Difluoroethylene

Keywords: carbene ligands • fluorinated ligands • homogeneous catalysis • metathesis • ruthenium

A new topology within the family of metallohelicates has been revealed by the crystal structure of the complex $[Mn_3(HL)_3]$ (see picture). The novel oxygen-based pentadentate ligand H_3L reacts with $Mn(OAc)_2$ to produce this unique triple-stranded helicate with an unprecedented asymmetric arrangement of the metal ions along the molecular axis. This helicate displays intramolecular magnetic coupling.



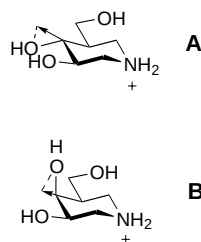
Angew. Chem. **2001**, *113*, 3552–3554

G. Aromí, P. Carrero Berzal, P. Gamez,
O. Roubeau, H. Kooijman, A. L. Spek,
W. L. Driessen, J. Reedijk* . 3444–3446

A Unique Asymmetric $[Mn_3^{II}]$ Triple-Stranded Helicate from a Symmetric Pentadentate Ligand

Keywords: chirality • helical structures • magnetic properties • manganese • supramolecular chemistry

A new answer to a 100-year-old puzzle is presented here, namely, why are galactosides hydrolyzed faster than glucosides? This disparity was long attributed to facilitation by axial OH groups of a conformational change en route to the transition state. However, the results presented here indicate that differences in the electron-withdrawing effects (represented by arrows) of equatorial (**A**) and axial OH groups (**B**) are decisive.



Angew. Chem. **2001**, *113*, 3555–3557

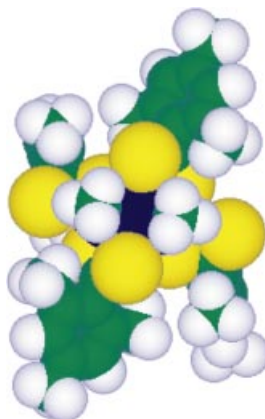
H. H. Jensen, L. Lyngbye,
M. Bols* 3447–3449

A Free-Energy Relationship between the Rate of Acidic Hydrolysis of Glycosides and the pK_a of Isofagomines

Keywords: amines • basicity • glycosides • hydrolysis • kinetics



Intramolecular S–Si interactions occur between the silicon and the sulfur atoms in the disilane $[Me(MesCS_2)_2Si]_2$, in which each silicon atom bears two [2,4,6-trimethyl(thiobenzoyl)]thio ligands. This results in a coordination number of seven for both silicon atoms (the picture shows a space-filling representation of the structure). The hexacoordinate monosilane $(MesCS_2)_2SiPh_2$ was also synthesized. Mes = mesityl = 2,4,6-trimethylphenyl.



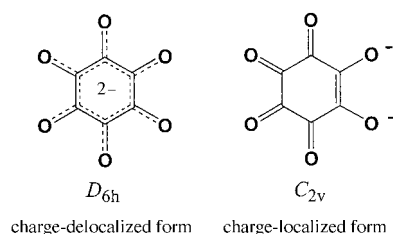
Angew. Chem. **2001**, *113*, 3558–3560

N. Kano, N. Nakagawa,
T. Kawashima* 3450–3452

A Disilane Containing Two Heptacoordinate Silicon Atoms and Dithiocarboxylate Ligands

Keywords: hypervalent compounds • silanes • silicon • S ligands

The nonbenzenoid aromatic D_{6h} and enediolate C_{2v} valence tautomers of the relatively unstable rhodizonate dianion (see picture) have been generated in situ and stabilized by hydrogen bonding with (3-hydroxyphenyl)urea and 1,1'-ethylenediurea, respectively, in two novel crystalline inclusion compounds.



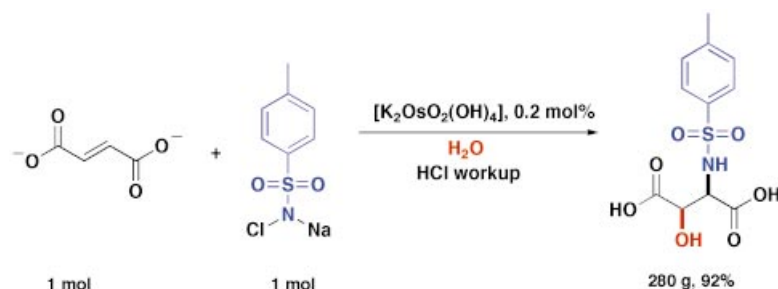
C.-K. Lam, T. C. W. Mak* .. 3453–3455

Generation and Stabilization of D_{6h} and C_{2v} Valence Tautomeric Structures of the Rhodizonate Dianion in Hydrogen-Bonded Host Lattices

Keywords: aromaticity • inclusion compounds • rhodizonate • tautomerism • urea

Angew. Chem. **2001**, *113*, 3561–3563

In water and in the absence of any ligands, salts of unsaturated carboxylic acids are readily and cleanly aminohydroxylated to provide vicinal hydroxyamino acid derivatives in high yields (see scheme).



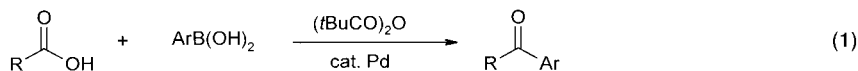
V. V. Fokin,*
K. B. Sharpless* 3455–3457

A Practical and Highly Efficient Aminohydroxylation of Unsaturated Carboxylic Acids

Keywords: amino acids • amino alcohols • homogeneous catalysis • osmium • oxidation

Angew. Chem. **2001**, *113*, 3563–3565

Mild and convenient: a palladium-catalyzed cross-coupling reaction between arylboronic acids and carboxylic anhydrides, which are generated in situ from the corresponding carboxylic acids, allows the efficient synthesis of a broad variety of functionalized aryl ketones [Eq. (1)].



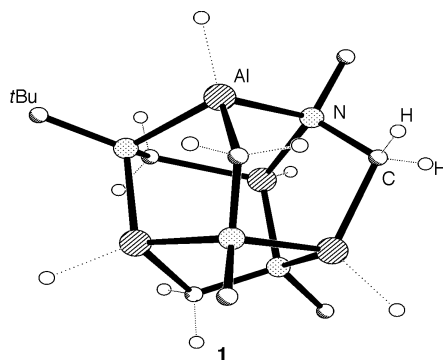
L. J. Goossen,* K. Ghosh .. 3458–3460

Palladium-Catalyzed Synthesis of Aryl Ketones from Boronic Acids and Carboxylic Acids or Anhydrides

Keywords: boronic acids • carboxylic acids • homogeneous catalysis • ketones • palladium

Angew. Chem. **2001**, *113*, 3566–3568

A significant difference in the arrangement of the atoms within the cage skeleton of the $Al_4C_4N_4$ polyhedron **1** was found in comparison with those of other known hexagonal-prismatic arrays of aluminum compounds containing a twelve-membered core. Compound **1** was prepared in good yield by the reaction of equivalent amounts of $H_3Al \cdot NMe_3$ and *tert*-butyl isocyanide in refluxing toluene.



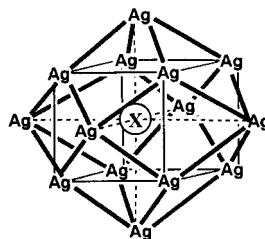
W. Zheng, A. Stasch, J. Prust,
H. W. Roesky,* F. Cimpoeșu,
M. Noltemeyer,
H.-G. Schmidt 3461–3464

A Polyhedral Aluminum Compound with an $Al_4C_4N_4$ Framework

Keywords: ab initio calculations • aluminum • cage compounds • isocyanides

Angew. Chem. **2001**, *113*, 3569–3572

A templating anion is a must for the formation of the cage compounds $[Ag_{14}(C\equiv C tBu)_{12}X]OH$ ($X = Cl, Br$), in which a rhombic dodecahedron of silver atoms surrounds the central X atom (see picture). The silver atoms are held together by a combination of bridging alkynyl groups (not shown) and argentophilic $Ag \cdots Ag$ interactions. In the absence of the templating anion insoluble polymeric materials are obtained.



D. Rais, J. Yau, D. M. P. Mingos,*
R. Vilar,* A. J. P. White,
D. J. Williams 3464–3467

Anion-Templated Syntheses of Rhombohedral Silver–Alkynyl Cage Compounds

Keywords: alkynyl ligands • cage compounds • metal–metal interactions • silver • template synthesis

Angew. Chem. **2001**, *113*, 3572–3575

A supramolecular triangle results, instead of the expected square, from the reaction of a compact, ditopic 90° platinum(II) center and the most rigid, aromatic linear linker, pyrazine (see picture). This unexpected species is characterized both in solution and the solid state.



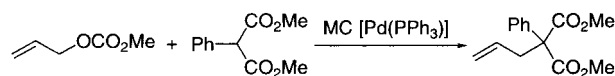
M. Schweiger, S. R. Seidel, A. M. Arif, P. J. Stang* 3467–3469

The Self-Assembly of an Unexpected, Unique Supramolecular Triangle Composed of Rigid Subunits

Keywords: N ligands • platinum • pyrazine • self-assembly • supramolecular chemistry

Angew. Chem. **2001**, *113*, 3575–3577

Buried alive! A triphenylphosphane palladium catalyst was immobilized onto/into a polymer by microencapsulation and the polymer-supported catalyst (MC[Pd(PPh₃)]) successfully used in several palladium-catalyzed reactions (one example is shown). The reactions proceeded in high yields, and the catalyst was recovered by filtration and reused. The air-sensitivity of the palladium complex is substantially suppressed by this immobilization.



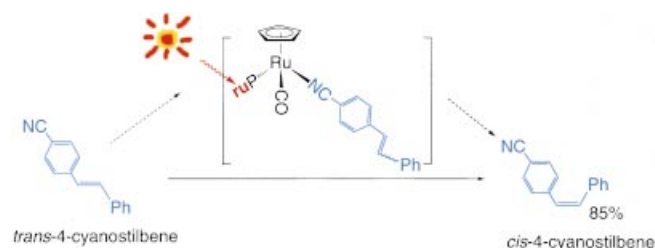
Angew. Chem. **2001**, *113*, 3577–3579

R. Akiyama, S. Kobayashi* 3469–3471

Microencapsulated Palladium Catalysts: Allylic Substitution and Suzuki Coupling Using a Recoverable and Reusable Polymer-Supported Palladium Catalyst

Keywords: allylic compounds • homogeneous catalysis • palladium • supported catalysts • Suzuki coupling

Intramolecular photosensitization by using the new visible-light-active *tert*-phosphane ligand (**ruP**) which changes a transition metal complex into a photoactive reaction center on coordination. The scheme shows the resulting intramolecular *trans* → *cis* photoconversion of a stilbene group.



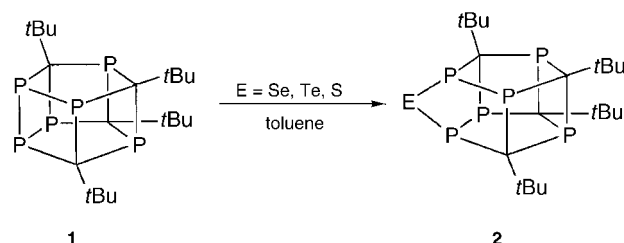
Angew. Chem. **2001**, *113*, 3580–3582

M. Osawa,* M. Hoshino, Y. Wakatsuki 3472–3474

A Light-Harvesting *tert*-Phosphane Ligand Bearing a Ruthenium(II) Polypyridyl Complex as Substituent

Keywords: energy transfer • light harvesting • P ligands • photoisomerization • ruthenium

Exclusive opening of the unique P–P bond with formation of **2** is observed when **1** is treated with carbene analogues [Eq. (1)]! This behavior is explicable by the release of strain resulting in a low-lying (20 kcal mol^{−1}) reactive singlet biradical. The HOMO and LUMO centred at the reactive sites and the thermodynamic stability of the products support this behavior.



Angew. Chem. **2001**, *113*, 3582–3585

M. M. Al-Ktaifani, D. P. Chapman, M. D. Francis, P. B. Hitchcock, J. F. Nixon,* L. Nyulászi* .. 3474–3477

The Hexaphosphapentaprismane P₆C₄tBu₄: A “Jaws-Like” Cage Molecule That Bites!

Keywords: ab initio calculations • cage compounds • chalcogens • insertion • phosphorus heterocycles



Supporting information on the WWW (see article for access details).

* Author to whom correspondence should be addressed



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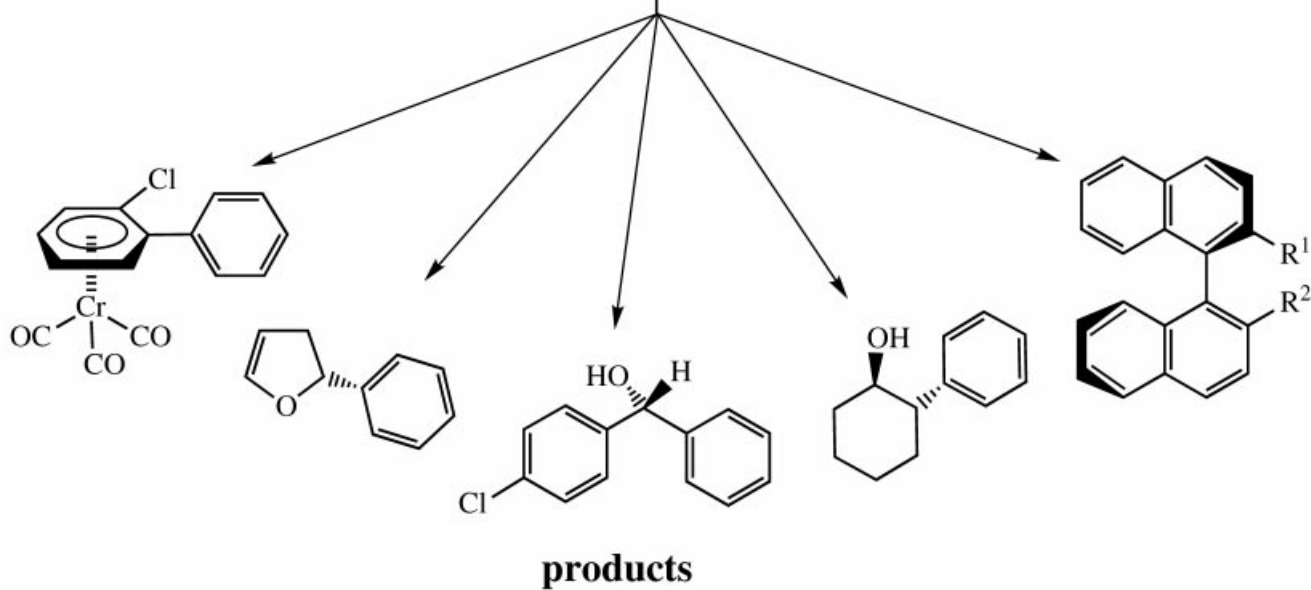
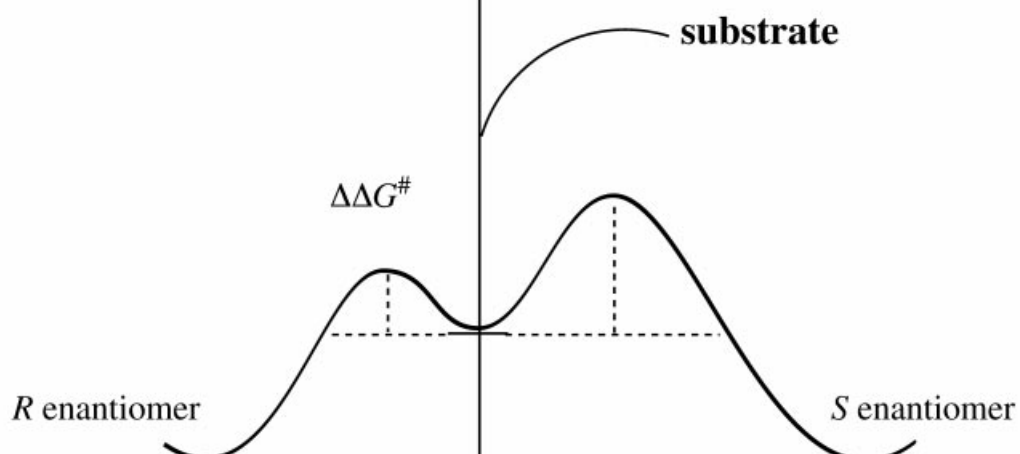
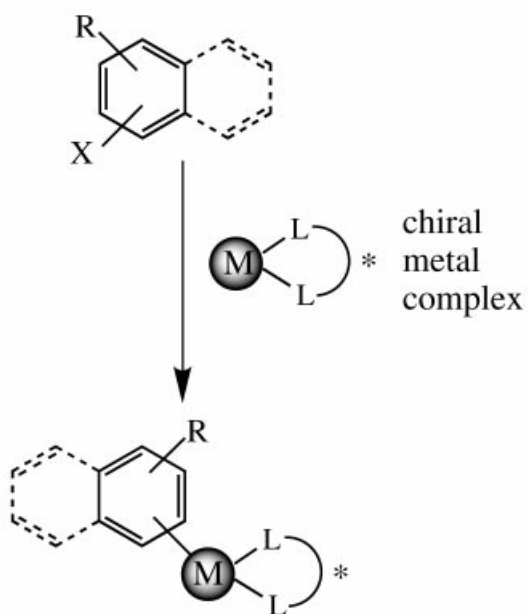
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Efficient stereochemical control in aryl transfer reactions can be achieved by the use of chiral metal complexes.



Catalyzed Asymmetric Arylation Reactions

Carsten Bolm,* Jens P. Hildebrand, Kilian Muñiz, and Nina Hermanns

*Dedicated to Professor K. Barry Sharpless
on the occasion of his 60th birthday*

Addition and substitution reactions with carbon nucleophiles are fundamental processes in organic synthesis, and the development of general catalytic asymmetric variants thereof is still a major challenge today. In contrast to enantioselective alkyl transfer reac-

tions, the corresponding arylations have not yet reached a high level of maturity. The existing protocols are either of no general applicability or are limited in terms of selectivity. This article summarizes established routes for catalytic asymmetric aryl transfer

together with the latest developments in this area. The scope and limitations of this reaction are discussed.

Keywords: arylation • asymmetric synthesis • homogeneous catalysis • transition metals

1. Introduction

The demand of chiral compounds for both pharmaceutical and agrochemical purposes has increased dramatically during the past decade.^[1] Although several approaches to enantio-merically pure chemicals exist—such as resolution or use of chiral auxiliaries—catalytic methods are by far the preferred choice, and they are also often regarded as being the most economical.^[2] Asymmetric catalysis involving chiral metal complexes plays a dominant role in this area, and hence many new and promising enantioselective processes utilizing main group or transition metals have been developed in recent years.^[3] Furthermore, significant progress with respect to selectivity and substrate scope has been achieved for both catalytic carbon–carbon and carbon–heteroatom bond formations. Within this context, enantioselective alkylations such as the asymmetric addition of dialkylzinc reagents to aldehydes^[4] and the palladium-catalyzed allylic alkylation^[5] belong to the most prominent examples, which have even

reached the status of test reactions for novel ligand designs. In light of these impressive achievements and considering the fact that nonasymmetric metal-catalyzed cross-coupling reactions^[6,7] are on the verge of becoming truly general processes, it is rather surprising that the catalytic, asymmetric transfer of sp^2 - and sp -hybridized carbon atoms has attracted much less attention.^[8] There are numerous reasons for the lack of asymmetric versions of these synthetically highly useful processes. For example, the synthesis of suitable transfer reagents is often difficult or coherent with insufficient functional group tolerance. Furthermore, the reactivity of many reagents is not suitable to allow for additional Lewis acid catalysis. Often, low temperatures are required for high enantioselectivity, but achieving efficient transmetalation under these conditions can be problematic.

Finally, the existing protocols for the identification of novel, highly enantioselective metal catalysts are still in their infancy. Thus, most of the known catalysts for the asymmetric transfer of sp^2 - and sp -hybridized carbon atoms rely on classical axial- or planar-chiral ligands such as BINAP or ferrocenes, respectively (abbreviations used in this review are explained in the appendix). Promising high-throughput screening (HTS) methods are being developed, but at the present stage they do not as yet offer general solutions.^[9,10]

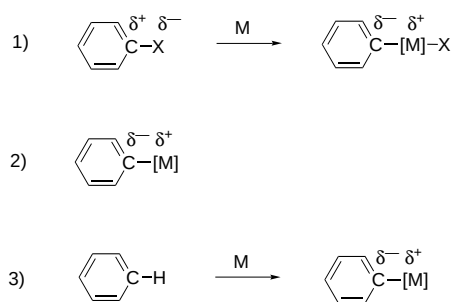
One of the key issues in aryl transfer reactions is the selective formation of an appropriate aryl–metal intermediate. In general, three major routes^[11] towards such species can be distinguished mechanistically depending on the type of aryl precursor (Scheme 1).

1) Generation of an aryl–metal compound by (formal) metal insertion into a C_{aryl} –X bond (X = halogen, alkoxy, carboxy, etc.). Such a process has the advantage that the

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Scheme 1. Mechanistically different routes towards aryl–metal intermediates capable of aryl transfer. For further information see the text.

fragment which is to be transferred is derived from common starting materials (for example, aryl halides) which often are commercially available or readily synthesized. The major mechanistic implication is that the metal catalyst participates in two steps: first, in an umpolung of the carbon atom which is to be transferred, and second, in the subsequent coupling with the other reactant.

2) Use of a preformed aryl–metal compound which is either the reactive species itself or serves as the precursor of another metal-containing compound obtained by transmetalation. In this case, the aryl precursor already contains a nucleophilic carbon atom and no umpolung is required. Examples of such a reaction type involve starting materials such as diarylzincs, aryltrialkylstannanes, and Grignard reagents which are transmetalated to palladium during the catalytic aryl transfer.

3) The formation of aryl–metal compounds by $C_{\text{aryl}}\text{--H}$ activation. Although such functionalizations have been known for some time,^[11, 12] important advances have only recently been achieved.^[13] At present, only a single application in asymmetric catalysis is known, in which an unactivated arene has been used in a reaction sequence involving a direct $C_{\text{aryl}}\text{--H}$ activation (see Section 2.2).^[14]

Asymmetric aryl transfer reactions have been known since the early 1970s when Consiglio and Botteghi,^[15] and Kumada and co-workers^[16] reported enantioselective Grignard cross-coupling reactions. Substantial advances involving other



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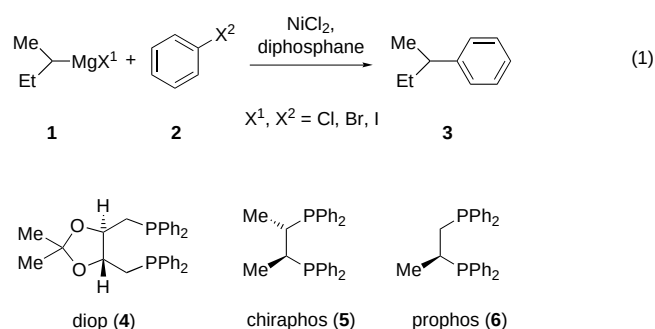
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important transformations, such as 1,2- and 1,4-additions or asymmetric ring openings of *meso*-epoxides, have only recently been made (see Sections 2.5, 4, 6). This article intends to highlight the most significant achievements in the development of catalytic asymmetric arylations.

2. Asymmetric Aryl Transfers in Cross-Coupling Reactions

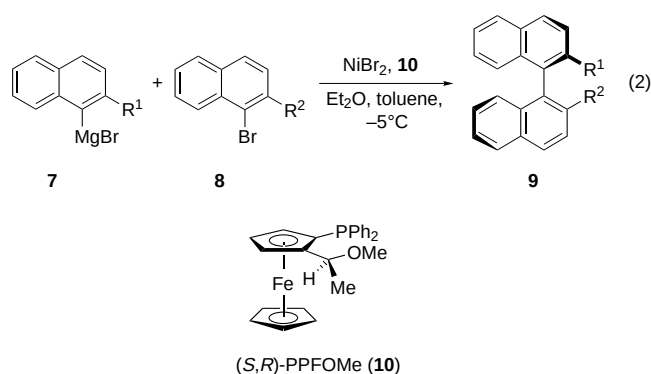
2.1. Grignard Cross-Coupling Reactions and Related Processes

Today, palladium- and nickel-catalyzed cross-coupling reactions are some of the most prominent C–C bond-forming reactions.^[6] Numerous reagents, such as aryl stannanes, boranes, and boronates, to name just a few, have been developed for efficient aryl transfers of this type. Grignard reagents were among the first to be used in asymmetric aryl cross-coupling reactions^[17] as developed by Consiglio and Botteghi,^[15] and Kumada and co-workers.^[16] For example, 2-phenylbutane (**3**) is formed in high yield when 2-butylnickel halide **1** is treated with halobenzene **2** and a nickel catalyst [Eq. (1)]. Asymmetric transformations have been



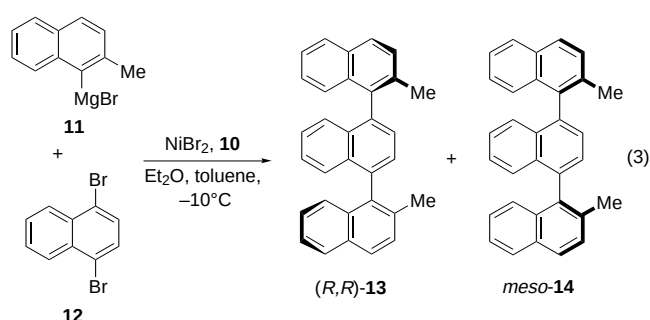
achieved with chiral diphosphanes such as diop (**4**; up to 17% *ee*), chiraphos (**5**; 43% *ee*), and prophos (**6**; up to 47% *ee*). Since **1** racemizes readily, the consumption of both enantiomers is possible, which allows for high conversion of this secondary alkyl Grignard reagent. The absolute configuration of **3** and the level of asymmetric induction both depend on the type of halide in **1** and **2**, with bromides being the substrates of choice.

A catalytic asymmetric synthesis of axial-chiral compounds—namely 1,1'-binaphthyl derivatives—became possible when aryl Grignard reagents were used. Early examples were hampered by low conversion and only very moderate enantioselectivity.^[17] A major breakthrough was achieved when Hayashi, Ito and co-workers performed the nickel catalysis in the presence of (phosphanylferrocenyl)ethyl methyl ether (**10**).^[18] Axial-chiral products **9** were obtained with high enantiomeric excesses (for example, for R¹, R² = Me: 95% *ee*) in moderate to good yields [Eq. (2)]. At low temperatures 1-chloronaphthalene could also be employed, and the product was formed in even higher enantiomeric excess relative to the corresponding bromide.

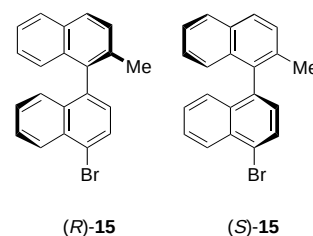


The ether moiety at the stereogenic center of **10** was crucial for the success of this asymmetric aryl transfer. Nickel complexes bearing structurally related bisphosphanes or P,N ligands displayed much lower activity. It is likely that the ether oxygen atom coordinates to the magnesium atom and guides the Grignard reagent into the appropriate position for transmetalation to the nickel center.

This type of cross-coupling could also be extended to the enantioselective synthesis of axial-chiral ternaphthalenes [Eq. (3)].^[19] Thus, (*R,R*)-**13** was obtained with a very high *ee* value (up to 95%) from 1,4-dibromonaphthalene (**12**).^[19b] However, its formation was accompanied by the production of achiral *meso*-ternaphthalene **14** (**13**:**14** = 86:14).



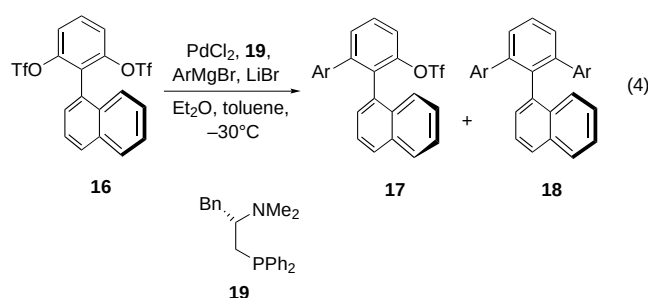
The stereochemistry and the product ratio of this catalysis were rationalized as follows: the asymmetric cross-coupling between **11** and **12** proceeds with similar enantioselectivity as observed for the corresponding monobromonaphthalenes in the synthesis of 1,1'-binaphthyls^[18] to afford (*R*)- and (*S*)-**15** in a ratio of 90:10. Since the newly formed stereochemical



element (a chiral axis) in **15** has only a minor effect on the second coupling and the reaction path, with the *R* enantiomer dominating again, the major enantiomer of **15** gives almost exclusively (*R,R*)-**13** and the minor one mainly *meso*-**14**. As a

consequence, this two-step coupling sequence leads to an increase in the enantiomeric excess of the final product and allows the synthesis of ternaphthalene (*R,R*)-**13** with a very high *ee* value.

An interesting alternative to the “conventional” asymmetric cross-coupling between two aryl moieties is the selective substitution of an enantiotopic group at a prochiral substrate. In 1995 Hayashi et al. described an elegant desymmetrization of biaryls such as **16** that led to axial-chiral **17** in both high yield (87%) and enantioselectivity (93% *ee*).^[20] The transformation was accomplished by selective low-temperature coupling of an arylmagnesium bromide with one of the enantiotopic triflate groups of **16**. A chiral palladium complex with (*S*)-phephos (**19**) served as the catalyst [Eq. (4)].^[21] As in

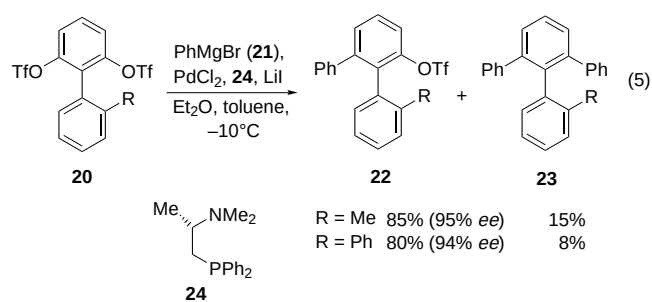


many other cross-coupling reactions, additives had a pronounced effect:^[22] lithium bromide was found to enhance both the yield and the asymmetric induction.^[23] Lithium iodide was equally as effective in many reactions, while the corresponding chloride proved unsuitable. Interestingly, the amount of additive only influenced the reaction rate, not the enantioselectivity. While equimolar quantities led to high product yields, the contrary was observed when more or less lithium salt was present.

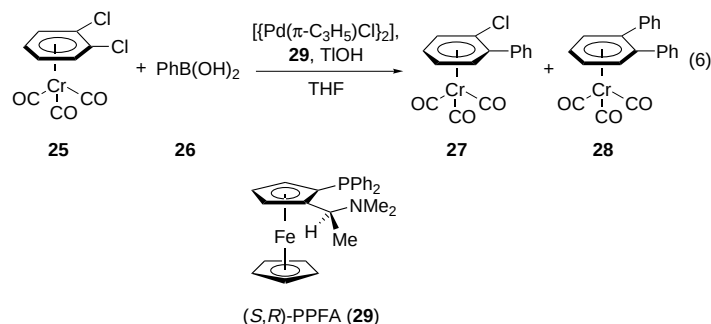
The catalysis also involves a kinetic resolution,^[24] which results in an increase in the enantiomeric excess of the initially formed **17**.^[25] Studies with racemic **17** showed that the minor enantiomer, (*R*)-**17**, formed in the first phenylation of **16**, with the palladium/(*S*)-phephos catalyst being consumed about five times faster than its enantiomer (*S*)-**17** to give achiral diarylated compound **18**.^[26] As a result, the enantiomeric excess of **17** is increased, although at the expense of the product yield.

While catalyses with *m*-tolylmagnesium bromide also led to highly enantioenriched biaryls, other Grignard reagents such as *o*- and *p*-tolylmagnesium halide or *p*-chlorophenylmagnesium halide were unreactive. Later, the authors disclosed that prochiral bi- and terphenyls could also be employed as substrates.^[27] In this case, P,N ligands derived from amino acids with smaller substituents at the stereogenic center were more effective, and a palladium catalyst containing (*S*)-alaphos (**24**) proved superior to other Pd^{II}/ligand combinations [Eq. (5)].

A range of other Grignard reagents including alkynylmagnesium halides^[28] were also found to be applicable, thereby extending the scope of this asymmetric cross-coupling involving the discrimination of enantiotopic groups.



Using the same principle, Uemura and Hayashi et al. studied the desymmetrization of planar-prochiral compounds such as **25**.^[29] Thus, when a palladium catalyst with (*S,R*)-PPFA (**29**) was used as the ligand, planar-chiral **27** was obtained with 69% *ee* together with a small amount of the achiral bis-coupling product **28** [Eq. (6)]. It is noteworthy that

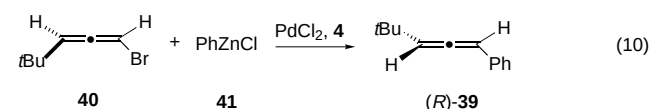
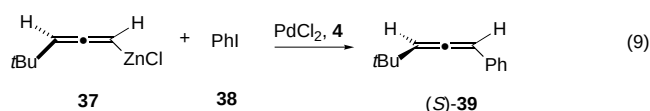
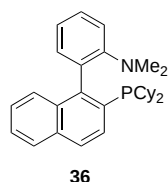
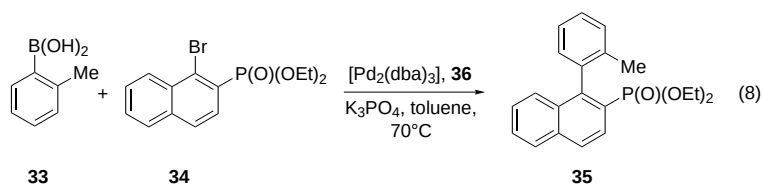
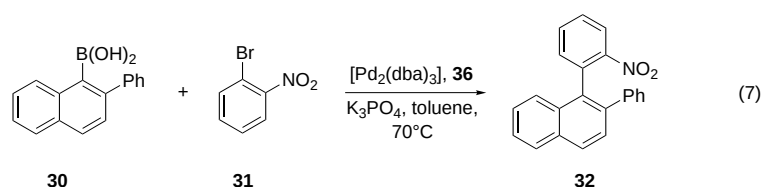


in this case the aryl transfer reagent was a boronic acid and that this reaction is therefore a rare example of an asymmetric Suzuki–Miyaura coupling.^[30] The reaction is sensitive to electronic effects, and the use of the more electron-rich 4-methoxyphenyl boronic acid caused a decrease in enantioselectivity together with a dramatic reduction in the reaction rate.

Examples of asymmetric Suzuki couplings of aryl boronates to give axial-chiral biaryls were very recently described by Cammidge and Crépy.^[31] The reactions closely resemble asymmetric Grignard cross-coupling reactions and rely on the same catalyst as reported by Hayashi, Uemura, et al. For example, use of PdCl₂/**29** in the coupling of the starting materials **7** and **8** (R¹ = R² = Me) afforded product **9** (R¹ = R² = Me) with up to 85% *ee*.

The first catalytic enantioselective synthesis of functionalized biaryls was reported by Yin and Buchwald almost simultaneously as the results of Cammidge and Crépy were published.^[32] Here, axial-chiral versions of Buchwald's highly active cross-coupling catalysts^[33] were employed to afford products **32** and **35** in up to 92% *ee* [Eqs. (7) and (8)]. Compound **36** proved the most effective in this transformation. The catalyst loading could be lowered to 0.2 mol% without deterioration of the *ee* value. The use of an aryl iodide and an aryl chloride gave almost identical results.

The catalytic synthesis of enantioenriched allenes was reported by van Koten, Elsevier, and co-workers [Eqs. (9) and (10)].^[34] When racemic **37** was coupled with phenyl iodide



(38) in a Negishi-type reaction catalyzed by a palladium complex with diop (**4**) as a chiral ligand the *S* enantiomer of **39** was formed with 25 % *ee*. The same product was obtained from the catalyzed coupling of bromoallene **40** and phenylzinc chloride (**41**). However, **39** now had the opposite configuration (and only up to 9 % *ee*). Reactions with magnesium or copper derivatives of **37** afforded *S*-configured **39**. Although in this early work the enantioselectivity was rather low, the use of other ligands might lead to further significant improvements.

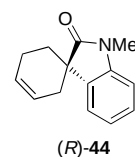
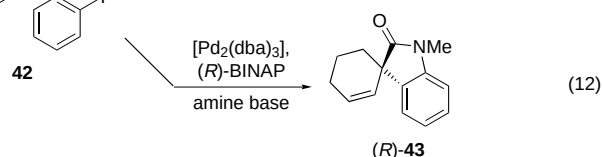
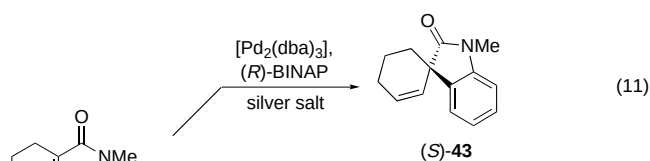
More than twenty-five years after Consiglio and Botteghi,^[15] and Kumada and co-workers^[16] demonstrated the concept, impressive enantioselectivities have been achieved in arylations involving palladium- and nickel-catalyzed cross-coupling reactions. In this context, the synthesis of biaryl derivatives using aryl halides and aromatic Grignard reagents are particularly noteworthy. However, even at this advanced stage, further progress is required. The development of more universal catalysts and truly general procedures that allow for the use of a broad range of aryl transfer reagents still remains a major challenge for future work. Moreover, the extension of the current cross-coupling concept of organomagnesium reagents to boronates, zinc reagents, or stannanes should provide the synthetic chemist with a useful portfolio of asymmetric cross-couplings. These should then allow for the transfer of an aryl group onto almost every substrate with excellent enantioselectivities.

2.2. Asymmetric Arylation by Heck Reactions

A very important aryl transfer reaction for both academic and industrial purposes is the palladium-catalyzed cross-coupling of aryl halides or sulfonates with olefinic double bonds which was independently discovered by Mizoroki et al.^[35] and Heck and Nolley.^[36] This transformation has emerged as one of the most useful tools for organic synthesis^[37] and was employed in the groundbreaking total syntheses of taxol by Danishefsky et al.^[38] and morphine by Overman and co-workers,^[39] to name just two of the vast number of examples.^[40] An important extension has been the development of asymmetric variants for both intra- as well as intermolecular couplings, which have again found numerous applications in natural product synthesis.^[41]

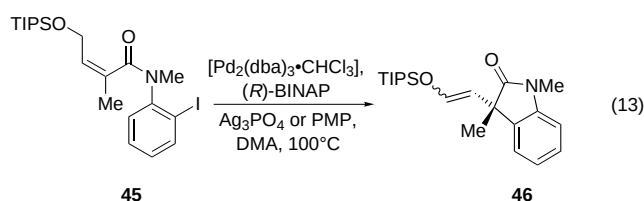
The first asymmetric Heck reaction was reported by Shibasaki and co-workers in 1989 when they described the synthesis of *cis*-decalins with moderate enantiomeric excesses (up to 46 %) through an intramolecular Heck-type cyclization.^[42] Almost simultaneously, Overman and co-workers published their results on asymmetric aryl/vinyl couplings.^[43] In this case, a trienyl triflate underwent polycyclization when subjected to a catalyst system consisting of palladium acetate and diop (**4**) to afford spirocyclic dienones. The enantioselectivities were similar to those achieved in the decalin synthesis by Shibasaki et al. Interestingly, both procedures were intramolecular^[44] and relied on an asymmetric delivery of an unsaturated moiety to the olefinic double bond to form a quaternary carbon center.^[45]

Recent efforts have focussed on the synthesis of spirooxindols, which are important building blocks for the construction of complex natural products. The intramolecular asymmetric Heck reaction allows a convenient approach to such heterocycles containing a stereogenic all-carbon quaternary center. If the cyclization of **42** was carried out under “conventional” conditions, that is, with a (*R*)-BINAP-modified palladium catalyst in the presence of a silver salt, the (*S*)-configured product, (*S*)-**43**, was formed with good enantioselectivity (80 % *ee*) in good yield (Eq. (11)).^[46]

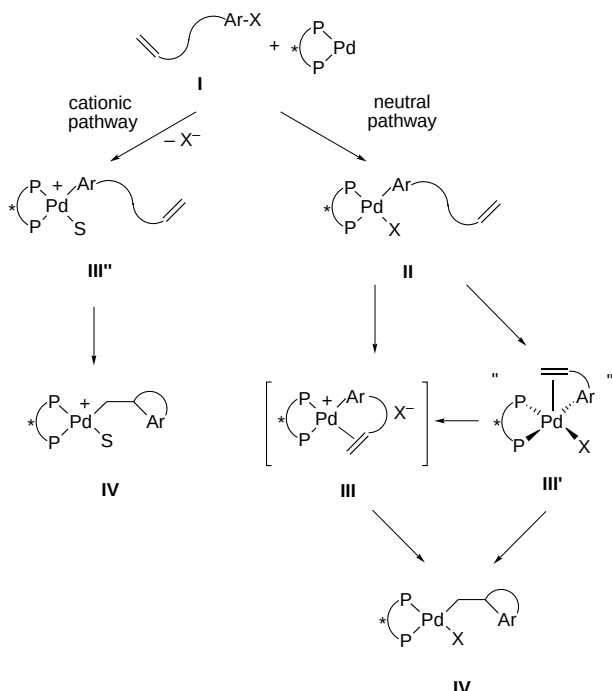


In the absence of an additive, the product has the opposite absolute configuration [Eq. (12)].^[47] This reaction path that yields (*R*)-**43** with the same catalyst ((*R*)-BINAP/[Pd₂(dba)₃]) can be enhanced by the addition of 1,2,2,6,6-pentamethylpiperidine (PMP).^[48] Additionally, a β -hydride elimination/re-addition/ β -hydride elimination sequence takes place resulting in a kinetic resolution of the initially formed enantioenriched spirooxindole **43** (for the analogous phenomenon in intermolecular Heck reactions, see Scheme 3). Thus, cyclization of **42** to (*R*)-**43** (45%, 89–95% *ee*) was accompanied by the formation of regioisomeric (*R*)-**44**. The latter product had an enantiomeric excess of only 31%, which indicates that the minor *S*-configured isomer of **43** had undergone a more rapid isomerization than its enantiomeric counterpart. As a consequence, this process leads to an enantiomeric enrichment of (*R*)-**43** at the expense of product yield.

As a result of an analogous kinetic resolution (*Z*)- α,β -unsaturated anilide **45** gave product **46** with high enantioselectivity (up to 92% *ee*) under both neutral and cationic conditions [Eq. (13)].^[41b, 49]



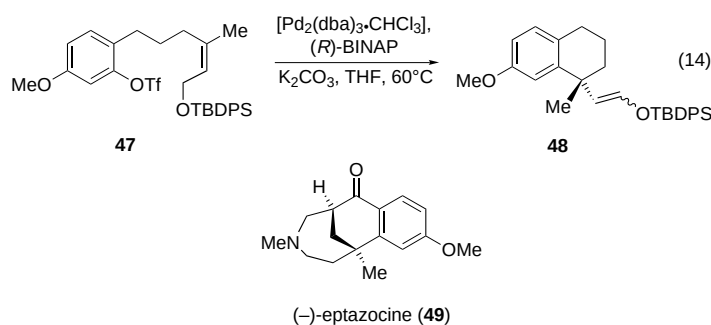
Scheme 2 summarizes the processes from a mechanistic point of view. In the cationic process the silver salt was suggested to serve as a halide scavenger as well as removing the halide from the palladium complex. The resulting cationic palladium intermediate **III'** is then characterized by its



Scheme 2. The possible mechanistic pathways of intramolecular Heck reactions (S = solvent).

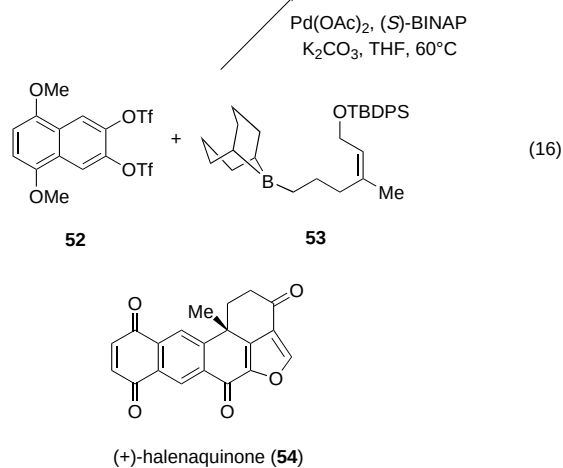
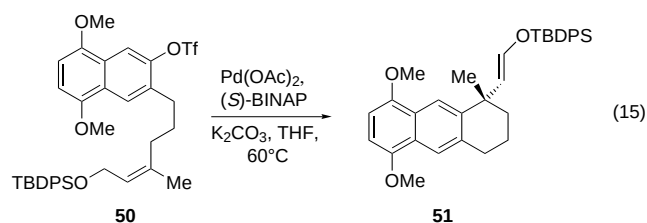
reduced tendency for ligand dissociation. As demonstrated by Hayashi et al. in related intermolecular Heck-type reactions (see below), the intermediacy of a four-coordinate species with a chelating BINAP ligand is essential for high enantioselectivity, and bidentate binding can only be ensured if the halide is efficiently removed. The basicity of the additive also has a pronounced effect on the yield and asymmetric induction of the reaction. Thus, weakly basic silver salts impede the cyclization, and the enantioselectivity is decreased significantly. While the cationic path apparently proceeds via **III'**, the enantiodiscriminating step of the neutral route was suggested to be either olefin coordination (**III/III'**) or insertion (**IV**), with halide displacement by the tethered alkene (**III**) being the most likely.

Asymmetric Heck processes can also be used in the synthesis of tetraline derivatives with quarternary stereogenic centers. For example, in the total synthesis of (–)-eptazocine (**49**) by Shibasaki and co-workers, tetraline core **48** was assembled in both high yield (90%) and excellent enantiomeric excess (90%) [Eq. (14)].^[50] Furthermore, the depicted *Z* alkene **47** gave much higher enantioselectivities than the *E* isomer, and the two diastereomers afforded opposite enantiomers of **48** in the Heck-type cyclization.^[44d]



The tetralines can also be formed by other cyclizations, which occur with exceptionally high enantioselectivity. Thus, in the total synthesis of (+)-halenaquinone (**54**), catalysis of the ring-closure of **50** by a BINAP-modified palladium complex gave key intermediate **51** with 87% *ee* and 78% yield [Eq. (15)].^[51] The same compound was obtained in almost identical enantiomeric excess (85%) in a “one-pot” tandem-type transformation by using **52** and **53** as starting materials in a Suzuki coupling/asymmetric Heck cyclization sequence [Eq. (16)].^[52]

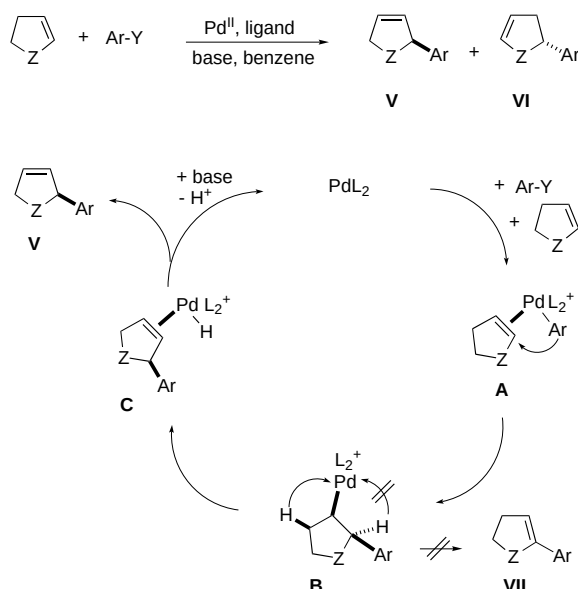
Intermolecular reactions between olefins and aryl sources have been devised mainly for cyclic substrates. The general idea is depicted in Scheme 3. Generation of a Pd⁰ species from an appropriate Pd^{II} precursor is followed by its oxidative addition into the arene–heteroatom bond (Ar–Y). After displacement of substituent Y, coordination of the olefin furnishes cationic Pd–olefin complex **A**. Regioselective aryl transfer with *syn* addition gives a pallada species **B**, which releases the metal by reductive β -hydride elimination to give to **C**. Since such a process must occur in a *syn* fashion, the hydrogen atom at the stereogenic center can not be removed and formation of achiral product **VII** is excluded. Instead, β -hydride elimination occurs at the remaining neighboring



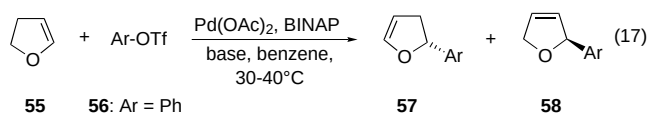
carbon atom giving rise to hydride–olefin complex **C**, which finally releases the desired chiral product **V**. The presence of base regenerates the Pd^0 catalyst. In many cases regioisomeric **VI** is the major product, and its formation will be discussed in detail later.

Enantioselective versions of such intermolecular Heck reactions were first reported by Hayashi and co-workers in 1991.^[53] Aryl triflates afforded 2-aryl-2,3-dihydrofurans **57** with enantioselectivities of up to 93% *ee* when 2,3-dihydrofuran (**55**) was employed as the olefinic substrate^[54] together with a catalyst derived from palladium acetate and using BINAP as a bidentate ligand [Eq. (17)].^[55] In contrast, the use of aryl iodides gave only racemic material, and it was proposed that, in this case, partial dissociation of the ligands from the neutral $[\text{Pd}\{\text{Ar}(\text{BINAP})\text{I}\}]$ intermediate took place during the reaction with the olefin, thus furnishing a reactive, albeit unselective, catalyst.

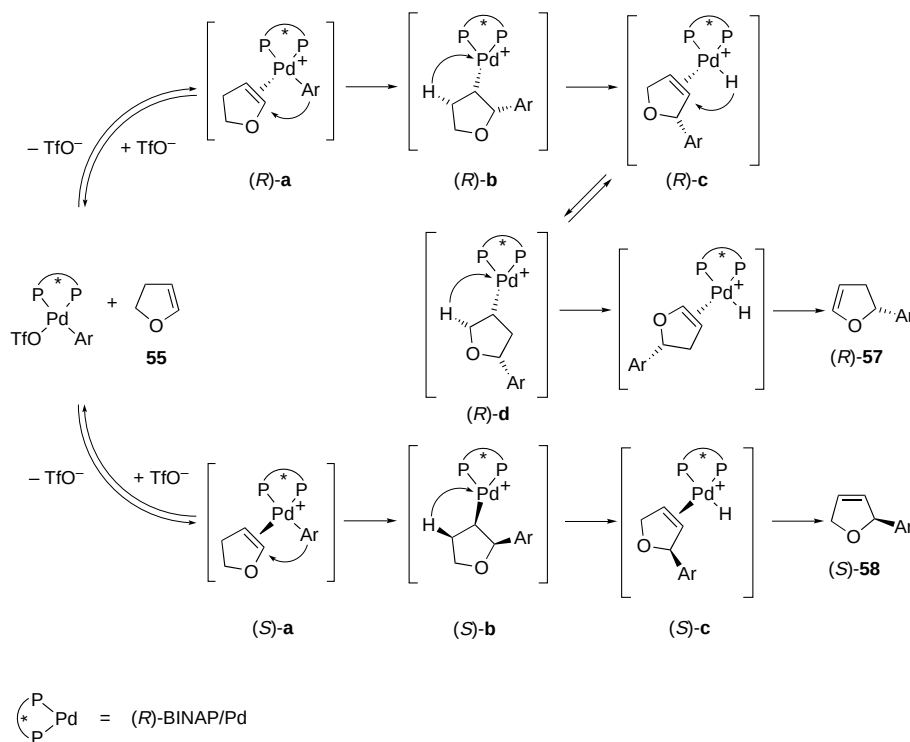
As well as **57**, regioisomeric 2-aryl-2,5-dihydrofurans **58** with opposite configuration at the stereogenic center were generated, and it was found that an enantiomeric enrichment of **57** occurred during the catalysis.^[56] The rationalization of such reaction behavior follows the mechanistic path^[57] depicted in Scheme 4 for the transfer of an aryl group onto 2,3-dihydrofuran (**55**). After oxidative insertion of an initially formed (*R*)-BINAP/palladium(0) intermedi-



Scheme 3. Mechanistic pathways of intermolecular Heck reactions.



ate into the aryl triflate bond, dissociation of the triflate anion generates a cationic palladium complex which is prone to olefin coordination. Coordination to either of the enantiotopic faces of the dihydrofuran results in the formation of two diastereomeric intermediates (*R*)-**a** and (*S*)-**a**.^[58] Delivery of the aryl group by addition to the respective double bond gives

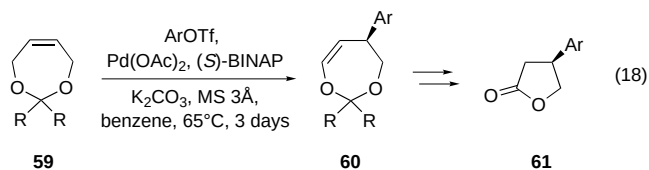


Scheme 4. Mechanistic pathway for the transfer of an aryl group onto **55**.

rise to (*R*)-**b** and (*S*)-**b** which both undergo β -hydrogen elimination to afford the diastereomeric coordination complexes (*R*)-**c** and (*S*)-**c**, respectively. The latter bears (*S*)-**58** as an olefinic ligand which can be directly released. In contrast, (*R*)-**c** undergoes selective hydropalladation to yield (*R*)-**d**. In an overall reversible process, β -hydrogen elimination of this intermediate finally leads to (*R*)-**57**. The different behavior of the diastereomeric complexes **c** is explained as follows: (*S*)-**c** suffers from severe steric interactions between the transferred aryl group and one of the phenyl groups on BINAP, which results in an enhanced rate for olefin decomplexation. Diastereomer (*R*)-**c** lacks this steric strain, and it can therefore more easily undergo addition to the coordinated olefinic double bond leading to (*R*)-**d** as the intermediate in the synthesis of (*R*)-**57**. Thus, the observed product distribution and the enantioselectivity of (*R*)-**57** are indicative of the stereochemical effectiveness of the chiral ligand BINAP.^[55] A high enantioselectivity in the formation of the major product ((*R*)-**57**) may be achieved since the two diastereomeric intermediates **c** undergo different subsequent reactions (isomerization of the major intermediate (*R*)-**c** versus ligand dissociation of (*S*)-**c**). Consequently, the authors refer to this transformation as a kinetic resolution process.

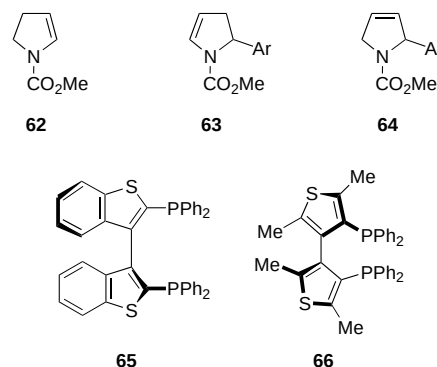
Further studies revealed that the choice of the base had a pronounced effect on the regio- and enantioselectivity. Thus, **57** (Ar = Ph; resulting from phenyl transfer from phenyl triflate **56**) was the exclusive product when substituted pyridines were present as bases during the catalysis. Unfortunately, the enantiomeric excess was only moderate (67% *ee* for 2,6-dimethylpyridine), with the *R* enantiomer being formed predominantly. The use of 1,8-bis(dimethylamino)-naphthalene (a proton sponge) led to a sharp increase in the enantioselectivity (>96% *ee*), but now the two isomeric dihydrofurans (*R*)-**57** and (*S*)-**58** (Ar = Ph) were formed in an unsatisfying ratio of 71:29. Furthermore, the *ee* value of the latter product was only 17%.^[56]

With the goal of obtaining products of the general type **60**, which could be regarded as masked chiral β -aryl- γ -butyrolactones **61**, 4,7-dihydro-1,3-dioxepins **59** have been employed as olefinic substrates in intermolecular Heck reactions [Eq. (18)].^[59] Thus, the enantioselective cross-coupling of **59**



with aryl triflates catalyzed by a BINAP-modified palladium complex furnished the 5-aryl-substituted products **60** with moderate to good enantiomeric excesses (up to 75% *ee*). The presence of a combination of 3-Å molecular sieves (MS) and potassium carbonate as a base was found to be of major importance for achieving this enantioselectivity. The use of 4- or 5-Å MS resulted in the yields dropping while the enantiomeric excess remained about the same. Further improvements of this transformation were subsequently reported by Pfaltz and co-workers (see below).^[60]

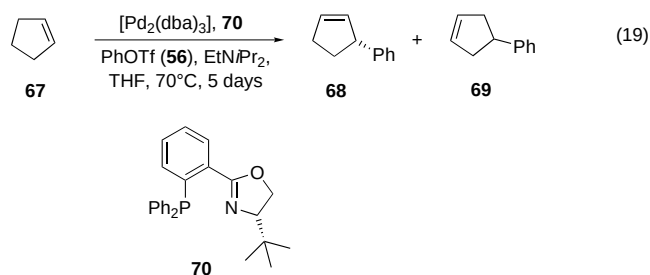
Arylations of dihydropyrroles by asymmetric Heck couplings were described by Ozawa and Hayashi,^[61] Hallberg and co-workers,^[62] and Tietze and Thede.^[40c] Ozawa and Hayashi reported that when 1-methoxycarbonyl-2,3-dihydropyrrole (**62**) was subjected to conditions similar to those for Pd/BINAP-catalyzed couplings of aryl triflates with dihydrofurans the corresponding arylated products **63** were obtained in moderate to good yield with an *ee* value of up to 83% (Ar = *p*-chlorophenyl). Again, a kinetic resolution-type process



afforded regioisomeric **64** (with a low *ee* value) containing the double bond in the 3,4 position. Tietze and Thede were able to show that the ratio between **63** and **64** was significantly improved (up to 31:1) when BITIANP (**65**) was used as a ligand, and that the enantioselectivity was raised to 93–95% *ee*.^[40c] Contrary to expectations, palladium catalysis with TMBTP (**66**) as the chiral ligand gave **64** as the predominant isomer (**63**:**64** = 1:4), although as a racemate.

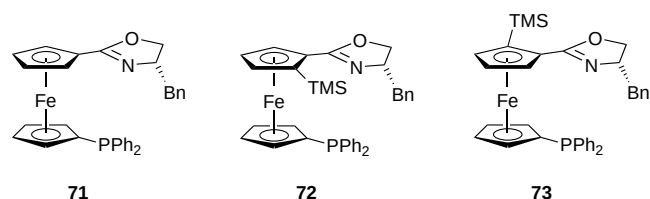
The new axial-chiral ligands **65** and **66** were also applied in the intermolecular phenylation of 2,3-dihydrofuran (**55**).^[40c, 63, 64] Tietze, Sannicolò, and co-workers found that ligand **65** was highly regio- and enantioselective, and afforded exclusively 2,5-dihydrofuran **58** (Ar = Ph) with 91% *ee*. Also, **65** proved suitable in transformations employing a number of aryl triflates (up to 96% *ee*) as well as cyclohexenyl triflate (86% *ee*). It is noteworthy that **66** was superior to **65** in intramolecular Heck reactions terminated by a silane group.^[64]

Substantial improvements in the intermolecular asymmetric Heck reactions were achieved early on by Pfaltz and co-workers, who employed *C*₁-symmetric P,N ligands containing oxazoline moieties such as **70**.^[60, 65] These ligands together with a Pd⁰ source showed remarkably enhanced levels of both enantioselectivity and the ratio of regioisomeric products.^[60] Aryl and vinyl triflates could be used together with cyclic olefins, dihydrofurans, and dioxepins. For example, the phenylation of 2,3-dihydrofuran (**55**) afforded 2-phenyl-2,5-dihydrofuran (**58**, Ar = Ph) with an excellent *ee* value (97%) and in 87% yield. The formation of regioisomer **57** (Ar = Ph) was not observed. The related vinylation with cyclohexenyl triflate took place with complete enantioselection under a variety of reaction conditions. Since these catalysts displayed decreased reactivity relative to Pd/BINAP complexes, they allowed the use of substrates such as cyclopentene (**67**), which afforded **68** with high enantiomeric excess (up to 91% *ee*) [Eq. (19)]. Furthermore, the regioselectivity was excellent,



and achiral **69** was only obtained in traces (**68:69** = 99:1 in THF).

An interesting extension of Pfaltz's work was recently disclosed by Hou and co-workers,^[66] who employed catalysts bearing ferrocenyloxazolines **71–73**. These ligands differ in

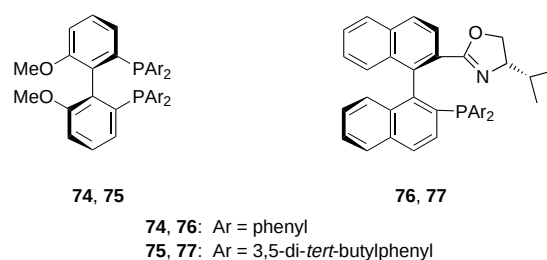


their substitution patterns at the upper cyclopentadienyl fragment and in their relative configurations of the chirality elements. Initial studies with compound **71** having a stereogenic center resulted in the exclusive formation of a 2,5-dihydrofuran (*R*)-**58** (Ar = Ph) with 77% *ee* after a relatively short reaction time of eight hours (at 60 °C). The introduction of planar chirality by adding substituents *ortho* to the oxazoline moiety improved the enantioselectivity. Thus, *ee* values of 84 and 92% were obtained with **72** and **73**, respectively. Interestingly, both products now differed in their absolute configuration, with a dominance of the *S* enantiomer in the former and the *R* isomer in the latter reaction. The authors attributed this result to the presence of the plane of chirality directing the behavior of the ligand during the catalysis.^[67, 68]

Pregosin and co-workers studied the Pd-catalyzed enantioselective intermolecular Heck reaction using ligands **74** and **75**.^[69] The enantioselection of **75** with bis(3,5-*tert*-butylphenyl) substituents at the phosphorus atom proved superior to **74**, which has a diphenylphosphanyl group. For example, the transfer of a phenyl group to 2,3-dihydrofuran (**55**) to give **57** (with Ar = Ph) occurred regioselectively with **75**, and afforded the product with excellent enantiomeric excess (> 98%) and in 65% yield. On the other hand, the enantioselectivity was only 84% *ee* (71% yield) when **74** was employed. However, presumably because of steric reasons, **75** displayed lower reactivity, and arylation at 40 °C was slow.

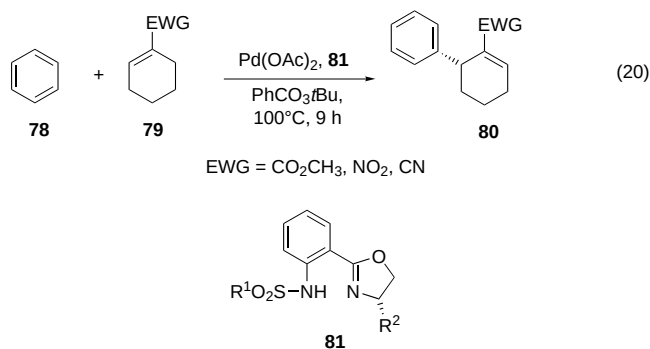
The same trend was observed by Pregosin, Albinati, and co-workers with ligands **76** and **77**; they found that the latter with the bis(3,5-*tert*-butylphenyl) substituents was superior and led to enhanced enantioselection (by 10–12% *ee*).^[70] The catalyses with ligands **76** and **77** proceeded at similar rates.

The superiority of the ligands with the sterically demanding *tert*-butyl group at the 3 and 5 position was attributed to an increased rigidity of the chiral cleft around the palladium



center. The authors also disclosed that the use of [Pd₂(dba)₃] in these catalyses led to a sharp decrease in the reaction rate. This result was attributed to the dba ligand reducing the nucleophilicity of the palladium catalyst by lowering the electron density at the metal through π back-bonding. Thus, this palladium(0) catalyst is almost unreactive towards oxidative addition to phenyl triflate (**56**) under the standard reaction conditions.^[71]

A mechanistically related aryl–olefin coupling, which involves the activation of an arene C–H bond, is the Fujiwara–Moritani reaction.^[12, 13a] Recently, Mikami et al. described the first example of an asymmetric version of this transformation.^[14] A catalyst derived from palladium acetate and sulfonamide **81** promoted this reaction in an enantioselective manner. Benzene (**78**) was chosen as the substrate for the C–H activation, and cyclic olefins were employed to ensure that achiral products were not formed (compare with **VII** in Scheme 3). With *tert*-butyl perbenzoate as the reoxidant, electron-deficient olefins **79** reacted at 100 °C to give arylated products **80** with up to 49% *ee* and yields of 6 to 33% yields [Eq. (20)]. Further improvements in the reaction might result from the use of other ligands. This asymmetric catalysis involves a C–H activation of an otherwise unreactive substrate,^[13] and hence it deserves particular attention.

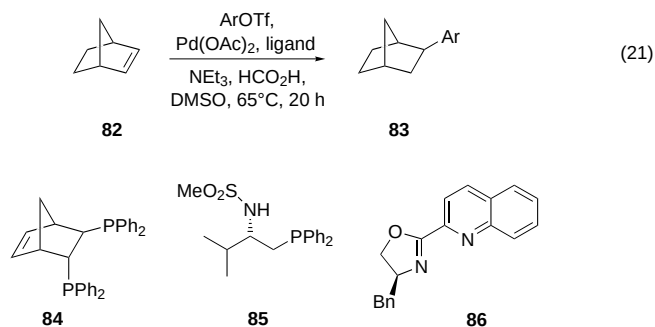


Intramolecular enantioselective Heck reactions appear to be much more mature than their intermolecular counterparts, which might—at least in part—be a consequence of the importance of such processes for the synthesis of natural products incorporating all-carbon quarternary stereocenters. Major progress has undoubtedly also been made in the intermolecular versions, but developments in that field still deserve particular attention. Often the availability of catalysts that provide sufficient reactivity, enantio-, and regioselectivity—especially for substrates other than dihydrofurans—is rather limited. Thus, future investigations will have to be

devoted to the identification of catalysts for intermolecular Heck reactions with broader applicability to different substrate classes and increased activity as well as enantioselectivity.

2.3. Hydroarylation of Bicyclo[2.2.1]hept-2-enes

An interesting example of a reductive cross-coupling was first reported by Brunner and Kramler in 1991.^[72] Norbornene (**82**) was treated with aryl iodides in the presence of a catalyst composed of palladium acetate and NORPHOS (**84**) to give *exo*-2-arylnorbornanes **83** [Eq. (21)]. At this early stage, the



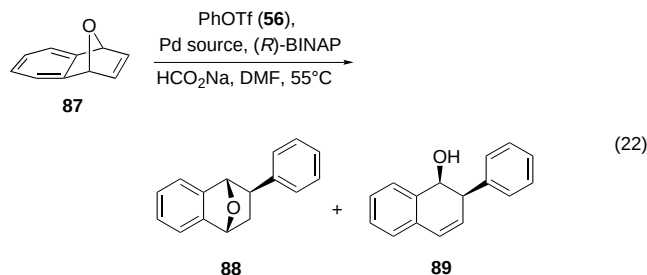
enantiomeric excesses were only moderate, with a maximum value of 41 %. Later, Achiwa and co-workers substantially improved the enantioselectivity to 74 % *ee* (**83** with Ar = Ph) by using phenyl triflate (**56**) and aminophosphane **85** as the ligand.^[73] Recently, Namyslo and Kaufmann^[74] reported a comprehensive study of this transformation and confirmed the superiority of **85** relative to bisphosphanes as well as other chelating P,N ligands. The use of phenyl nonaflate (nonaflate = nonafluorobutane sulfonate) gave the product in 47 % yield and with an enantiomeric excess of 86 %. More conventional leaving groups afforded the enantioenriched aryl norbornane with lower selectivity. Replacement of triethylamine by piperidine or a proton sponge did not result in any change in the enantioselectivity.

Although several aromatic and heteroaromatic arenes can be used in the asymmetric hydroarylation, the yields generally suffer from side reactions which lead to relatively large amounts of hydrodehalogenated aryl halides or reduced aryl sulfonates. The hydride source, usually formic acid, is responsible for the formation of an intermediate aryl–palladium hydride, which after reductive coupling gives rise to the reduced aromatic compounds.

In another recent report Zhou and co-workers^[75] described the use of $[\text{Pd}_2(\text{dba})_3]$ and the chiral ligand quinolinyl oxazoline **86**. The results achieved with this catalyst were comparable to those obtained by Achiwa with phenyl iodide (**38**) as an aryl source. Contrary to other reports in this field, the enantioselectivity was temperature dependent, and the best results were achieved at room temperature.^[76]

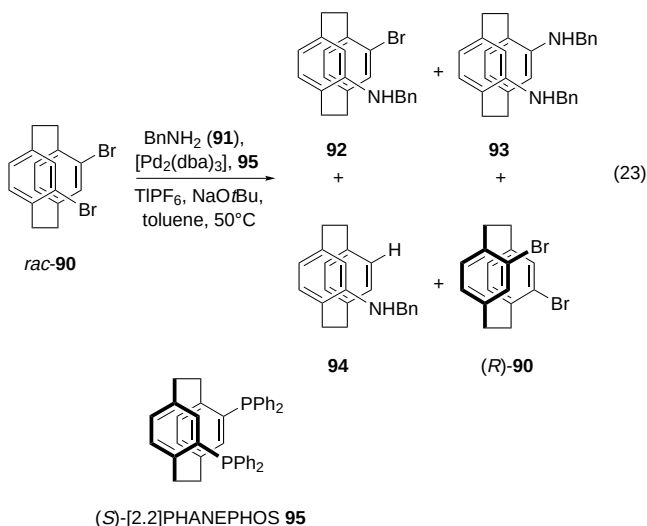
Moinet and Fiaud studied the hydrophenylation of 7-oxybenzonorbornene (**87**) catalyzed by chiral palladium complexes.^[77] BINAP proved to be the most suitable ligand. Again phenyl triflate (**56**) was superior to phenyl iodide (**38**) when

used as the aryl source and afforded the hydroarylated product **88** with 64 % *ee* [Eq. (22)]. Moreover, 15 % of the corresponding ring-opened alcohol **89** (83 % combined yield of **88** and **89**) was formed with a remarkable 96 % *ee*.



2.4. Asymmetric Arylations of Amines

Another interesting aryl transfer reaction in which a primary amine serves as the aryl acceptor was recently disclosed by Rossen, Pye, et al.^[78] An enantioselective variant of the Hartwig/Buchwald coupling^[79] was developed for the kinetic resolution of racemic 4,12-dibromo-[2.2]paracyclophane (**90**). Thus, when *rac*-**90** was treated with benzylamine (**91**) in the presence of 2 mol % of a palladium catalyst containing (*S*)-[2.2]PHANEPHOS (**95**)^[80] as the chiral ligand a product mixture consisting of monoaminated paracyclophane **92**, a small amount of diamine **93**, a dehalogenated derivative **94**, and recovered starting material **90** was obtained [Eq. (23)]. The determination of the enantiomeric excess of **90** revealed that a kinetic resolution had taken place. Thus, at 50 °C the rate of reaction for the amination of (*S*)-**90** was three to four times higher than for (*R*)-**90**. The use of a palladium complex derived from BINAP gave lower overall rates.

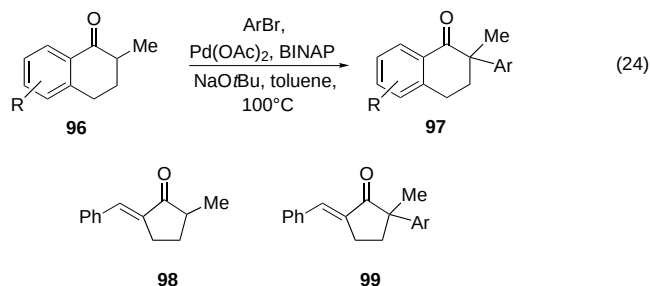


The addition of thallium hexafluorophosphate resulted in a significant rate enhancement, and by allowing the conversion to reach 79 %, an *ee* value of 93 % was obtained for the remaining (*R*)-**90**. An *ee* value of 99.9 % could be achieved at about 90 % conversion. The mechanism of the Hartwig/Buchwald amination reaction is proposed to proceed via insertion of palladium into the arene–halogen bond, followed

by coordination of the amine to the resulting complex, and, finally, reductive release of an aryl amine from the metal complex. This sequence of events is analogous to the proceedings in other coupling reactions, and hence the described kinetic resolution may be regarded as an asymmetric transfer of a paracyclophane onto an amine catalyzed by a chiral palladium complex. However, unlike in the other cases described in this review, the chirality-inducing step does not consist of a mere stereodiscriminating transfer reaction, but rather of a prior enantiodiscriminating aryl activation.

2.5. Asymmetric α -Arylations of Carbonyl Compounds

Palladium-catalyzed α -arylations of ketone enolates were first reported by Kuwajima and Urabe^[81] in 1982 and then by Migita and co-workers^[82] in 1984. They used silylenol ethers and enol acetates in their studies. About a decade later Palucki and Buchwald^[83] and Harmann and Hartwig^[84] independently reinvestigated this useful transformation and developed protocols for the catalytic introduction of aryl moieties into the α -position of ketones. These methods rely on the use of palladium/bisphosphane complexes which have proven to be excellent catalysts for related amination reactions. After having first employed racemic BINAP in the α -arylation, Buchwald and co-workers introduced an asymmetric version using the same catalyst (10–20 mol %) with enantiopure BINAP [Eq. (24)].^[85] Enantiomerically

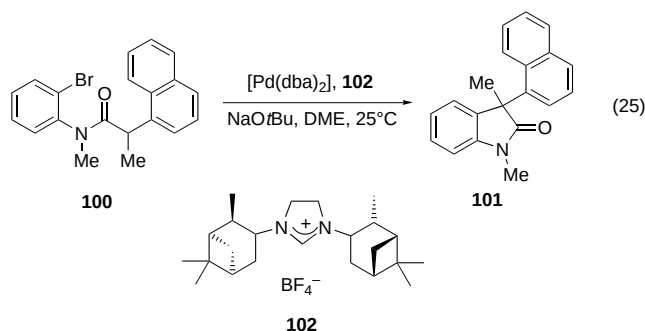


enriched products **97** with all-carbon quaternary centers were obtained from α -monomethyl-substituted tetralones **96** and a twofold excess of aryl bromides. Unfortunately, the asymmetric induction remained only moderate to good (61–88% *ee*).

A suprising fact was revealed when α' -blocked α -methyl-cycloalkanones such as **98** were employed. In this case, the enantioselectivity was highly dependent on the ring size of the substrate, and cyclopentanones were α -arylated with significantly higher enantioselectivities than their six-membered counterparts. Aryl bromides with *meta* and *para* substituents could be used to furnish α,α -disubstituted ketones **99** with high enantioselectivities (up to 98% *ee*) and excellent yields. For reasons still to be revealed, a number of other aryl and vinyl bromides led to racemic products.^[85]

Recently, Lee and Hartwig reported on asymmetric syntheses of oxindoles by intramolecular α -arylation of amides.^[86] Optically active heterocyclic carbenes served as the ligands in the palladium catalysts which gave α,α -disubstituted oxin-

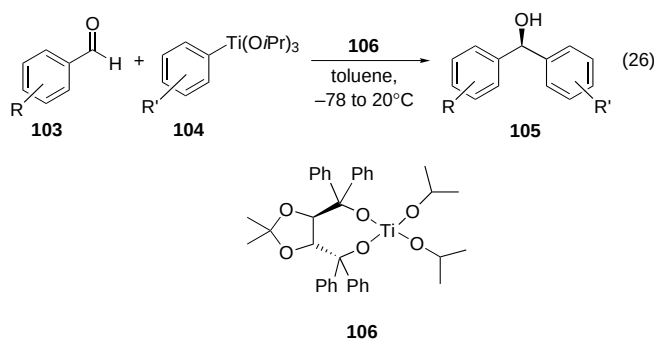
doles with substantial enantioselectivity. For example, **101** was obtained from **100** in 93% yield with 67% *ee* by using [Pd(dba)₂] and **102** as the chiral ligand (5 mol % each) and with sodium *tert*-butoxide as the base [Eq. (25)]. The enan-



tioselectivity could be increased to 76% *ee* by lowering the reaction temperature (from 25 to 10°C) and increasing the catalyst amount to 10 mol %. Optically active phosphane ligands were also tested, but the respective palladium catalysts gave only products with lower *ee* values.^[87]

3. Asymmetric Aryl Addition to Carbonyl and Heterocarbonyl Groups

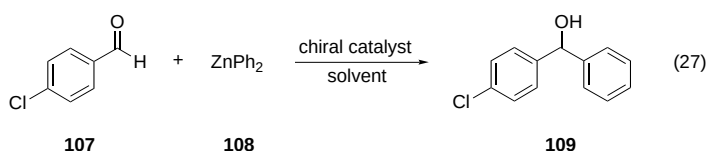
Whereas a stoichiometric enantioselective aryl transfer onto aldehydes was reported by Seebach et al. as early as 1985,^[88] it took until 1994 before a catalyzed version of this reaction, which employed titanium–TADDOLate complex **106** as the chiral catalyst, was described by the same group [Eq. (26)].^[89] The aryltitanium reagents **104**, which were used



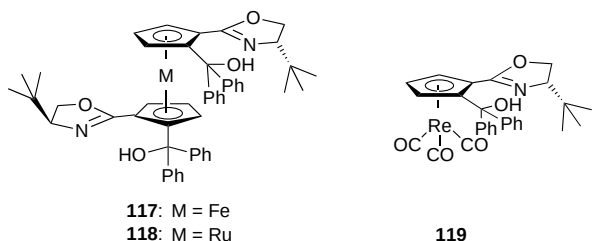
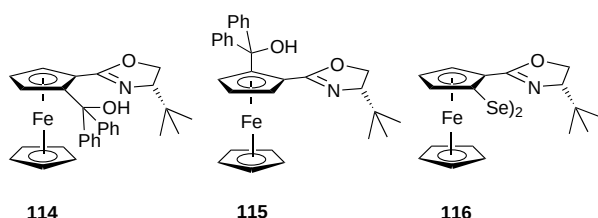
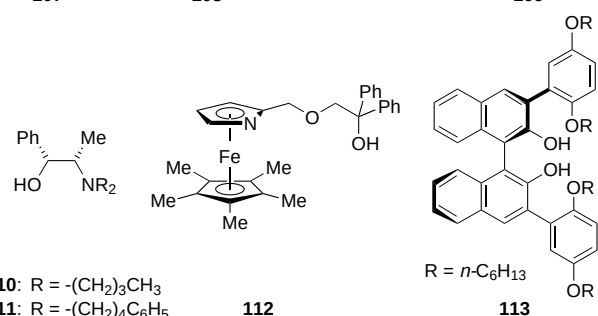
as the aryl sources, were prepared by treatment of the appropriate aryl Grignard or aryllithium reagents with chlorotriisopropoxytitanium. Since the inorganic magnesium/lithium salts had a detrimental effect on the catalysis, they had to be removed by centrifugation followed by filtration.^[90] The resulting soluble titanium reagents were then applied to the catalyzed aldehyde additions. However, the major disadvantages of this reaction were the relatively high catalyst loading of 20 mol % and the fact that substituted aromatic titanium reagents (**104** with $R' \neq H$) afforded products with very low enantioselectivities or even as racemic mixtures. The method also proved effective for aliphatic aldehydes, and even acetaldehyde gave the corresponding product with

97% *ee*. Both electron-poor and electron-rich aromatic substrates showed remarkable levels of enantioselection (up to 96% *ee*) with phenyl derivative **104** ($R' = H$).

An alternative asymmetric transfer of an aryl group to an aldehyde was described by Soai et al., who reported on the use of a zinc species generated in situ from zinc dichloride and phenylmagnesium bromide.^[91] Excess of stoichiometric amounts of *N,N*-dibutylnorephedrine (**110**) served as the chiral modifier giving enantiomerically enriched products with up to 82% *ee*.^[92] Surprisingly, the face selectivity of the process was altered with respect to that of the closely related dialkylzinc additions. It is important to note, that isolated diphenylzinc reacted very sluggish in the aryl transfer. Its use in asymmetric aldehyde additions was first described by Fu and co-workers [Eq. (27)].^[93] In an asymmetric catalysis using 3 mol% of chiral azaferrocene **112** the reaction of 4-chlorobenzaldehyde (**107**) with diphenylzinc (**108**) afforded the corresponding diarylmethanol **109** with 57% *ee* in nearly quantitative yield at room temperature.



110: $R = -(CH_2)_3CH_3$
111: $R = -(CH_2)_4C_6H_5$



The report by Fu and co-workers initiated intense research in this area and shortly thereafter two other catalytic asymmetric arylation of aldehydes were described which relied on structurally very different ligands. Huang and Pu reported successful applications of catalysts formed in situ from diorganozincs and chiral binaphthols (BINOLs), such as

113, which gave enantiomerically enriched alcohols with up to 94% *ee* in the phenylation of aldehydes.^[94] By comparing the absolute configurations of these products with those from related diethylzinc additions^[95] it was found that the stereochemical course of both transformations was identical. Furthermore, the authors reported a dependence of the enantioselectivity on the substrate concentration. Hence, the products had higher enantiomeric excesses in more dilute solutions. This behavior was explained by the fact that under these conditions the uncatalyzed transfer of the phenyl group to give racemic alcohols was less efficient. The reaction conditions were optimized for individual substrates, and significant differences were found. For example, several product alcohols could be obtained in a satisfying manner at room temperature in toluene with a relatively low catalyst loading (5 mol%), while reactions with other substrates required a solvent change or even the presence of methanol. In some cases an initial addition of diethylzinc to the binaphthol ligand generated a more active catalyst.

The chiral BINOLs were also incorporated into chiral polymers, which catalyzed the asymmetric phenylation of aldehydes to afford products with up to 92% *ee*.^[96] These results were comparable to those achieved with the low molecular weight BINOL-derived catalysts.

Since diphenylzinc itself is capable of adding to aldehydes, the final *ee* value of the product is usually lowered by a relatively strong uncatalyzed background reaction. With the goal to further activate the catalyst by ligand modification, Huang and Pu refined the BINOL structure and introduced electron-withdrawing substituents such as fluorine. As a result, more active catalysts were obtained, and as hoped for, the rate enhancement of the catalyzed pathway became more dominant and led to higher enantioselectivities (up to 95% *ee* for 4-chlorobenzaldehyde).^[97]

At the same time, Bolm and Muñiz described a catalyst system based on the chiral ferrocene **114**.^[98] The use of 5 to 10 mol% of the catalyst in toluene at 0°C led to a smooth addition of (isolated) diphenylzinc to various aldehydes to give secondary alcohols with good enantioselectivities. Aromatic substrates afforded synthetically interesting diarylmethanol compounds with up to 88% *ee*.^[98b] In addition, a range of aliphatic aldehydes were converted into the corresponding enantiomerically enriched benzyl alcohols with enantioselectivities of up to 75% *ee*. Asymmetric amplification studies illustrated that the actual catalytic species was monomeric. It is again important to note that the absolute stereochemistry of the product suggested an analogous reaction path as in the related diethylzinc addition with **114**, which had been investigated by the same authors earlier.^[99]

Ferrocene **114** is *S* configured at the stereogenic center and has an *R_p* configuration at the plane of chirality (subscript p).^[100] Comparative studies with its *S_p*-configured diastereomer **115** revealed how well-balanced the stereogenic elements of the ligand had to be in order to achieve high enantioselectivity.^[68] Thus, the addition of $ZnPh_2$ (**108**) to aldehyde **107** in the presence of the ferrocene **115** gave diarylmethanol **109** with only 9% *ee*.^[101] This result was again attributed to the significant uncatalyzed background reaction between $ZnPh_2$ and the aldehyde giving a racemic product. As

had previously been shown in alkylation reactions,^[102] the relative rate enhancements by the two diastereomers **114** and **115** are distinctively different and thus, the competitive unselective background reaction becomes more important in the catalysis with the less active catalyst **115** and leads to a reduction in the enantiomeric excess of the final product.

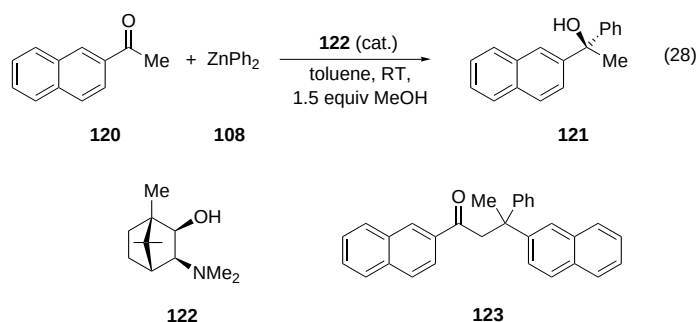
Efforts to overcome the problem of the parallel unselective addition of ZnPh_2 led to the development of an improved protocol, which relied on a modified phenyl transfer reagent. Thus, when isolated ZnPh_2 was replaced by a zinc reagent formed in situ by combining ZnPh_2 with ZnEt_2 in a ratio of 1:2, the enantioselectivity of the aryl transfer catalyzed by **114** was significantly improved to 90–98% *ee* for a wide spectrum of substrates.^[103, 104] Three more aspects are particularly noteworthy: Firstly, the reaction temperature could be raised from 0 to 10 °C without loss of enantioselectivity. Secondly, *ortho* substituents were better tolerated in aromatic aldehydes. For example, compounds such as 2-bromobenzaldehyde gave greatly improved enantioselectivities (91 versus 73% *ee*). Thirdly, the amount of the relatively expensive diarylzinc reagent ZnPh_2 was reduced to 0.65 equivalents (versus 1.5 equiv), which indicates that both phenyl groups could now be activated and then transferred to the aldehydes, while the product alcohols were still obtained in almost quantitative yields.

Under these improved conditions the less-active S,S_p -configured ferrocene **115** directed the addition of the modified phenylzinc reagent to 4-chlorobenzaldehyde (**107**) and yielded (*S*)-**109** with 68% *ee* rather than 9% *ee* as before. Using a 1:1 mixture of the diastereomeric ferrocenes **114** and **115** gave (*R*)-**109** with 91% *ee*.^[105]

Early attempts to improve the activity and enantioselectivity of the catalysts led to the development of other metallocenes such as ferrocenes and ruthenocenes **116**–**118**.^[106–108] Although in several reactions a good asymmetric induction in the aryl transfer was achieved (for example, the use of 5 mol% of **116** and **117** gave **109** with 84 and 94% *ee*, respectively), the overall catalyst performance was at best equal to that of ferrocene **114**. Other metal catalysts were prepared and tested,^[109] and, finally, a major breakthrough was achieved by the introduction of cyrhetrene **119**.^[110] The enantioselectivities in the phenyl transfer to aldehydes were further improved and *ee* values of up to 99% were reached in the formation of diarylmethanols by application of 2–10 mol% of this chiral Re complex. Furthermore, the use of **119** enabled the reaction to be carried out with a decreased catalyst loading; the enantiomeric excesses remained remarkably high even with less than 5 mol% of **119**. For example, use of only 2 mol% of **119** gave **109** with 96% *ee*, as compared to the previously obtained 97% *ee* with 10 mol% of **114**.^[103] Clearly, cyrhetrene **119** is an outstanding catalyst for the transfer of a phenyl group to an aldehyde.^[111]

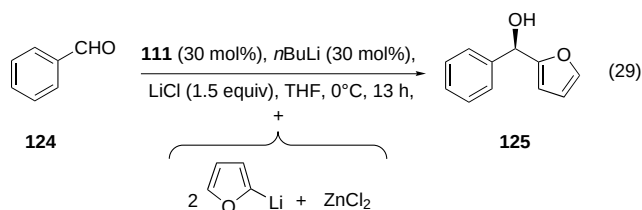
In the aryl transfers discussed previously the aldehydes have served as reaction partners. Attempts to use other carbonyl compounds as aryl acceptors have only been described to a limited extent. An early example was reported by Dosa and Fu, who developed an asymmetric 1,2-addition of diphenylzinc (**108**) to ketones catalyzed by Noyori's DAIB

(**122**). The corresponding tertiary alcohols were obtained with good to high enantioselectivities [Eq. (28)].^[112] Since the use of isolated diphenylzinc (**108**) and **120** afforded relatively



large quantities of **123** (60%), derived from an aldol condensation reaction followed by 1,4-addition, the desired product **121** was only obtained in low yield (26%; 64% *ee*). It was found that the reaction yielding **123** could be efficiently suppressed by the addition of methanol (1.5 equiv relative to the ketone). Both the chemical yield and enantioselectivity of **121** were improved (58% yield, 72% *ee*). Presumably, a less reactive zinc alkoxide was formed, which underwent 1,2-addition more selectively.^[122, 113]

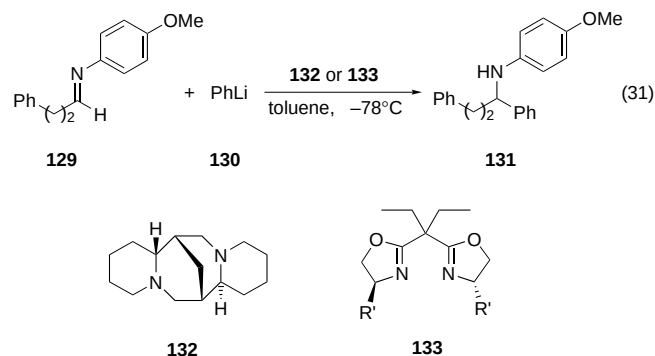
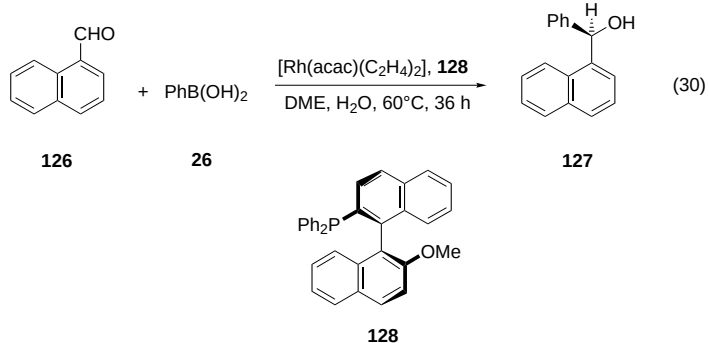
Heteroaromatic groups can also be transferred to aldehydes. Thus, *N,N*-disubstituted norephedrine derivative **111** proved to be the best auxiliary for an enantioselective furylation of benzaldehyde (**124**), as described by Soai and Kawase [Eq. (29)].^[114] A complex reaction protocol had to be



developed to obtain reasonable results: this included the supposed in situ formation of di(2-furyl)zinc from 2-furyllithium and zinc dichloride, as well as a pretreatment of **111** with one equivalent of *n*-butyllithium and five equivalents of LiCl. Thus, alcohol **125** was obtained with 30% *ee* by using 30 mol% of **111** and using benzaldehyde (**124**) as the substrate. Stoichiometric quantities of **111** were required to improve the enantioselectivity to 72% *ee*.

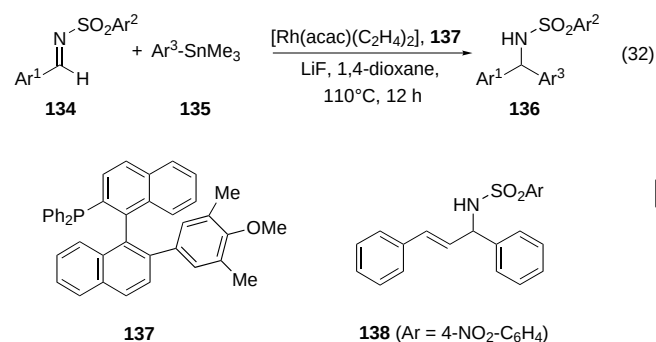
Miyaura and co-workers described an asymmetric 1,2-addition of phenylboronic acid (**26**) to 1-naphthaldehyde (**126**) using (*S*)-MeO-MOP (**128**) as a chiral ligand [Eq. (30)]. Contrary to the analogous 1,4-additions (see Section 4), only a moderate enantioselectivity was achieved to give **127** with 41% *ee* (78% yield).^[115]

Heterocarbonyl compounds can also be applied as aryl acceptors. An asymmetric addition of phenyllithium (**130**) to imine **129** in the presence of substoichiometric quantities of sparteine (**132**) [Eq. (31)] was reported by Denmark et al.^[116–118] When reactions of organolithium reagents with imines were studied it was found that both sparteine (**132**) as



well as bisoxazolines **133** were suitable chiral ligands for the synthesis of optically active amines. The former gave significantly higher enantioselectivities in the transfer of a phenyl group from **130** onto **129**. Unfortunately, a major decrease in enantioselectivity was observed when substoichiometric quantities of the chiral ligand were used. Thus, the addition of **130** to imine **129** in the presence of one equivalent of **132** yielded **131** with 82 % *ee* (99 % yield). When 0.2 equivalents of **132** were used, the same product had only 39 % *ee* (97 % yield).^[119]

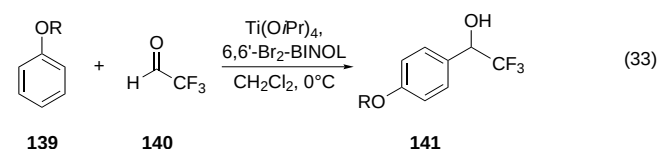
The first catalytic asymmetric addition of aryl stannanes to imines was described by Hayashi and Ishigedani [Eq. (32)].^[120] This transformation is rhodium-catalyzed in a



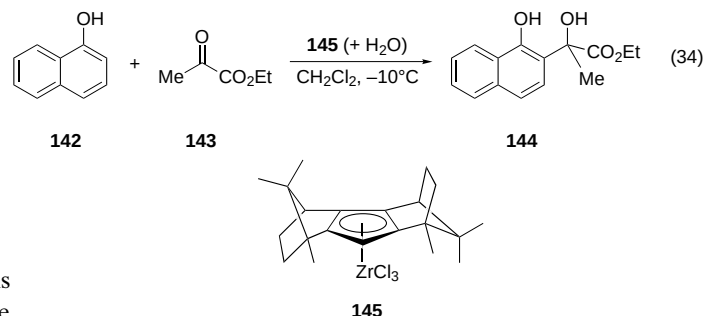
similar way to related reactions, such as conjugate additions and aldehyde arylations, however, here organostannanes are utilized as aryl sources instead of arylboronic acids. Rhodium catalysts with monophosphane (MOP) ligands such as **137** (3 mol % of Rh and 6 mol % of ligand) were superior to complexes bearing chelating bisphosphanes such as BINAP or diop (**4**).^[121] The imines were used in the form of *N*-

alkylidenesulfonamides **134**, which yielded the corresponding products **136** with enantioselectivities that depended on the substituents Ar^2 at the sulfonamide group. Compounds bearing a *p*-nitrobenzene sulfonyl (nosyl) group were among the best in terms of both enantioselectivity (up to 96 % *ee*) and yield. Furthermore, the resulting sulfonamides could easily be deprotected to give the corresponding optically active diaryl-methylamines. Chiral allylamines could also be obtained by this method. Thus, when the sulfonamide derived from cinnamyl aldehyde was used, the phenylation occurred smoothly to give allylic amine **138** with 93 % *ee* and in 77 % yield. In this study only aryl and α,β -unsaturated imines were used. It remains to be established whether the protocol is also applicable to simple aliphatic imines.^[122]

The aryl transfer reactions discussed so far in this section have involved the formation of organometallic intermediates with aryl–metal bonds. Conceptionally different are Friedel–Crafts-type additions of electron-rich arenes onto catalyst-activated carbonyl or heterocarbonyl compounds. An early result employing stoichiometric amounts of a chirally modified aluminum reagent in the enantioselective *ortho*-hydroxy-alkylation of phenol with trichloroacetaldehyde (up to 80 % *ee*) was reported by Casiraghi and co-workers.^[123, 124] Recently, a modification of this approach was used by Mikami and co-workers in asymmetric Friedel–Crafts reactions of phenol ethers **139** and fluoral (**140**). The use of 10 mol % of a titanium catalyst bearing 6,6'- Br_2 -BINOL as the ligand afforded 1-aryl-2,2,2-trifluoroethanols **141** with up to 90 % *ee* [Eq. (33)].^[125, 126] Even the use of only 1 mol % of the catalyst led to relatively high enantioselectivities. Asymmetric activation^[127] allowed the efficiency of the catalyst to further increase.



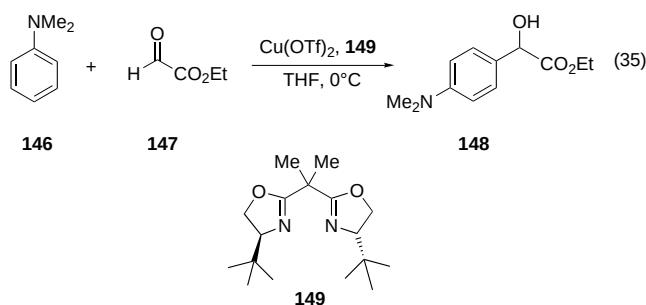
Erker and van der Zeijden reported in 1990 that 1-naphthol (**142**) reacted with ethyl pyruvate (**143**) under catalysis with camphor-derived zirconium complex **145** to give product **144** in an asymmetric manner [Eq. (34)].^[128] With only 1 mol % of



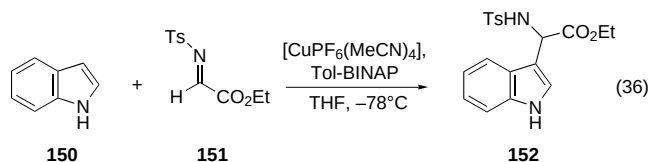
the catalyst, 70 % of **144** having 27 % *ee* was obtained after 2 h at room temperature. The enantioselectivity was conversion dependent, and hence decreased during the reaction. When the temperature was lowered to -10°C and, most remark-

ably, when a well-defined amount of water was added, the *ee* value of **144** acutely increased. Thus, the *ee* value of the product reached 89 % at 70 % conversion using a ratio of **142**:**143**:**145**:H₂O of 100:500:5:27. At 90 % conversion **144** had 84 % *ee*.

This Friedel–Crafts chemistry was recently extended by Jørgensen and co-workers who found that bisoxazoline–copper(II) complexes catalyzed the enantioselective Friedel–Crafts reaction between *N,N*-dimethylaniline (**146**) and ethyl glyoxylate (**147**) [Eq. (35)].^[129] Exclusive *para*-substitution took place on ligand **149** to give **148** with up to 94 % *ee*. Substituted *N,N*-dimethylanilines, furans, and other related compounds also worked well and afforded the corresponding products with moderate to good enantioselectivities.

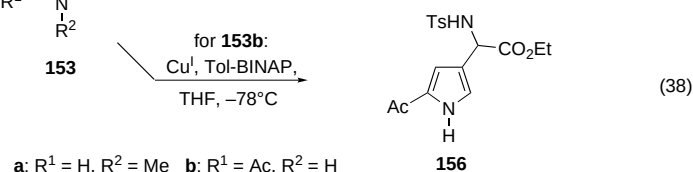
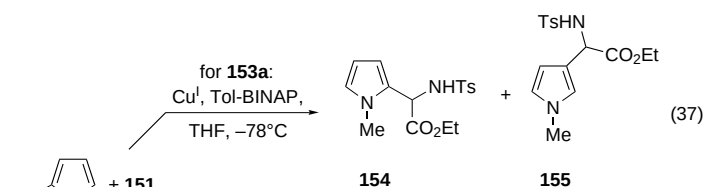


The catalyzed asymmetric addition of heteroaromatic nucleophiles onto the *N*-tosylimine **151** derived from ethyl glyoxylate was developed by Johannsen.^[130] The catalyst system [CuPF₆(MeCN)₄]/Tol-BINAP^[131] is very efficient, and the novel *N*-tosylamino acid esters were obtained with high enantioselectivity and good yield with a catalyst loading of only 1 mol %. For example, catalyzing the reaction of indole (**150**) with imine **151** afforded product **152** with 96 % *ee* and 89 % yield [Eq. (36)]. Whereas indoles reacted selectively

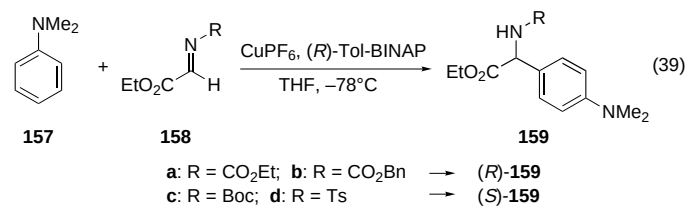


at the 3 position, pyrroles **153** showed less regioselectivity. Thus, in the reaction with *N*-methylpyrrole (**153a**), an approximately 1:1 mixture of the 2- and 3-substituted products **154** and **155** (having 84 and 56 % *ee*, respectively) was formed in a total yield of 89 % [Eq. (37)].^[132] The regioselectivity problem can be delineated by introduction of a deactivating acetyl group in the 2 position of the pyrrole ring. Thus, the addition of **151** to **153b** took place with high selectivity to give 3-substituted pyrrole **156** with 94 % *ee* and 76 % yield [Eq. (38)].

Jørgensen and co-workers used the Cu^I/Tol-BINAP catalyst system for the asymmetric addition of electron-rich aromatic compounds to α -imino esters.^[133] Optimization of the reaction protocol (5 mol % of catalyst in THF at –78 °C) allowed the synthesis of **159a** with 96 % *ee* and in 75 % yield from *N,N*-dimethylaniline (**157**) and imine **158a** [Eq. (39)]. The *N*-ethoxycarbonyl group of **159a** could then be cleaved to



a: R¹ = H, R² = Me b: R¹ = Ac, R² = H



a: R = CO₂Et; b: R = CO₂Bn → (R)-**159**
 c: R = Boc; d: R = Ts → (S)-**159**

liberate the corresponding *N*-unprotected amino acid ester. The imine protecting group had a pronounced effect on the absolute configuration of the product: when (*R*)-Tol-BINAP was used as a ligand, *N*-alkoxy- and *N*-benzyloxycarbonyl-protected imines of **158** gave the *R* enantiomer of the corresponding addition products **159**, which is in contrast to more bulky *N*-protecting groups such as Boc or tosyl (Ts), which led to the formation of the *S* enantiomer of **159**. On the basis of an observed drastic temperature dependence of the enantioselectivity it was proposed that two competitive coordination modes were responsible for the switch of mechanistic pathways leading to opposite asymmetric induction.

The examples of catalyzed asymmetric aryl transfer reactions onto carbonyl compounds summarized in this review illustrate that significant progress has been achieved in recent years. However, several factors still need improvement. For example, in the case of arylzinc reagents the catalysis is still hampered by the fact that phenyl transfers from diphenylzinc have been predominantly studied. The applicability of other arylzinc reagents still needs to be demonstrated. Thus, at this stage it may be concluded that the rhodium-catalyzed 1,2-additions employing aryl boronic acids and stannanes have a wider scope despite the problems related to cost and potential toxicity of reagents. However, in many cases, the aryl transfer reagent has to be employed in large excess, and therefore further developments are desirable for the improvement of the catalyst efficiency. Since this process is closely related to rhodium-catalyzed conjugate additions (see Section 4), it should follow that progress will be achieved for both transformations almost simultaneously.

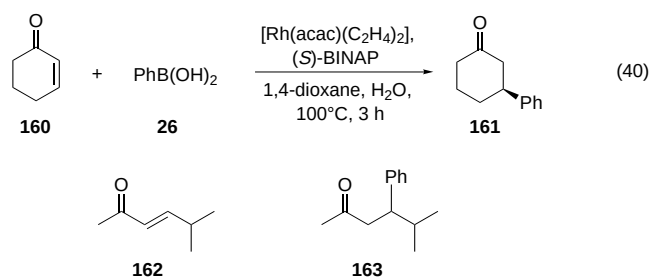
Very impressive results have recently been reported in the area of Friedel–Crafts-type aryl transfers. The catalysts used in these reactions for the activation of the carbonyl or heterocarbonyl compounds are simple and lead to high enantioselectivities. Improvements are still required regarding the regioselectivity, because often potentially highly useful

products are obtained as regioisomeric mixtures. Here, also, new catalyst systems can be expected to offer solutions to overcome the currently existing limitations.

4. Conjugate Addition Reactions and Allylic Substitutions

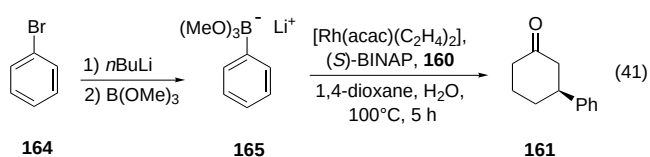
Achieving high enantioselectivities in catalyzed asymmetric 1,4-additions of nucleophiles to α,β -unsaturated carbonyl compounds has been a longstanding goal.^[134] Most significant progress has been made with respect to nickel- and copper-catalyzed conjugate additions to enones using dialkylzincs and, in certain cases, with organomagnesium reagents. Only recently, a novel rhodium-catalyzed process has been developed, which appears to be of high generality with respect to reagents, substrates, and selectivity.

Hayashi, Miyaura and co-workers described the highly enantioselective 1,4-addition of alkenyl- and arylboronic acids to enones catalyzed by a rhodium(i)/BINAP catalyst.^[135] For example, phenylboronic acid (**26**) and cyclohexenone (**160**) reacted smoothly to give ketone **161** with 97% *ee* and in >99% yield [Eq. (40)]. Linear *trans* enones such as **162** also



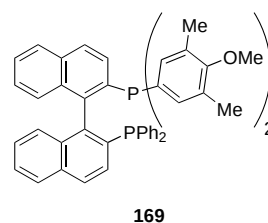
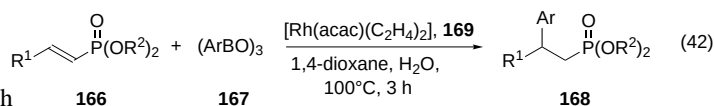
added **26** with excellent enantioselectivity to give **163** with 97% *ee* and 82% yield. No 1,4-addition was observed in the absence of the rhodium catalyst. Since boronic acids undergo decomposition under the standard reaction conditions (dioxane/ H_2O , 100°C), they had to be employed in a relatively large excess (often fivefold) to obtain high product yields. The mechanism of this process was proposed to involve an aryl–rhodium intermediate, which after insertion into the enone double bond rearranges to a rhodium–enolate. Hydrolysis and transmetalation releases the product and regenerates the catalytically active aryl–rhodium species.

Given the fact that arylboronic acids are readily generated from the corresponding aryl halides, it appeared desirable to directly employ the latter. This is indeed possible by an in situ sequence including lithiation of an aryl bromide (for example, phenyl bromide (**164**)), subsequent formation of the corresponding arylborate **165** with trimethylborate, and 1,4-addition catalyzed by the chiral rhodium/BINAP complex [Eq. (41)].^[136, 137] Moreover, the excess of arylating reagent



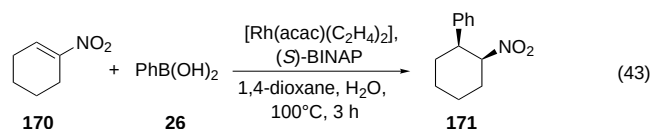
could be significantly lowered from 5 to 2.5 equivalents with this new method. Interestingly, while *p*-methoxyphenyl boronic acid did not react under the original conditions because of the competing decomposition, the use of 4-bromoanisole in accordance with the new protocol gave both high yields and excellent asymmetric induction in the addition to cyclic enones. The catalysis also displayed a pronounced dependence on the amount of water present. Thus 0.5 equivalents were sufficient to keep it at an acceptable rate whereas almost no addition (4%)^[138] was observed under anhydrous conditions. With 10 equivalents of water (relative to the aryl bromide) the enantioselectivity remained at a very high level (98% *ee*), but the yield was significantly lowered (19%).^[138] It should also be noted that in this in situ process, substrate to catalyst ratios of up to 1000:1 still afford almost complete enantioselection (99% *ee*). The species responsible for transmetalation to rhodium is suggested to be either $\text{Li}[\text{ArB}(\text{OMe})_2\text{OH}]$ or $\text{ArB}(\text{OMe})(\text{OLi})$ as determined in control experiments using phenyl dimethoxyboronate and lithium hydroxide. Both protocols can also be applied in the conjugate addition to α,β -unsaturated esters.^[139] Again, as described for enones, the one-pot method proved superior and prevented the hydrolysis of the boronic acid from occurring—the process responsible for the low product yields. Surprisingly, when lactones were employed, the in situ method largely failed, whereas the use of arylboronic acids led to smooth catalysis.

Unsaturated phosphonates **166** can also be employed as substrates in 1,4-additions. When used together with arylboroxines **167** and a rhodium complex containing **169** they afford the corresponding β -arylated phosphonates **168** in good to high yield with excellent enantioselectivities [Eq. (42)].^[140]



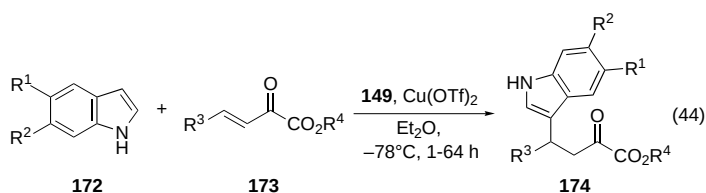
Attempted use of arylboronic acids under the previously described conditions proved less efficient. This occurs as a consequence of a reduced catalyst activity in the presence of relatively large quantities of water, which served as the cosolvent. On the other hand, utilizing arylboroxines **167** and a strictly limited amount of water (only one equivalent of water with respect to the boron compound) led to an improvement in both the yield and enantiomeric excess.

Recently, Hayashi et al. demonstrated that arylboronic acids could also be added enantioselectively to α -substituted 1-nitroalkenes using rhodium catalysis.^[141] For example, the reaction between **26** (5 equiv) and 1-nitrocyclohexene (**170**) in the presence of 3 mol % of $[\text{Rh}(\text{acac})(\text{C}_2\text{H}_4)_2]/\text{BINAP}$ at 100°C in dioxane/water led to the formation of **171** with up to 99% *ee* [Eq. (43)]. The diastereoselectivity was also high,



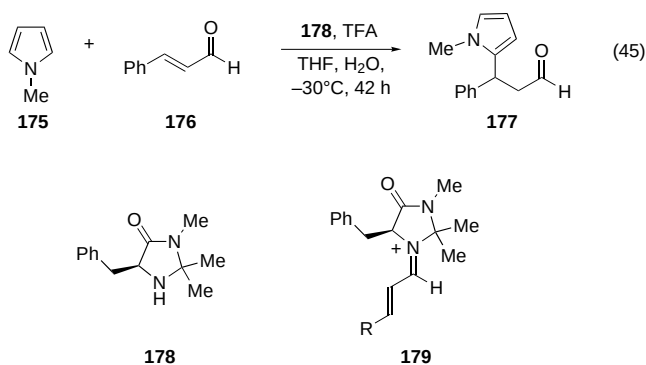
with the thermodynamically less-stable *cis* isomer being preferred (*cis:trans* = 87:13). Base treatment of the *cis*-rich mixture resulted in equilibration and afforded a *trans:cis* ratio of 97:3 without changing the *ee* value.

Metal-catalyzed Friedel–Crafts-type additions of electron-rich arenes onto unsaturated carbonyl compounds are mechanistically very different. Jørgensen and co-workers described such a process using a combination of the bisoxazoline ligand **149** and copper(II) triflate as the catalyst (2–10 mol %) [Eq. (44)].^[142] The addition occurred with excellent enantio-



selectivities (up to >99.5% *ee*) with indols **172** and β,γ -unsaturated α -ketoesters **173** as substrates, and afforded products **174** in high yields. Other heteroaromatic compounds, such as furans and anisoles, also gave the corresponding Friedel–Crafts products, but the *ee* values remained below 90%.

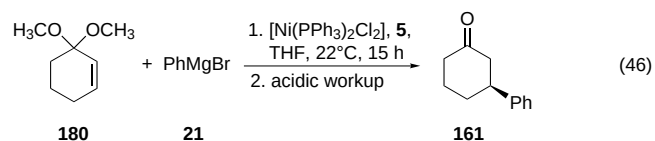
In this context the organocatalytic addition of pyrroles to α,β -unsaturated aldehydes developed by Paras and MacMillan is also of interest.^[143] A smooth reaction between N-substituted pyrroles such as **175** and enals occurred in the presence of 20 mol % of imidazolidinone **178** and TFA as cocatalyst to afford the 1,4-addition products with up to 97% *ee* [Eq. (45)]. The utility of the protocol was demon-



strated in the reaction of cinnamaldehyde (**176**) and **175** on a 25-mmol scale, where **177** was obtained with 93% *ee* and in 87% yield. The sense of asymmetric induction was readily explained by the directing effect of the proposed intermediate iminium ion **179**.

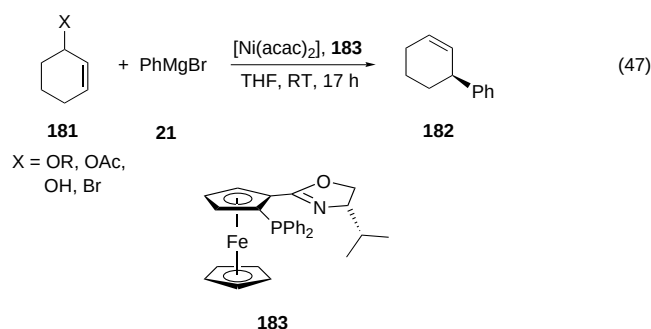
Enantiomerically enriched 3-phenylcyclohexanone (**161**) can also be obtained from phenylmagnesium bromide (**21**)

and cyclohexenone ketal **180** [Eq. (46)].^[144] For this allylic substitution type reaction, Hoveyda et al. developed a protocol which makes use of a nickel complex containing chiraphos (**5**) as the chiral ligand. The product was obtained (after



hydrolysis of the intermediate enol ether) with good enantioselectivity (83% *ee*) and satisfying yield (67%) under optimized reaction conditions. The enantiomeric excess was strongly dependent on the reagent and additive. Thus, the enantioselectivity was significantly lower (**161** with 66% *ee*) in the catalysis when phenylmagnesium chloride was used as the aryl transfer reagent. The presence of an additional 10 mol % of triphenylphosphane was essential for obtaining high asymmetric induction in several other reactions. Lower enantioselectivities were observed with 5 and 20 mol % of this additive. At present, the precise role of the triphenylphosphane is unclear and hence general trends concerning beneficial effects of Lewis basicity or the structure of other phosphane additives can not be deduced.

Asymmetric allylic arylations starting from 3-substituted cyclohexenes **181** and phenylmagnesium bromide (**21**) have recently been described by Uemura and co-workers [Eq. (47)].^[145] By using a combination of [Ni(acac)₂] and



ferrocene **183** as the chiral catalyst 3-phenylcyclohexene (**182**) was obtained with an *ee* value of up to 88% (for **181** with X = Br). Other aryl Grignard reagents could also be applied, and afforded products with a maximum *ee* value of 95% (from the reaction of **181** (X = OPh) and 2-naphthylmagnesium bromide).

Uemura and co-workers also demonstrated that arylboronic acids could be used in a nickel catalysis to afford the allylic substitution products with moderate enantiomeric excesses (**182** with 53% *ee*) in good yields.^[146] In this case the catalytic system required the addition of DIBAL and KOH to be active. It was proposed that the initial mechanistic step involves a transfer of an aryl group from a boron to a nickel atom. All attempts to employ palladium-derived catalysts failed and gave **182** in very low yield.

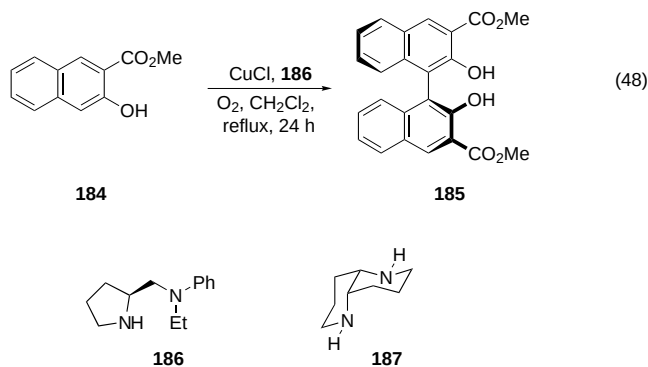
As noted before, the development of efficient catalytic processes to achieve enantioselective conjugate additions and allylic substitutions has been a long-standing goal in asym-

metric catalysis. For alkylations, the achievements in copper-catalyzed conjugate additions of diorganozinc reagents and palladium-catalyzed alkylations marked the first major steps towards a general solution of these problems. On the basis of these successful reactions, improvements of other asymmetric addition reactions including the transfer of aryl groups can now be expected. The emergence of the rhodium-catalyzed 1,4-addition of aryl moieties to α,β -unsaturated carbonyl compounds, which allows the transfer of various sp^2 -hybridized groups in a highly enantioselective fashion, already represents an important breakthrough in this area. This transformation is characterized by low catalyst loading as well as the utilization of readily available ligands and substrates. Simple compounds such as boronic acids serve as convenient aryl transfer reagents, of which many are either commercially available or easily prepared from inexpensive starting materials. At present, the only drawback of this method is that often relatively large quantities of the aryl source have to be employed. With the development of more active catalysts and new reaction protocols these negative aspects can also be expected to be eliminated in the very near future.

5. Enantioselective Oxidative Coupling of Naphthols

The oxidative coupling of naphthols is a widely used method for the synthesis of binaphthols. Since the latter and their derivatives belong to the most successful chiral ligands in asymmetric catalysis,^[55, 96] an enantioselective catalytic version of this coupling reaction appeared most desirable. Early achievements have been described by Smrcina et al.,^[147] and further developments along the same lines have recently been reported by Nakajima et al.^[148] Both research groups used copper complexes containing chiral amino ligands. For example, when substituted naphthol **184** was oxidatively coupled the corresponding biaryl **185** was formed in good yield [Eq. (48)]. The enantioenriched product had an *ee* value of 78 % when a combination of copper(I) chloride and proline-based diamine **186** was used, while significantly lower enantioselectivities resulted when (–)-sparteine (**187**) was used as a ligand.^[149]

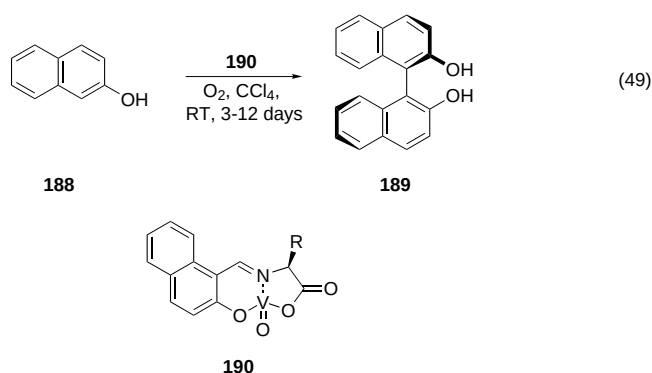
The ester substituent on the naphthol was essential to achieve a good asymmetric induction in the coupling sequence. This behavior was explained by a necessary coordination of the copper catalyst in a chelate fashion to the substrate. The enantiodiscrimination is then believed to



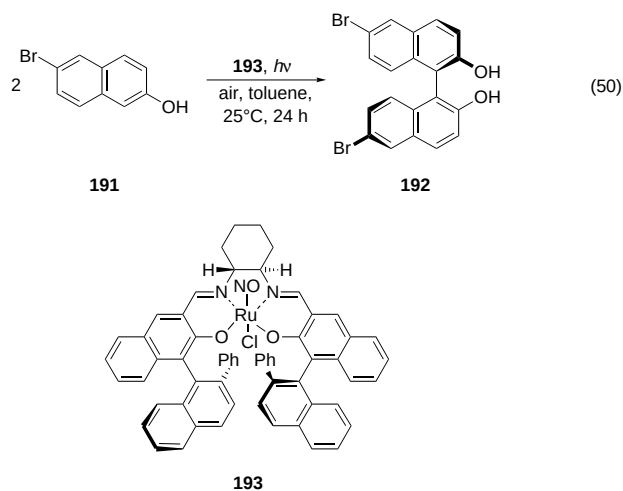
proceed by keto–enol tautomerism after oxidative carbon–carbon bond formation between the two naphthol units.

Recently, copper-catalyzed oxidative biaryl coupling reactions have also been investigated by Yang and Kozlowski, who used 1,5-diazadecalin (**187**) as a ligand.^[150] Enantioselectivities of up to 93 % *ee* were achieved in the coupling of **184** when 10 mol % of a 1:1 mixture of a copper(I) or copper(II) source and **187** were employed. Again, the ester substituent in the 3 position proved essential, and other substrates lacking this moiety gave lower enantioselectivities.

Chen and co-workers found that oxovanadium(IV) complexes **190** could be used as catalysts for the enantioselective oxidative coupling of 2-naphthols.^[151] For example, the dimerization of 2-naphthol (**188**) in the presence of 10 mol % of **190** (with R = *i*Pr) gave **189** with 62 % *ee* in 94 % yield [Eq. (49)]. Other substrates afforded substituted coupling products with enantioselectivities in the range of 10–68 % *ee*.



Another approach towards the asymmetric oxidative coupling of 2-naphthol derivatives was recently reported by Katsuki and co-workers.^[152] A photopromoted aerobic coupling occurred when salen–ruthenium complex **193** was used as the catalyst, and gave 2,2'-binaphthols with moderate to good enantioselectivities.^[153] In contrast to various other systems, substituents at the 3 position of the 2-naphthols were not required. The best results were achieved with compounds bearing electron-withdrawing groups at C6. For example, the asymmetric coupling of 6-bromo-2-naphthol (**191**) in the presence of 5 mol % of **193** afforded **192** in 30 % yield with 71 % *ee* [Eq. (50)]. With 2-naphthol (**188**) itself, the corre-

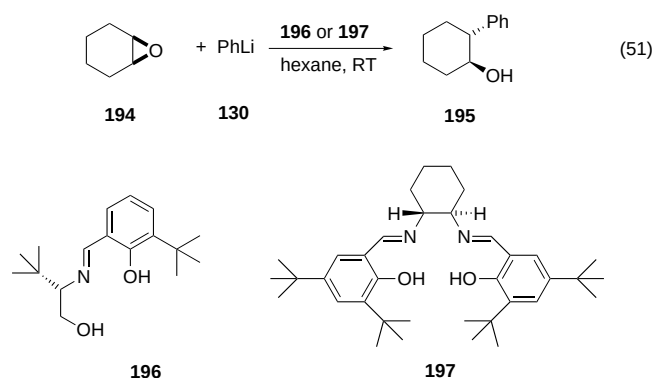


sponding product **189** was obtained with 65% yield and 57% *ee*.

It is noteworthy that these coupling reactions do not involve one of the species outlined in Scheme 1. Instead, aryl radicals are involved at the stage prior to dimerization which generate the binaphthylene upon formal combination.

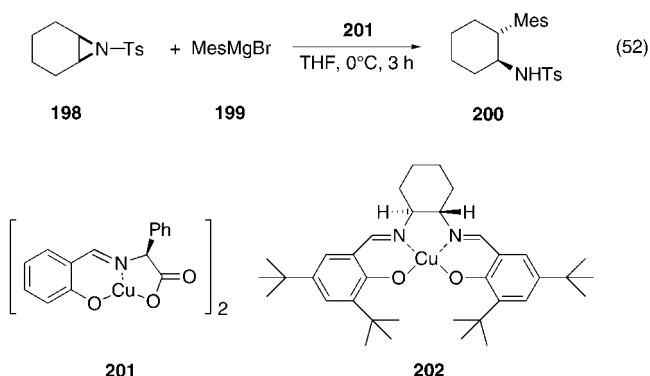
6. Asymmetric Ring Opening of Epoxides and Aziridines

A desymmetrization^[154] of *meso*-epoxides such as **194** by means of enantioselective ring opening using phenyllithium (**130**) was developed by Oguni et al. [Eq. (51)].^[155] The



catalysts used in this study were either the tridentate Schiff base **196**, or salen ligand **197**, which afforded the corresponding alcohol **195** with enantioselectivities of up to 90% *ee*. Surprisingly, the reaction of the same substrate with alkyl-lithium reagents resulted in both low yields and enantioselectivities. It was suggested that the catalytically active species in the arylation was either a lithium alkoxide or a lithium amide which was initially formed by attack of phenyllithium on the imine moieties of the ligands.

Recently, Müller and Nury described a related transformation that utilized *meso*-aziridines as starting materials [Eq. (52)].^[156] Aryl Grignard reagents were found to add to



N-sulfonylaziridine **198** under copper catalysis to afford the corresponding products with moderate enantiomeric excesses. Whereas the transfer of the phenyl group from PhMgBr (**21**) occurred with low enantioselectivities (best result: 29% *ee*),

the catalyzed addition of mesitylmagnesium bromide (**199**) by 10 mol% of **201** gave **200** with 72% *ee*. Surprisingly, the absolute configuration of the phenyl addition product with **202** as the catalyst proved opposite to the one expected from the related alkyl Grignard addition.

Particularly noteworthy is the fact that these transformations described by Oguni et al.^[155] and Müller and Nury^[156] represent rare examples of asymmetric openings of epoxides and aziridines with carbon nucleophiles.^[154, 157, 158]

7. Conclusion and Outlook

Although the great potential of catalytic asymmetric arylations is apparent, transformations of such type have only recently started to emerge as useful tools in organic synthesis. Many limitations still have to be overcome before these catalyses reach the same remarkable level of maturity as their corresponding counterparts in alkylation chemistry. Until now, general catalysts have only been found for a small number of reactions, and hence it is of no surprise that these rely on readily available ligands such as BINAP or related compounds. A major obstacle in the development of efficient asymmetric arylation reactions is the lack of practical aryl transfer reagents. Further studies can be expected to be devoted towards their improvement in order to broaden the scope of this reaction. Since many reactions are asymmetric variants of well-established viable cross-coupling reactions (or at least utilize substrates commonly used in those transformations), it is foreseeable that achiral cross-coupling variants will most likely have a major impact on the development of novel asymmetric aryl transfer reactions.

Appendix: Abbreviations

acac	= acetylacetonate
alaphos	= (2-dimethylaminopropyl)diphenylphosphane
BINAP	= 2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl
BINOL	= 1,1'-binaphthalene-2,2'-diol
BITIANP	= 2,2'-bis(diphenylphosphanyl)-3,3'-dibenzo[<i>b</i>]-thiophene
Boc	= <i>tert</i> -butoxycarbonyl
chiraphos	= 2,3-bis(diphenylphosphanyl)butane
Cy	= cyclohexyl
DAIB	= 3- <i>exo</i> -dimethylaminoisoborneol
dba	= dibenzylidene acetone
DIBAL	= diisobutylaluminum hydride
diop	= 2,3- <i>O</i> -isopropylidene-2,3-dihydroxy-1,4-bis(diphenyl-phosphanyl)butane
DMA	= <i>N,N</i> -dimethylacetamide
DME	= 1,2-dimethoxyethane
DMSO	= dimethylsulfoxide
EWG	= electron-withdrawing group
MeO-MOP	= 2-diphenylphosphanyl-2'-methoxy-1,1'-binaphthyl
Mes	= mesityl = 2,4,6-trimethylphenyl
NORPHOS	= 5,6-bis(diphenylphosphanyl)-2-norbornene

[2.2]PHANEPHOS	= 4,12-bis(diphenylphosphanyl)-[2.2]paracyclophane
phephos	= 2-dimethylamino-1-diphenylphosphanyl-3-phenylpropane
PMP	= 12,2,6,6-pentamethylpiperidine
PPFA	= 1-(2-diphenylphosphanylferrocenyl)ethyl-dimethylamine
PPFOMe	= 1-(2-diphenylphosphanylferrocenyl)ethyl methyl ether
propfos	= 1,2-bis(diphenylphosphanyl)propane
TADDOL	= $\alpha,\alpha,\alpha',\alpha'$ -tetraphenyl-1,3-dioxolane-4,5-dimethanol
TBDPS	= <i>tert</i> -butyldiphenylsilyl
Tf	= triflate = trifluoromethanesulfonyl
TFA	= trifluoroacetic acid
TIPS	= triisopropylsilyl
TMBTP	= 4,4'-bis(diphenylphosphanyl)-2,2',5,5'-tetramethyl-3,3'-bithiophene
TMS	= trimethylsilyl
TolBINAP	= 2,2'-bis-(di- <i>p</i> -tolylphosphanyl)-1,1'-binaphthyl
Ts	= tosyl = toluenesulfonyl

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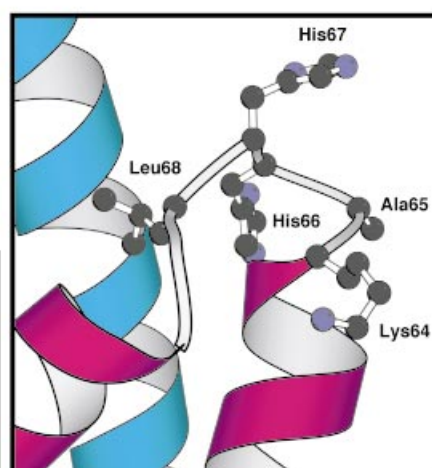
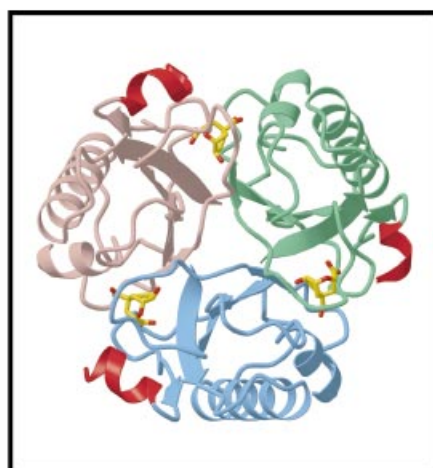
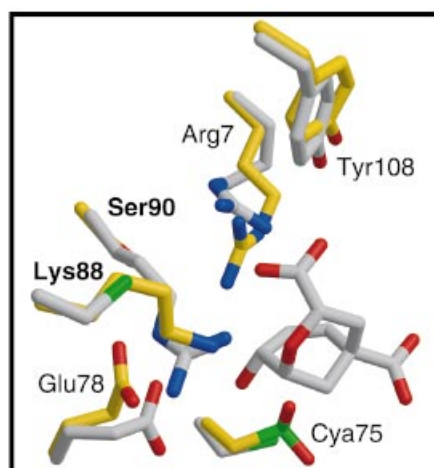
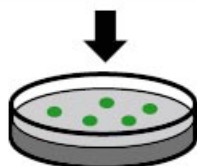
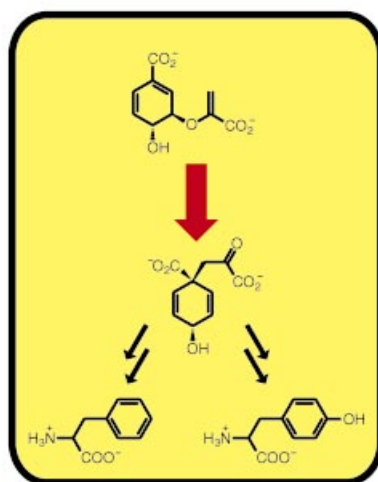
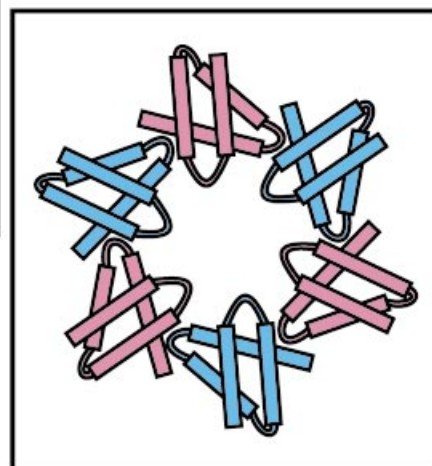
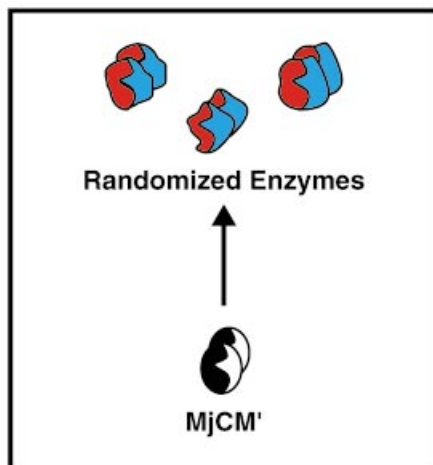
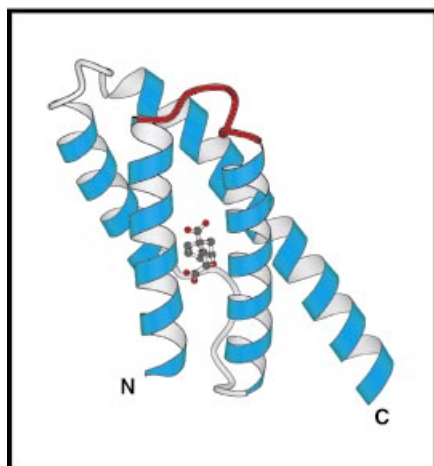
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Investigating and Engineering Enzymes by Genetic Selection

Sean V. Taylor, Peter Kast, and Donald Hilvert*

Natural enzymes have arisen over millions of years by the gradual process of Darwinian evolution. The fundamental steps of evolution—mutation, selection, and amplification—can also be exploited in the laboratory to create and characterize protein catalysts on a human timescale. In vivo genetic selection strategies enable the exhaustive analysis of protein libraries with

10^{10} different members, and even larger ensembles can be studied with in vitro methods. Evolutionary approaches can consequently yield statistically meaningful insight into the complex and often subtle interactions that influence protein folding, structure, and catalytic mechanism. Such methods are also being used increasingly as an adjunct to design, thus

providing access to novel proteins with tailored catalytic activities and selectivities.

Keywords: directed evolution • enzyme catalysis • enzymes • genetic selection • protein design • protein structures

1. Introduction

Evolution is the slow and continual process by which all living species diversify and become more complex. Through recursive cycles of mutation, selection, and amplification, new traits accumulate in a population of organisms.^[1] Those that provide an advantage under prevailing environmental conditions are passed from one generation to the next. Since ancient times, man has exploited evolution in a directed way to produce plants and animals with useful characteristics. The cross-breeding of individuals with favorable traits successfully harnesses sexual recombination, one of the most powerful evolutionary strategies to generate new variants. From these crossings, progeny with improved features are chosen for additional breeding cycles, thus channeling the course of development.

Biologists and chemists have recently begun to use evolutionary strategies to tailor the properties of individual molecules rather than whole organisms. An array of methods has been developed to generate diversity in populations of molecules. Depending on the experiment, mutagenesis might entail degenerate oligonucleotide-directed or error-prone DNA synthesis,^[2, 3] shuffling of mutant DNA fragments,^[4, 5] or combinatorial syntheses of chemical compound libraries.^[6]

From the resulting molecular ensembles, desirable members must be identified by selection or screening procedures. In the amplification step, the self-replicating properties of the evolving molecules can be exploited (for example, if these are nucleic acids). Alternatively, more of the desired compound can be prepared by large-scale chemical synthesis.

In practice, the sorting step is the most critical part of any laboratory evolution experiment. How can rare but useful variants be efficiently isolated from complex mixtures of less desirable molecules? A chemist seeking the optimal octameric peptide inhibitor for a particular enzyme might start by making systematic substitutions at a single site in a known peptide. If allowed substitutions are restricted to the 20 standard amino acids, assaying the 20 different octameric peptides individually to find the highest affinity inhibitor would be relatively trivial. Simultaneously randomizing two positions in the peptide would generate 400 (20^2) different variants. Screening and testing such a library would be substantially more involved, but still manageable. However, it would be a daunting task indeed to identify the best inhibitor in a combinatorial library of fully randomized octameric peptides having 20^8 ($=2.6 \times 10^{10}$) individual members—unless an extremely efficient and sensitive sorting procedure were available.

Genetic selection is perhaps the most powerful technique currently available for analyzing large populations of biomacromolecules. In this review we focus on its use as a tool in mechanistic enzymology and protein design. Because numerous genetic selection systems have been developed, only a small fraction of which can be covered here, the reader is also referred to other recent reviews on this topic.^[7–17]

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2. Selection versus Screening

Identifying interesting variants in large combinatorial libraries can be accomplished either by assaying all the members individually or by applying conditions that allow only variants of interest to appear. Unfortunately, the terms “screening” for the former strategy and “selection” for the latter are often confounded in the literature. The basic difference between these approaches is illustrated schematically in Figure 1.

In general, screening strategies require an active search of all variants in the library. This approach is depicted in

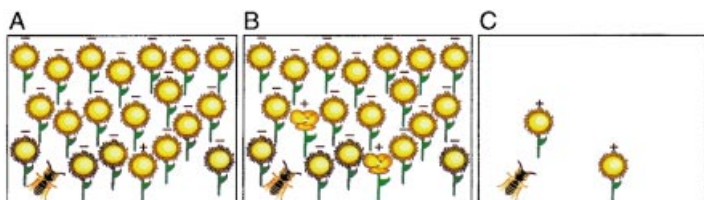


Figure 1. Different search modes for finding nectar-producing flowers (marked with +). A) Random screening: The bumblebee must check each flower individually to differentiate producers from the large number of nonproducers (marked with –). B) Facilitated screening: The bee can distinguish nectar-producing from nonproducing flowers on the basis of their distinct phenotypes. C) Selection: The bee only encounters the desired flowers because nectar production was a prerequisite for plant growth in this case.

Figure 1 A and B from the point of view of a bumblebee. Desirable nectar-producing flowers must be detected against a large background of flowers unable to produce nectar (undesired library members). If desired and undesired variants are difficult to distinguish, screening must be conducted one at a time in a “random” or “blind” fashion (Figure 1 A). A “facilitated screening” strategy is sometimes possible if the desired variants have a distinctive appearance or “phenotype” (Figure 1 B). Nevertheless, irrelevant library members are always present, which lowers the signal-to-noise ratio and which compete for resources.

In contrast, selection strategies exploit conditions favoring the exclusive survival of desired variants, thereby mimicking the culling process associated with true Darwinian evolution. Figure 1 C shows how the bee easily encounters nectar-producing flowers under selective conditions that eliminate the flowers that do not produce nectar.

2.1. Classical Solutions to the Sorting Problem

Selection and/or screening techniques are widely used in molecular biology, particularly for the cloning of DNA fragments or genes into circular DNA molecules called vectors, which can be replicated inside bacteria by the cellular DNA synthesis machinery.^[18, 19] Cloning involves enzymatic

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ligation of the ends of the insert fragment with the corresponding complementary ends of an appropriately cut vector.^[20] The resulting circular molecule, a plasmid, is then transferred into bacteria (usually the species *Escherichia coli*) by a process called transformation. During transformation, only a small percentage of cells successfully take up DNA. To select for these transformed bacteria, the cell suspension is plated onto agar medium in petri dishes that contains a particular antibiotic. Since the vector also carries a gene that specifies resistance against this antibiotic, only those cells that have received the original vector or the new plasmid survive, multiply, and form a colony on the agar medium. Because every one of the approximately 10^7 cells in the colony contains identical DNA, they all belong to the same clone.

After this very effective initial selection step, the molecular biologist is left with the problem of finding those clones that have the desired plasmid rather than the original vector molecule lacking the inserted gene.^[19] Depending on the complexity of the cloning strategy and the efficiency of the ligation step, relatively few of the antibiotic-resistant clones may actually possess the desired DNA fragment. The examples in Figure 2 show how an *in vivo* selection approach, which employs a positive-selection cloning vector, is superior to either random or facilitated screening in detecting clones with the desired genetic makeup (or “genotype”).

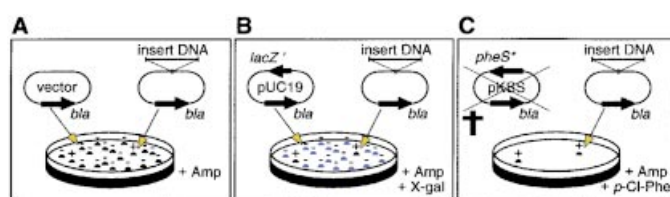


Figure 2. Search strategies for differentiating colonies with plasmids containing an inserted gene (marked with +) from those with only vector DNA (marked with –) after plating on nutrient agar plates following transformation.^[20] The *bla* gene encodes β -lactamase, which renders transformants resistant to the antibiotic ampicillin. A) Random screening: The phenotype of all colonies is identical. DNA from many randomly picked clones must be isolated and analyzed to find the desired plasmid. B) Facilitated screening: The cloning vector pUC19^[223] carries a fragment (*lacZ'*) of the *lacZ* gene encoding β -galactosidase. The encoded polypeptide (the α -fragment of the enzyme) can associate with the product of a cellular *lacZ* fragment to form functional β -galactosidase,^[224] which cleaves 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) resulting in the precipitation of a blue indigo derivative.^[225] Vector-containing colonies consequently have a blue color on agar plates containing X-gal. In contrast, the desired plasmid-containing clones form colorless colonies because the cloned gene was inserted into the coding region for the α -fragment, thereby abolishing β -galactosidase activity. The clones with a white colony phenotype (shown here as black spots) must be picked from the background of blue colonies to find the correct genotype. C) Selection: The positive-selection vector pKSS carries a mutant *pheS* gene (*pheS**), which confers relaxed substrate specificity to the encoded phenylalanyl-tRNA synthetase α subunit.^[226] In the presence of the phenylalanine analogue *p*-chlorophenylalanine, bacteria expressing the mutant *pheS* gene on pKSS cannot survive because incorporation of the analogue into cellular protein causes general failure of protein function. However, transformed cells can form colonies on nutrient agar plates containing ampicillin and *p*-chlorophenylalanine if the conditionally lethal *pheS** gene on pKSS is destroyed by insertion of a cloned DNA fragment. Thus, all colonies growing after plating the transformation mixture have the correct genotype.

2.2. Advantages and Limitations of Selection

The main advantage of selection over screening is that many more library members can be analyzed simultaneously. This is because uninteresting variants are never seen. As a consequence, surveying libraries is much faster and can be carried out with higher throughput. In the best screening protocols currently available, which take advantage of fluorogenic or chromogenic substrates,^[21] the maximum number of library members that can be assayed is about 10^5 .^[22] In contrast, up to 10^{10} clones can be assessed in a single experiment using genetic selection in *E. coli* cells. This number is an upper limit based on transformation efficiencies obtainable with conventional protocols.^[23] The absolute limit, at least in principle, is only dependent on the scale of the transformation.

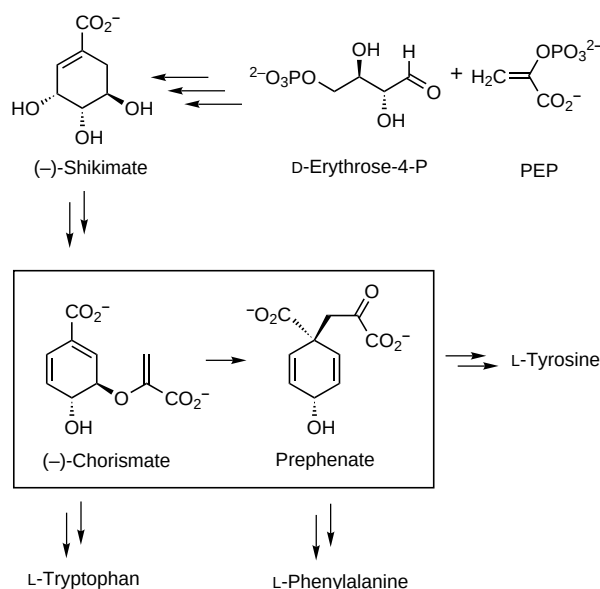
Nevertheless, it is challenging to develop suitable selection strategies for every desired catalytic task. In particular, *in vivo* selection schemes may be difficult or even impossible to devise if enzymes that work in non-natural environments, such as organic solvents, are desired.^[24, 25] Coupling the target reaction to survival in the selection step may require development of complex, nontrivial and intelligent assays. With every selection system, and in particular with very complex ones, there always exists the possibility that viable, but unanticipated or undesired solutions to the posed survival problem will surface.^[26, 27] If these false positives become too abundant, an efficient screening step may be necessary, or the system may need to be redesigned to eliminate this unwanted background by introduction of an additional selection step.

It is in the realm of very large combinatorial libraries that selection rather than screening gains crucial importance. As the focus shifts from randomizing an eight-residue peptide to a 100 amino acid protein (the typical size of a small functional domain), the number of sequence permutations rises to an astronomical 20^{100} . The ability to assay even a tiny fraction of this sequence space in directed molecular evolution experiments demands selection, even though initial development of an appropriate system may be considerably more involved than the setup of a screening procedure.

3. Genetic Selection of Novel Chorismate Mutases

The utilization of evolutionary strategies in the laboratory can be illustrated with proteins that catalyze simple metabolic reactions. One of the simplest such reactions is the conversion of chorismate to prephenate (Scheme 1), a [3,3] sigmatropic rearrangement. This transformation is a key step in the shikimate pathway leading to aromatic amino acids in plants and lower organisms.^[28, 29] It is accelerated more than a million-fold by enzymes called chorismate mutases.^[30]

Chorismate mutases have been intensively studied because of the scarcity of other enzyme-catalyzed pericyclic reactions. Mutases from different organisms exhibit similar kinetic properties, though they may share little sequence similarity.^[30] This dissimilarity extends to their tertiary and quaternary structures, as shown by comparison of chorismate mutases from *Bacillus subtilis* (BsCM),^[31, 32] *E. coli* (EcCM),^[33] and the



Scheme 1. Shikimate pathway for the biosynthesis of aromatic amino acids in plants and lower organisms. The [3,3] sigmatropic rearrangement of chorismate into prephenate is shown in the box. PEP = phosphoenolpyruvate.

yeast *Saccharomyces cerevisiae* (ScCM)^[34, 35] (Figure 3). BsCM, a member of the AroH class of chorismate mutases, is a symmetric homotrimer, packed as a pseudo- α/β -barrel. It has three identical active sites located at the subunit interfaces (Figure 3 A). In contrast, EcCM and ScCM (Figure 4 B and C, respectively) are distantly related homodimeric members of the *all- α* -helical AroQ class of chorismate mutases.^[36, 37] Homology searching in sequence databases has yielded other proteins that belong to each of these structural classes.^[36–38] Catalytic antibodies with modest chorismate mutase activity have also been generated.^[39–41] They have typical immunoglobulin folds, as shown in Figure 3 D for antibody 1F7.^[42]

The architectural diversity that nature exploits to promote this relatively simple chemical transformation is impressive and raises many intriguing questions. How do the individual enzymes work? What similarities and what differences are present? Why are natural chorismate mutases 10^4 times more effective than the catalytic antibody? Can mechanistic insights be used to improve the latter? More generally, what are the underlying chemical determinants of each structure? The individual catalytic domains of the BsCM, EcCM, and antibody proteins are each approximately 100 residues long. Why does the polypeptide adopt a mixed α/β conformation in one case, an *all- α* -helical structure in another, and an *all- β* topology in the third? The relationship between sequence, structure, and function can be rapidly and effectively examined by using random mutagenesis and genetic selection in vivo.

3.1. The Selection System

Over the past decade we have engineered several strains of yeast^[43, 44] and *E. coli*^[45, 46] that lack chorismate mutase. A typical bacterial selection system is depicted in Scheme 2. It is

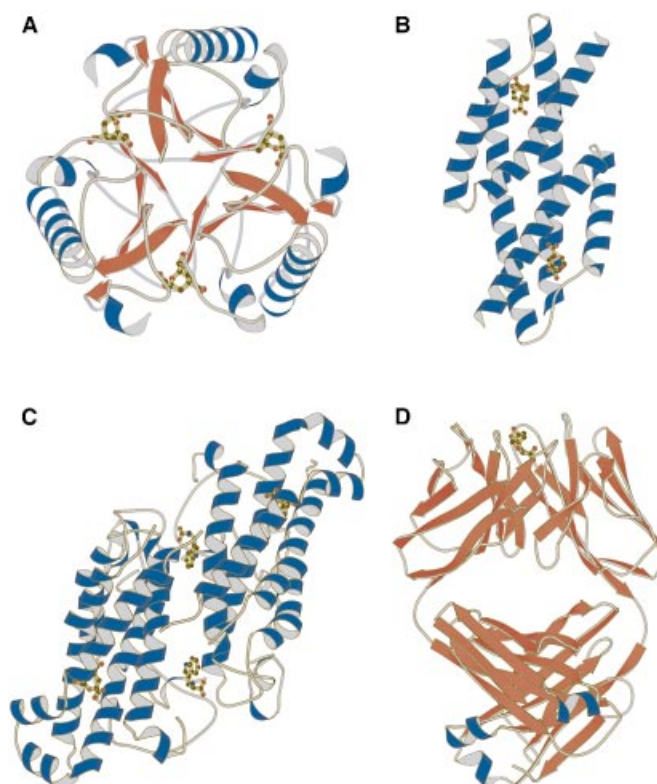
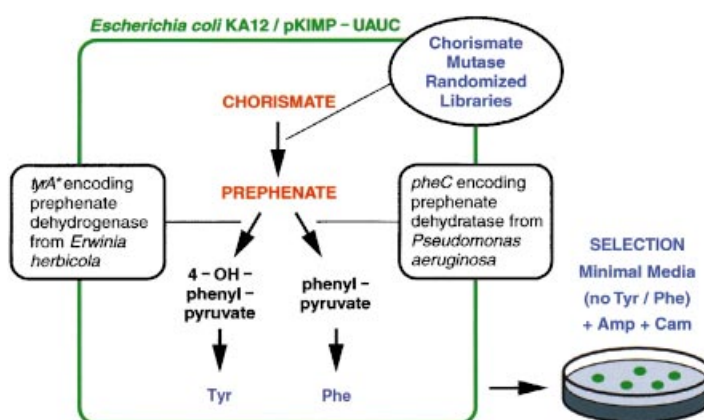


Figure 3. Ribbon diagrams of three naturally occurring chorismate mutases and a catalytic antibody. Helices are shown in blue and β -sheets in red. A transition state analogue inhibitor (1, Scheme 3) is shown in a ball-and-stick representation at each active site, with carbon atoms black and oxygen atoms red. A) Monofunctional chorismate mutase from *B. subtilis* (BsCM).^[31, 32] B) Chorismate mutase domain of the bifunctional chorismate mutase/prephenate dehydratase from *E. coli* (EcCM).^[33] C) Allosterically regulated chorismate mutase from the yeast *S. cerevisiae* (ScCM).^[34] Tryptophan, which activates the enzyme, is shown bound at the allosteric sites located at the dimer interface. D) Catalytic antibody 1F7,^[42] which was generated by using 1 as a hapten.^[39]



Scheme 2. An engineered *E. coli* selection system lacking endogenous chorismate mutase activity.^[45] The genes encoding the bifunctional enzymes chorismate mutase/prephenate dehydrogenase and chorismate mutase/prephenate dehydratase were deleted, and monofunctional versions of the dehydrogenase and dehydratase were supplied on plasmid pKIMP-UAUC. Potential chorismate mutases from random gene libraries are evaluated on the basis of their ability to complement the genetic defect and allow the cells to grow in the absence of added tyrosine (Tyr) and phenylalanine (Phe). The library plasmid and pKIMP-UAUC carry genes for ampicillin (Amp) and chloramphenicol (Cam) resistance, respectively.

based on *E. coli* strain KA12,^[46] which has deletions of the chromosomal genes for both bifunctional chorismate mutases (chorismate mutase/prephenate dehydrogenase and chorismate mutase/prephenate dehydratase). Monofunctional versions of prephenate dehydratase^[47] and prephenate dehydrogenase^[48] from other organisms are supplied by the plasmid pKIMP-UAUC, which leaves the cells deficient only in chorismate mutase activity.^[45]

As a consequence of the missing enzyme, KA12/pKIMP-UAUC bacteria are unable to grow in the absence of exogenously added tyrosine and phenylalanine. Their metabolic defect can be thought of as an interrupted circuit. Supplying the cells with an additional plasmid encoding a natural chorismate mutase can repair this genetic defect. Alternatively, a mutant of the natural enzyme or even an unrelated polypeptide can be introduced into the cell. If the protein is able to catalyze the conversion of chorismate to prephenate, the cells will be able to produce their own tyrosine and phenylalanine and grow under selective conditions (that is, without tyrosine and phenylalanine supplementation). If the protein does not accelerate the rearrangement, the cells will not grow at all. As a consequence, attention can be focused on functional clones without wasting time and resources on nonproductive members of the library. Additionally, the linkage of the desired phenotype (catalyst-enabled growth under selective conditions) with the responsible genotype (the gene encoding the catalyst) is automatic in this system, and allows easy isolation, diversification, and further evolution of interesting library variants.

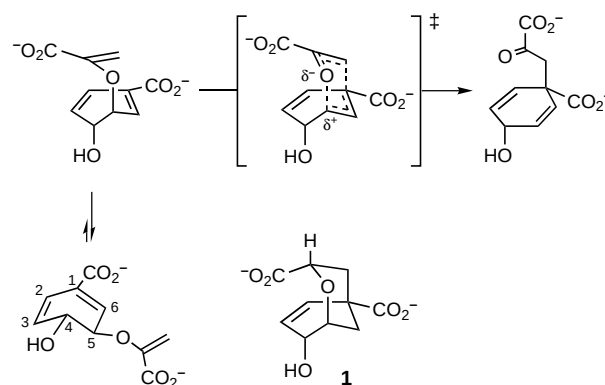
Since misplacement of catalytic residues by even a few tenths of an angstrom can mean the difference between full activity and none at all, enzymatic activity represents an extremely stringent criterion for selection. Insightful answers to many of the questions posed in the previous section can, therefore, be obtained by searching intelligently designed protein libraries for active chorismate mutases. The utility of this strategy in areas ranging from mechanistic enzymology to protein design is best illustrated through a few examples.

3.2. Mechanistic Studies

3.2.1. Active-Site Residues

Our understanding of the chorismate rearrangement derives largely from mechanistic studies of the uncatalyzed reaction^[49–52] and from computation studies.^[53–59] In order to rearrange, chorismate, which normally has a pseudo-diequatorial conformation in solution,^[51] must first adopt a pseudodixial conformation (Scheme 3). It then reacts exergonically^[60] via a transition state with a chairlike geometry.^[50] The process appears to be concerted but asynchronous, with formation of the C–C bond lagging behind cleavage of the C–O bond.^[49]

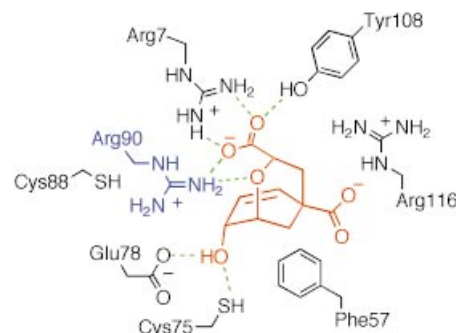
The transition state for the enzymatic reaction has been shown to have a chairlike geometry as well,^[61] and conformationally constrained compounds that mimic this structure, such as the oxabicyclic dicarboxylic acid **1** (Scheme 3), are good inhibitors of chorismate mutase enzymes.^[62–64] How a



Scheme 3. Chorismate prefers a pseudodiequatorial conformation in solution. It must adopt a disfavored pseudodixial conformation to reach the pericyclic transition state. The conformationally constrained oxabicyclic dicarboxylic acid **1**, which mimics the transition state, is a potent inhibitor of natural chorismate mutases.^[62] Antibodies raised against this compound also catalyze the reaction, albeit 100 to 10000 times less efficiently than their natural counterparts.^[39, 41]

protein might stabilize this high-energy species has been a matter of some debate. Recently, heavy atom isotope effects were used to characterize the structure of the transition state bound to BsCM.^[65] A very large ¹⁸O isotope effect at O(5) (ca. 5 %), the site of bond cleavage, and a small, normal ¹³C isotope effect at C(1) (ca. 0.6 %), the site of bond formation, show that the rearrangement step is significantly rate determining for this enzyme. These findings also confirm that the enzymatic reaction proceeds through a concerted but asymmetric transition state. Comparison with theoretical isotope effects obtained by Becke3LYP/6-31G* calculations^[65] suggests, however, that the enzymatic transition state might be more highly polarized than its solution counterpart, with more C–O bond cleavage and less C–C bond formation.

Examination of the residues that line the active site of BsCM (Scheme 4)^[31, 32] suggests a plausible explanation for the greater polarization of the bound transition state. The positively charged guanidinium group of Arg90 is located within hydrogen-bonding distance of the ether oxygen atom of the bound substrate, where it can stabilize the partial negative charge that builds up at this site in the transition



Scheme 4. Schematic representation of the BsCM active site. An extensive array of hydrogen bonding and electrostatic interactions is used to bind transition state analogue **1** (red). The side chain of Arg90 (blue) is oriented so that its guanidinium group is placed within hydrogen-bonding distance of the ether oxygen atom of the ligand.

state. The contribution of such a residue to catalysis would normally be investigated by site-directed mutagenesis. For instance, Arg90 might be replaced with lysine or methionine to examine what happens when the guanidinium is replaced with a different cation or when the positive charge is removed completely. However, since a selection system is available, all 20 natural amino acids can be evaluated at position 90 simultaneously. Those amino acids that yield a functional enzyme can be quickly identified by sequencing the genes able to complement the chorismate mutase deficiency. When this experiment was performed, the results were dramatic.^[45] Only arginine at position 90 yielded an active enzyme. Even the conservative replacement of arginine with lysine gave an enzyme that was inactive *in vivo*. Kinetic characterization of representative mutants showed that removal of the arginine side chain reduces catalytic efficiency by five to six orders of magnitude.^[38]

If the role of Arg90 is electrostatic stabilization of the developing negative charge on the ether oxygen atom of chorismate in the transition state, one might wonder why complementation was not observed when arginine was replaced with another positively charged residue such as lysine. One possibility is that the Arg90Lys variant is produced poorly in *E. coli*. The growth phenotype depends on the total amount of chorismate mutase activity present in the cell, which is determined by the specific activity of the catalyst and also its concentration. If the catalyst is active but present only at extremely low concentrations, no growth will be observed. While the BsCM mutants appear to be produced at comparable levels within the selection system,^[45] exceptions are possible.

A potentially more interesting explanation is that the active site of the enzyme places intrinsic structural constraints on possible substitutions. The lysine side chain is shorter than that of arginine and it is conceivable that it cannot reach far enough into the active site to place its ammonium group within hydrogen-bonding distance of the critical ether oxygen atom. If this is true, additional mutations at other positions within the active site might allow replacement of Arg90 without loss of function.

To test this idea, we simultaneously mutated two residues—Arg90 and Cys88—within the binding pocket of BsCM.^[45] The side chain of Cys88 is about 7-Å distant from the bound ligand, but it lies against the side chain of Arg90 and might influence the conformation of the latter (Scheme 4). Combinatorial mutagenesis of these two residues gives 400 variants, which can be rapidly evaluated in the chorismate mutase deficient selection strain. The results of this experiment proved quite informative (Figure 4).^[45] They showed first that Cys88 is not essential for catalysis. It can be replaced by large, medium-sized, and small amino acids; as long as an arginine residue is present at position 90, an active enzyme is obtained. Active double mutants with an additional Arg90Lys mutation were found if a small residue (glycine or alanine, for example) replaces Cys88, which shows that the guanidinium group is not crucial for catalysis. Perhaps the most interesting result to emerge from this study is that a cation at position 90 can be eliminated altogether, provided that Cys88 is replaced with a lysine residue.

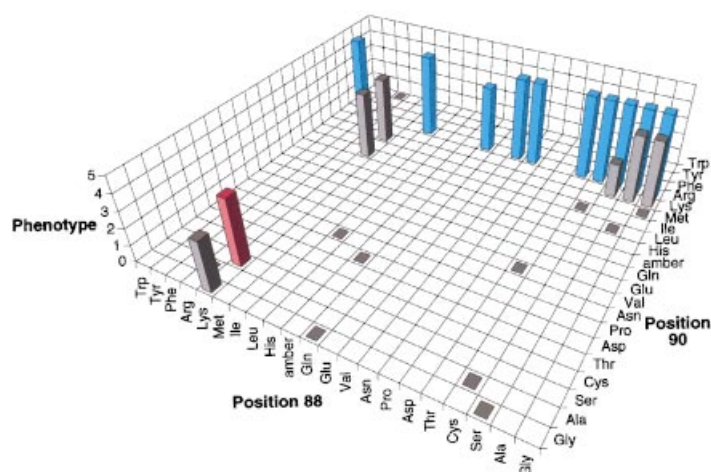


Figure 4. Outcome of a combinatorial mutagenesis and selection experiment involving simultaneous randomization of positions 88 and 90 in BsCM.^[45] The amino acids are ordered according to their side-chain volume. Active variants that were sequenced and a few inactive controls are shown with their *in vivo* activity, or phenotype. The phenotype is reflected by colony size, which is rated from 0 (no growth) to 5 (wild-type growth). Blue columns show variants with wild-type Arg90. The red column is variant Cys88Lys/Arg90Ser, which was analyzed in more detail (see Figure 5).

Kinetic studies on the Cys88Lys/Arg90Ser double mutant show that a lysine residue at position 88 restores three of the five to six orders of magnitude in catalytic efficiency lost upon removal of the arginine side chain at position 90.^[38] Crystallographic studies of this double mutant show that the side chain of Lys88 extends into the active site and orients its ammonium group, like the guanidinium group of Arg90 in the wild-type enzyme, within hydrogen-bonding distance of the ether oxygen atom of the bound ligand (Figure 5).^[38] Thus, combinatorial mutagenesis and selection resulted in a redesigned active site, which afforded a novel solution to the chemical problem of catalyzing the chorismate mutase reaction.

The results of these selection experiments are mechanistically significant insofar as they support a critical role for a cation in the mechanism of chorismate mutase. No active catalysts were found that lacked a cation in the vicinity of the

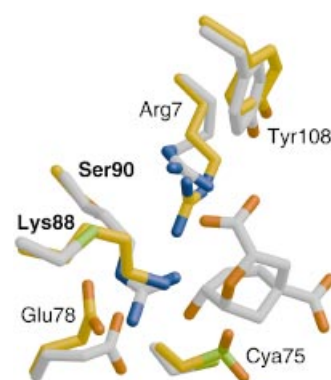
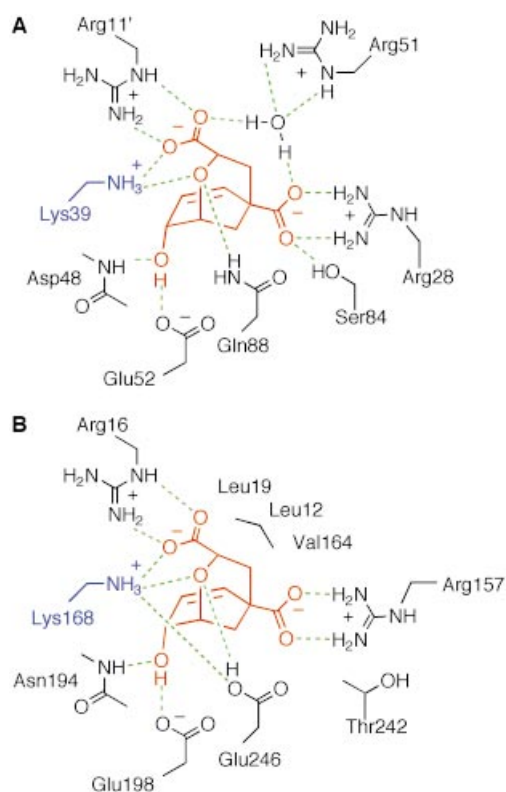


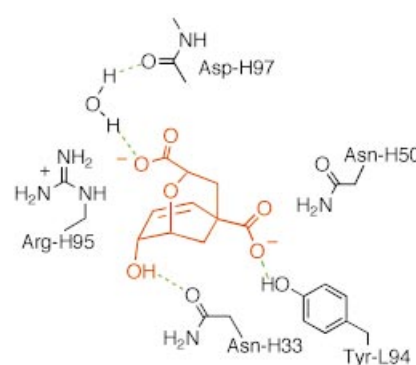
Figure 5. Superposition of residues from unbound Cys88Lys/Arg90Ser (carbon atoms in yellow, residues labeled) and wild-type BsCM complexed with inhibitor **1** (grey carbon atoms).^[38] Cys75 is partially oxidized to the sulfinate (Cys75) in the crystal of the mutant.

substrate's ether oxygen atom. The conclusion that a cation is crucial for high chorismate mutase activity can thus be made with much greater confidence than would have been possible following a conventional mutagenesis experiment in which only single substitutions were considered.^[66] Of course, we cannot exclude the possibility that other, equally effective arrangements of catalytic groups lacking a cation might be found in the future through more extensive mutagenesis experiments. However, it is worth noting that chorismate mutases from both *E. coli*^[33] and yeast^[34, 35] have a cationic lysine residue in an equivalent position in their respective active sites (Scheme 5), even though their tertiary structures are otherwise unrelated to BsCM. This lysine residue has been shown by mutagenesis to be catalytically essential in EcCM.^[67, 68]



Scheme 5. Schematic representation of the active sites of EcCM (A) and ScCM (B) complexed with transition state analogue **1**. The lysine counterparts of Arg90 in BsCM are shown in blue.

In this context, it is also interesting that the 10^4 -fold less-efficient catalytic antibody 1F7^[39, 40] lacks a comparable strategically placed cation (Scheme 6).^[42] This fact is not terribly surprising given the structure of the transition state analogue used to generate this catalyst (see Scheme 3).^[39] Nevertheless, it does suggest possible strategies for producing antibodies with much higher activity. For example, additional negative charges could be designed into a hapten to elicit new antibodies that contain the catalytically essential cation. Alternatively, a cation might be directly engineered into the 1F7 active site by using site-directed mutagenesis. A third approach that utilizes selection may ultimately be the most effective strategy for optimizing antibody activity, however.



Scheme 6. Schematic representation of the active site of catalytic antibody 1F7 with bound **1**. Arg-H95 is not within hydrogen-bonding distance of the ligand's ether oxygen atom.^[42]

The Fab fragment of 1F7 has already been shown to function in the cytoplasm of a chorismate mutase deficient yeast strain.^[43, 44] The catalytic antibody, when produced at a sufficiently high level, is able to replace the missing enzyme and weakly complement the metabolic defect. Conceivably, therefore, it can be placed under selection pressure to identify variants that have higher catalytic efficiency. Preliminary results from such experiments appear quite promising.^[69]

3.2.2. Random Protein Truncation

Identifying appropriate residues for mutational analysis is difficult when structural information for a protein is lacking. In such cases, random mutagenesis coupled with selection can provide a powerful means of analysis. We have used such an approach to examine how the 17 C-terminal residues of BsCM contribute to enzyme efficiency.^[70]

BsCM is 127 amino acids long. The first 115 residues adopt a well-defined structure, which ends in a 3_{10} helix near the active site (Figure 3A).^[31, 32] Residues beyond this point are not seen in X-ray structures of the free enzyme,^[31] which implies that they are highly disordered or have multiple accessible conformations. Some ordering of the C-terminus is observed crystallographically upon binding of the ligand to BsCM.^[31] In addition, ligand-induced conformation changes have been inferred from Fourier-transform infrared (FT-IR) spectroscopic studies.^[71] These observations raise the possibility that the flexible C-terminal tail might play some role in enzyme function. For example, it might serve as a lid to the active site that sequesters the substrate from bulk solution and/or contributes stabilizing interactions to the transition state.

To address these questions we developed a strategy involving random C-terminal truncation of the enzyme followed by selection of functional clones in the KA12/pKIMP-UAUC system.^[70] By using a polymerase chain reaction (PCR) procedure with a partially randomized oligonucleotide, we constructed two gene libraries in which the BsCM codons corresponding to residues 116–127 or residues 111–127 (including the crystallographically observed 3_{10} helix) were randomized. The libraries were designed to optimize the frequency of stops at each mutagenized codon. In this way, a nested set of randomly truncated proteins was

created. Since only functional genes survive selection, dispensable portions of the encoded proteins can be rapidly and directly identified. Non-native sequences are also explored by this method, so residues that might fulfill a crucial structural or mechanistic role can also be discovered.

After construction and transformation into the bacterial selection strain, both libraries contained 10^4 – 10^5 members. Essentially all members of the library in which the 12 C-terminal residues were mutagenized were viable on selection medium. These results demonstrate that none of the 12 C-terminal residues are absolutely required for enzymatic activity. They can be mutated and the last 11 residues can even be removed without significantly impairing activity *in vivo*. In contrast, when the last 17 residues were mutagenized, only about 25% of the library was able to grow on selection plates. The additional five amino acids form a well-defined 3_{10} helix and provide contacts with the rest of the protein and its ligands. Sequencing data show that mutations are tolerated at these positions, although a clear preference for wild-type Lys111, Ala112, Leu115, and Arg116 is observed. Moreover, active enzymes shorter than 116 amino acids were not found. Apparently, residues in the 3_{10} helix cannot be removed without killing the enzyme.

Kinetic characterization of several selected BsCM variants shows that truncation or mutation of the C-terminal tail has little effect on the turnover number (k_{cat}) of the enzyme (Table 1). When chorismate is bound to the active site of the variants, it is converted into prephenate nearly as efficiently as with wild-type BsCM. However, a substantial reduction in the $k_{\text{cat}}/K_{\text{m}}$ value is evident (Table 1). This finding indicates that the C-terminus, while not directly involved in the chemical transformation of bound ligand, does contribute to enzymatic efficiency by uniformly binding the substrate and transition state.

Table 1. Catalytic parameters of truncated BsCM variants.^[70]

BsCM variant	C-terminal amino acid sequence	k_{cat} [s^{-1}]	$k_{\text{cat}}/K_{\text{m}}$ [$\text{M}^{-1}\text{s}^{-1}$]
5–8	KAVVLR	26	2.8×10^3
1–3	NSNVLRP	30	2.0×10^3
V-7	KAVVLLT	26	1.6×10^3
5–11	KAVVLRPN	23	5.6×10^3
wild-type	KAVVLRPDLSTKNTL	46	6.9×10^5

3.3. Structural Studies

Selection methods are ideally suited for examining factors that influence protein structure and stability. The informational content of protein sequences is notoriously non-uniform, with many residues being highly tolerant to a wide range of substitutions, whereas others cannot be altered without dramatic consequences for folding or function.^[72, 73] Analysis of protein structure is thus a combinatorial problem requiring efficient methods for the simultaneous evaluation of many different alternative sequences. An experimentally accessible population of 10^{10} molecules is miniscule compared with the 20^{100} ($=10^{130}$) possibilities for a polypeptide with a length of 100 amino acids. Nevertheless, libraries of this size can provide statistically meaningful insights into intrinsic second-

dary structural preferences in proteins,^[74, 75] constraints on segments that link secondary structural elements,^[76–78] and optimal packing arrangements of residues in the interior of a protein.^[79–82]

3.3.1. Constraints on Interhelical Loops

The role of interhelical turns in determining protein structure has been explored in relatively simple four-helix-bundle proteins by generating combinatorial libraries and screening them for functional variants. For example, Hecht and co-workers mutagenized a three-residue interhelical turn in cytochrome *b*-562.^[76] All 31 variants isolated from the random library adopted stable nativelike structures as judged by the characteristic red color associated with heme complexes of cytochrome *b*-562. Similar results have been obtained with mutagenized turns in Rop,^[77, 83] a dimeric RNA-binding four-helix-bundle protein, although detailed analysis of individual mutants showed some variation in thermostability.^[84] Collectively, these studies suggest that there are relatively few constraints on sequence or length in interhelical segments.

How general are such conclusions? Do they apply to more complex proteins? Are there more elusive features that influence turn preferences? Selection for catalytic activity, an extremely sensitive probe of structural integrity, has the potential to reveal more subtle sequence preferences than simple screening protocols. We tested this premise using the EcCM enzyme (Figure 3B) as a template.^[85] The two identical polypeptides that make up this helical bundle protein consist of three helices joined by two loop segments (Figure 6A).^[33] The L2 loop connecting the H2 and H3 helices lies farthest from the active site and was targeted for analysis.

Initially, three solvent-exposed residues in the turn (Ala65–His66–His67, Figure 6B) were randomized and catalytically active variants identified by selection *in vivo*. The starting ensemble contained 8000 (20^3) distinct members, of which more than 63% were found to complement the chorismate mutase deficiency of the bacterial host strain, albeit with widely varying growth rates. The high percentage of complementing clones clearly confirms that there are few restrictions on this loop segment. Nevertheless, the range of growth rates observed for complementing clones suggests that substitutions in the turn can affect protein production, stability, or catalytic activity. In fact, careful sequence comparison uncovered a statistically meaningful preference for hydrophilic residues at these solvent-exposed positions in the variants affording the fastest growth. Although proline-containing turns with alternative backbone conformations are also tolerated, such sequences were only found in partially active clones.

Randomization of the same Ala65–His66–His67 tripeptide in combination with Lys64, which is part of the adjacent H2 helix (Figure 6), enabled investigation of the extent to which proximal secondary structure influences the allowable loop substitution patterns. A library containing all possible 160000 (20^4) members was constructed and subjected to selection. Again, a large fraction of these sequences ($>50\%$) could functionally replace the native sequence, which shows

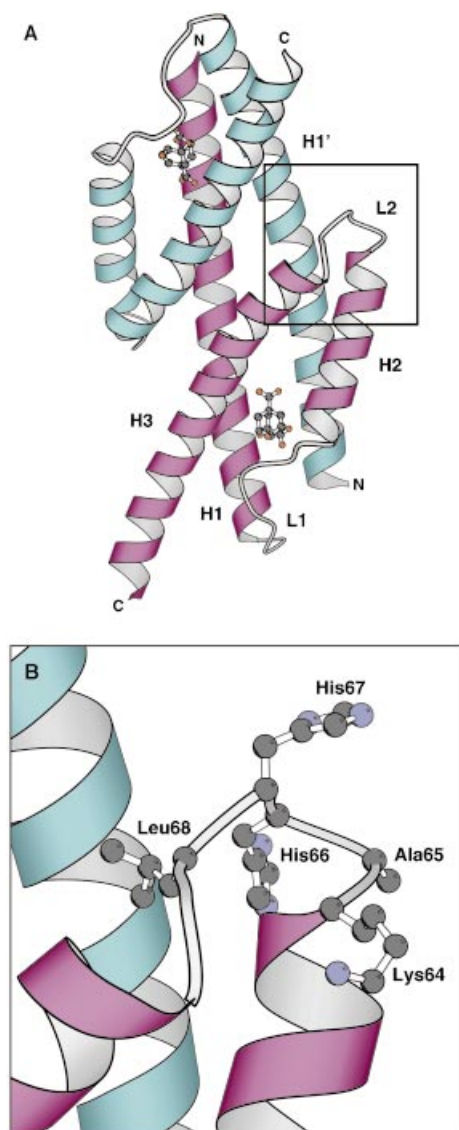


Figure 6. A) Ribbon structure and B) L2 loop of EcCM. Random mutagenesis of the L2 loop followed by selection for chorismate mutase activity *in vivo* showed little sequence constraint on solvent-exposed turn residues, aside from a modest bias in favor of hydrophilic amino acids.^[85] In contrast, long-range tertiary contacts impose a strict requirement for hydrophobic aliphatic amino acids at position 68.

that the secondary structure at position 64 does not limit the range of side chains allowed at this site, and that its randomization does not affect residue preferences in the region of the neighboring turn.

Quite different results were obtained when the buried turn residue Leu68 was randomized together with Ala65–His66–His67. Unlike the other turn residues, the side chain of Leu68 is buried at the interface between L2 and H1' (helix H1 of the other subunit) and thus sequestered from solvent (Figure 6). In this case, only 6–7% of the 160 000 possible sequences complemented the chorismate mutase deficiency, and sequence analysis indicated an extremely strict requirement for hydrophobic aliphatic amino acids at position 68. Thus, in contrast to the weak influence of a residue involved in a secondary structure element, long-range tertiary interactions between residues in an interhelical turn and residues else-

where in the protein can impose strong constraints on allowable sequence substitutions.

The EcCM study indicates the key advantages of genetic selection over screening methods. First, much larger libraries can be evaluated with little extra effort. Screening 10^5 individual clones in the absence of a convenient spectroscopic assay would be an arduous undertaking, but selection readily sorts the entire mixture into active, partially active, and inactive enzymes. Second, the greater stringency imposed by the requirement that selected variants be catalytically active allows nonobvious features to be discerned, such as the statistically meaningful preference for hydrophilic residues at solvent-exposed positions, or the identification of turns with altered backbone conformations. These finely tuned details of protein structure might be missed with less stringent assay criteria.

3.4. Altering Protein Topology

All proteins are composed of a limited set of secondary structure elements. Yet, we are just beginning to learn how such building blocks can be combined to yield well-defined tertiary and quaternary structures. Evolutionary strategies, which allow the simultaneous evaluation of huge numbers of alternatives on the basis of a selectable phenotype, such as catalytic activity, can greatly aid such efforts.

3.4.1. New Quaternary Structures

The re-engineering of existing protein scaffolds represents a manageable first step toward the larger goal of designing functional enzymes *de novo*. Alteration of the oligomerization state of various proteins, for example by converting monomers into multimers^[86] or multimers into monomers,^[87–92] has received particular attention. Such systems may shed light on the evolutionary origins of multimeric proteins.^[93, 94]

Converting an oligomeric protein into a catalytically functional monomer is particularly difficult when the individual polypeptides must be untangled, as in the intricately entwined homodimeric EcCM.^[33] Nevertheless, it seemed possible that the insertion of a flexible “hinge–loop” into the long H1 helix that spans the EcCM dimer would alter the enzyme’s quaternary structure. The important role of hinge–loops in determining the oligomerization state of a variety of proteins has been noted previously.^[93, 94] Although the active sites of EcCM are constructed from residues contributed by both polypeptide chains, such a segment might allow the N-terminal half of H1 to bend back on itself, thus displacing the second polypeptide, to yield a monomeric and catalytically active four-helix-bundle (Figure 7). Depending on the loop length and composition, other oligomeric states could also conceivably be populated.

To test these possibilities, random segments of four to seven residues were inserted into the middle of the EcCM H1 helix.^[95] The individual libraries (designated L4, L5, L6, and L7) have a maximum theoretical diversity of 160 000 (20^4), ca. 3.2×10^6 (20^5), ca. 6.4×10^7 (20^6), and ca. 1.28×10^9 (20^7) distinct members, respectively. In each case, transformation of

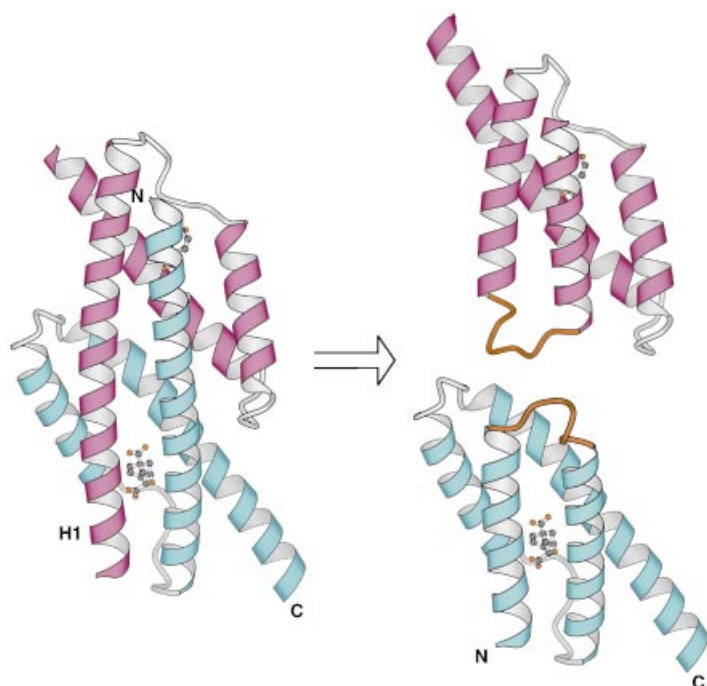
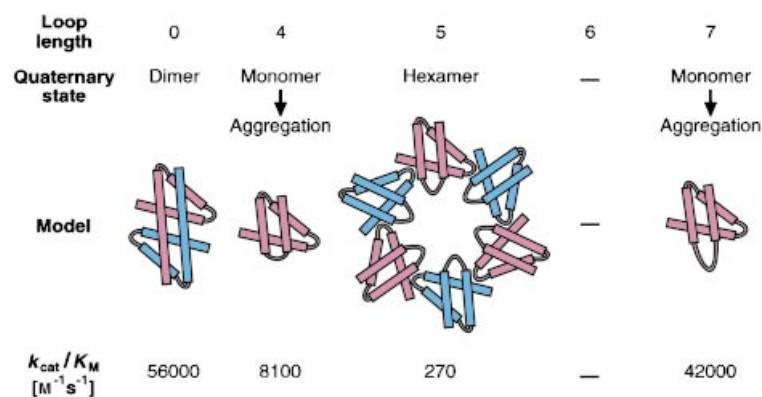


Figure 7. Topological conversion of dimeric EcCM into a monomer. Insertion of a flexible loop into the dimer-spanning H1 helix would allow the N-terminal portion of the helix to bend back on itself, thus displacing the other polypeptide to form a functional four-helix-bundle catalyst.

chorismate mutase deficient bacteria yielded roughly 10^7 clones, to give fully diverse and redundant coverage of the L4 and L5 libraries, about 10 % sequence coverage of the L6 library, and about 1 % coverage of the L7 library.

No functional clones were found in the L6 library under selection conditions, but 0.05 %, 0.002 %, and 0.5 % of the L4, L5, and L7 libraries, respectively, complemented the genetic defect. Several active enzymes from the libraries were characterized biochemically (Scheme 7). Insertion of 4 or 7 amino acid segments into the middle of the H1 helix yielded monomeric enzymes with near wild-type activity, but these proteins were unstable and tended to aggregate. In contrast, insertion of a five amino acid segment (Cys-Phe-Pro-Trp-Asp) yielded a well-behaved hexameric chorismate mutase (as



Scheme 7. Models for the structural organization of hinge-loop variants of EcCM showing the number of residues in the inserted hinge loop, the oligomeric state of active variants, and experimentally determined $k_{\text{cat}}/K_{\text{M}}$ values.^[95]

judged by analytic ultracentrifugation) that is about 200-fold less active than the wild-type protein.

The properties of the hexameric species, when compared with those of the monomers, suggest that protein stability is an important driving force in the evolution of oligomeric proteins.^[95] However, additional work is needed to elucidate how the subunits are organized and how quaternary structure influences function.

On a more general note, loop length appears to play a more important structural role than might have been anticipated from other experiments.^[83] The low percentage of active proteins recovered in these selection experiments indicates that relatively few loop sequences permit a change in the quaternary structure without affecting the structure of the active site. Moreover, the scarcity of functional clones (1 out of every 50000 L5 library members, for example) also means that it would have been extremely difficult to find active enzymes by screening with an in vitro chorismate mutase assay alone.

3.4.2. Stable Monomeric Mutases

Although the topological conversion of the EcCM homodimer to a monomer preserves the enzyme active site, it yields a much less stable protein. A cluster of hydrophobic residues between the two active sites appears to stabilize the dimer,^[37] but these interactions would be lost in the monomer. Exposure of these buried apolar groups to solvent presumably favors aggregation as well. Since the highly charged binding pocket is located in the interior of the helical bundle,^[33] the monomer itself lacks a conventional hydrophobic core and, hence, the usual driving force for protein folding.^[96]

A thermostable dimer was therefore considered as an alternative starting point for the design of a stable monomeric mutase. A large number of EcCM sequence homologues, some from thermophilic organisms, are known. For example, the hyperthermophilic archaeon *Methanococcus jannaschii* produces a chorismate mutase (MjCM) that is 25 K more stable than EcCM.^[37] Despite a sequence identity of only 21 %, six prominent residues that line the active site are strictly conserved and the two enzymes have comparable activities. Since the hydrophobic core of MjCM is very similar to that of EcCM, interactions distant from the dimer interface must be responsible for its additional stability. These same interactions were expected to stabilize the desired monomer.

Topological variants of MjCM were prepared by inserting a flexible loop into the middle of the H1 helix,^[97] as described in the previous section for EcCM (see Figure 7). In this case, though, the turn segment was modeled on a known helix–turn–helix motif in *E. coli* seryl-tRNA synthetase.^[98] Two residues in helix H1 (Leu22 and Lys23, numbered according to the homologous EcCM sequence) were duplicated and six residues of random sequence were inserted between the repeated amino acids. Two point mutations (Leu22a to Glu and Ile79 to Arg) were also included as an element of negative design to disfavor dimer formation and to minimize aggre-

gation of the monomers by reducing the exposed hydrophobic surface area. This design yields a library with approximately 6.4×10^7 (20^6) individual members. The library was constructed at the genetic level, used to transform the chorismate mutase deficient *E. coli* strain ($>10^8$ transformants), and evaluated by selection.

In contrast to the selection experiments described above which were carried out on solid media, the library was grown in liquid culture lacking tyrosine and phenylalanine. This process allows a direct competition for resources and amplification of the small fraction of the original library containing a functional chorismate mutase (0.7%). After three days of selection, more than 80% of the clones in the library complemented the genetic defect, which corresponds to over a 100-fold enrichment.

Selection greatly facilitates the search for a tractable monomer by eliminating inactive or weakly active clones. However, function, not topology, is the basis for selection. For this reason, representative clones were subsequently screened by size-exclusion chromatography. Only one of the 26 clones analyzed in this way had a retention time expected for a monomer (Figure 8). This protein had the six-residue insert

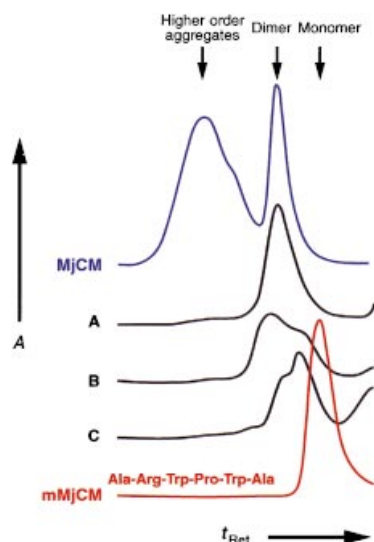


Figure 8. Analytical size-exclusion chromatography distinguishes between dimeric and monomeric chorismate mutases.^[97] Wild-type MjCM (top trace) was found to be a mixture of dimer and higher order aggregates. Most selected variants (for example, traces A–C) were dimers or mixtures of dimers and monomers. Only one of 26 variants tested (mMjCM, bottom trace) eluted as a monomer. It had the six amino acid insert shown. Analytical ultracentrifugation confirmed that this protein is monomeric in solution.

Ala-Arg-Trp-Pro-Trp-Ala. Its monomeric nature was confirmed by analytical ultracentrifugation. It is also highly helical, undergoes cooperative thermal and chemical denaturation ($\Delta G_U(\text{H}_2\text{O}) = 2.7 \text{ kcal mol}^{-1}$), and has significant catalytic activity. Its k_{cat} value for the rearrangement of chorismate is identical to that of MjCM, while its K_m value is elevated only threefold.^[97]

The combination of selection (>100 -fold enrichment) and screening (1 in 26) shows that fewer than 0.05% of the possible turn sequences are capable of yielding well-behaved, monomeric proteins. This result again contradicts the simple

expectation that most interhelical turn sequences are functionally equivalent.^[76] It also underscores in dramatic fashion the advantage of the selection approach. Individual screening of 1000–10000 proteins to find the one with the desired activity and topology would represent a major experimental undertaking.

Another important advantage of selection is exemplified by the observation that cells harboring the monomer grow much more effectively in the absence of tyrosine and phenylalanine than cells containing the wild-type MjCM dimer.^[97] Although the precise reasons for this difference are unknown, it may reflect the fact that the MjCM dimer forms catalytically inactive aggregates when overproduced in *E. coli*, whereas the monomer shows no tendency to do so (Figure 8). Not only does the process of genetic selection maximize catalytic activity, but problems associated with protein misfolding, aggregation, or toxicity are simultaneously minimized. This feature may be of value in the production of biocatalysts for commercially practical applications, where stable, easily produced enzymes are essential.

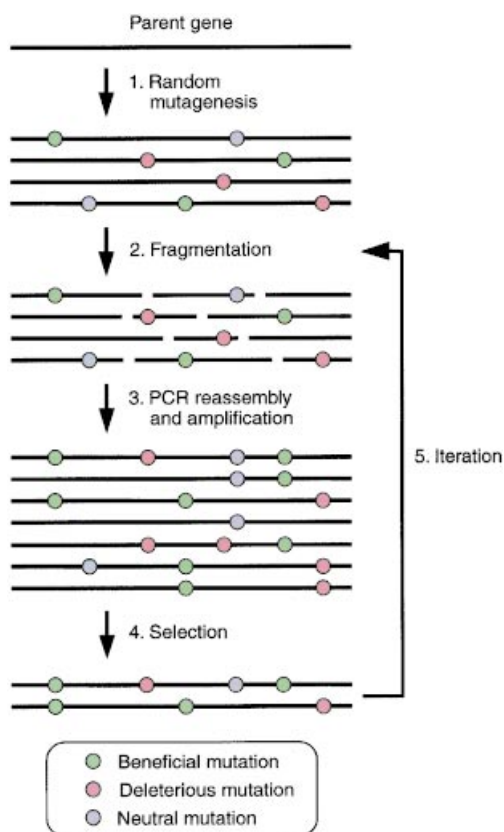
3.5. Augmenting Weak Enzyme Activity

True Darwinian evolution involves multiple cycles of mutation and selection. This process can be mimicked in a laboratory setting to optimize the properties of an inefficient enzyme. Such an endeavor is often termed directed evolution.^[10]

The hexameric but weakly active chorismate mutase^[95] described in Section 3.4.1 has been improved in this way.^[99] Mutations were introduced into the gene encoding the hexamer subunit by DNA shuffling (Scheme 8),^[5, 100] which mimics sexual recombination in vitro. Improved variants were selected, as before, by their ability to complement the chorismate mutase deficiency in bacteria. Plasmid DNA was isolated from the fastest growing cells and the entire procedure was repeated.

After two rounds of mutation and selection, several clones were found that grew at wild-type levels. The corresponding enzymes were isolated and characterized biochemically.^[99] One variant, which contained three mutations (Ser15Asp/Leu79Phe/Thr87Ile), had a 9-fold improvement in the k_{cat} value and a 35-fold improvement in the k_{cat}/K_m value. Size-exclusion chromatography also showed that it is still considerably larger than the wild-type dimer, but more detailed biophysical studies will be needed to determine its precise oligomerization state and subunit organization. This catalyst was subjected to a further round of mutagenesis and selection, and additional clones with somewhat improved k_{cat}/K_m values have been identified. Interestingly, many of these are missing the C-terminal histidine tag, which had been introduced to facilitate purification, plus an additional five to eight amino acids. Eliminating the C-terminal extension of the H3 helix could conceivably improve packing interactions between the subunits in the higher order oligomer.

In principle, directed evolution procedures could be repeated indefinitely until any desired activity has been attained. At some point, however, the catalyst will be



Scheme 8. DNA shuffling.^[5, 100] Techniques such as error-prone PCR can be used to mutagenize a parent gene. The resulting pool of homologous genes contains beneficial, deleterious, or neutral point mutations. Treatment of this pool with DNaseI gives rise to a set of random fragments. Novel recombinations accompany the reassembly of the random fragments into full-length genes. Selection for variants with improved function provides the starting point for another round of shuffling. Point mutations arise during the recombination process itself, which makes every round a genuine Darwinian evolution cycle.

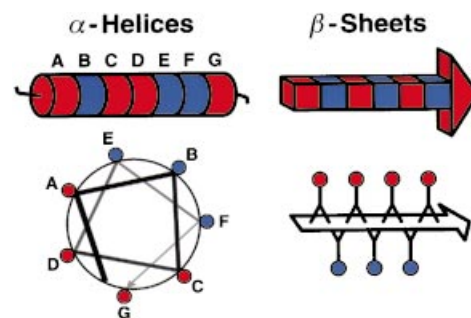
sufficiently active that the host cell grows like the wild-type strain, thus making selection for further improvement difficult. This is true for the modified hexamer even though it is still an order of magnitude less efficient than the homodimeric MjCM.^[37] Since total activity depends on the catalyst concentration as well as specific activity, reducing the available catalyst concentration can further increase selection pressure. In practice, intracellular protein concentrations can be lowered in a variety of ways, for example, by the use of low copy plasmids,^[101] weak promoters^[102], and inefficient ribosome binding sites.^[103]

3.6. Protein Design

The *de novo* design of *functional* proteins represents a far more ambitious goal than topological redesign of an existing enzyme. A number of small protein scaffolds have been successfully designed from first principles,^[104–110] often with the aid of computer algorithms.^[111, 112] Nevertheless, conferring function to these molecules—the ability to recognize another molecule with high selectivity or to catalyze a chemical reaction—remains an enormous challenge.

In principle, the same selection methods that have been successfully used to characterize the mechanistic and structural determinants of enzymes and to alter their topology should aid the design of new protein catalysts. For example, one could imagine directly selecting active catalysts from large random protein libraries.^[113] However, sequence space is infinitely vast. A library with the mass of the earth (5.98×10^{27} g) would contain at most 3.3×10^{47} different sequences. This number represents but a miniscule fraction of the total diversity available to even a small protein, yet far exceeds what is accessible to experimental investigation. Unless catalysts are present in ensembles of random variants at an unexpectedly high frequency, even the most sensitive and efficient selection procedure will be fruitless.

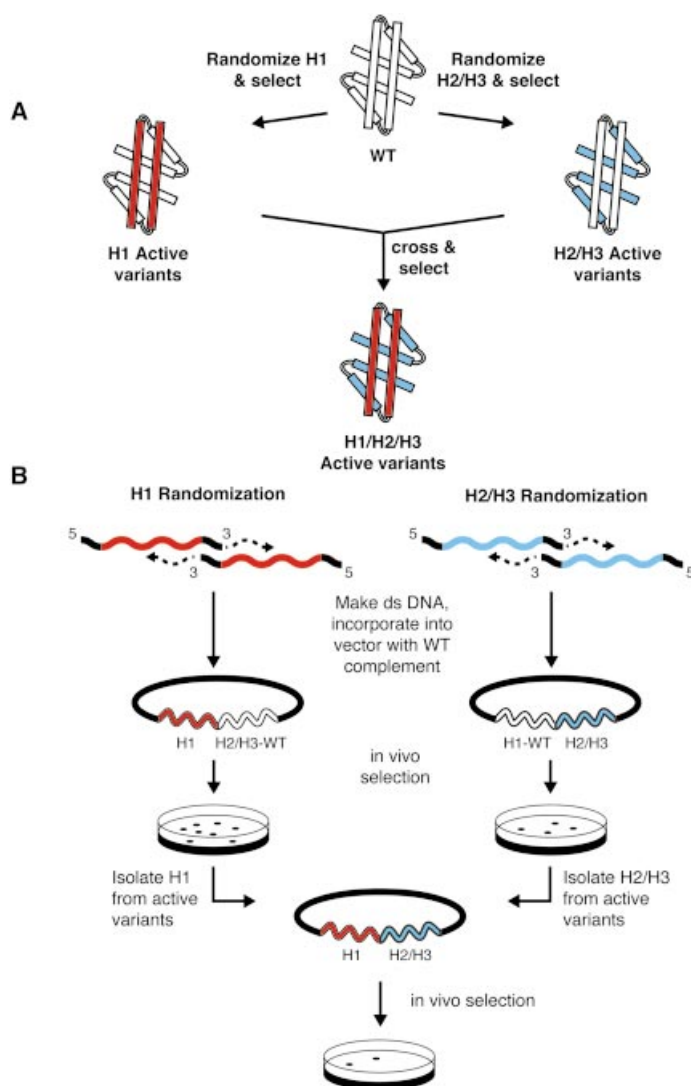
An attainable compromise between these two approaches might utilize basic structural information, such as the sequence preferences of helices and sheets or the tendency of hydrophobic residues to be buried in the protein interior, to design focused random libraries from which functional catalysts can be selected. Binary patterning of polar and nonpolar amino acids,^[75, 114, 115] for example, is a potentially general strategy for protein design that exploits the non-random distribution of amino acids in folded structures (Scheme 9). It has been successfully exploited to create compact helical bundles that share many of the properties of native proteins, such as protease resistance and cooperative folding.^[116–118]



Scheme 9. Amphiphilic secondary structures. Amphiphilic helical or β -sheet structures can be designed by specifying the locations of polar (red) and nonpolar (blue) residues through simple binary patterns.^[227]

We have systematically replaced the secondary structural elements in a homodimeric chorismate mutase (Scheme 10) with binary-patterned units of random sequence to explore the feasibility of such an approach for the design of active catalysts. Genetic selection was then used to assess the catalytic capabilities of the proteins in the resulting libraries, to provide quantitative information about the robustness of this particular protein scaffold and insight into the subtle interactions needed to form a functional active site.^[119]

In the first stage of our experiment modules corresponding to the H1 and H2/H3 helices were combined individually with the appropriate complementary wild-type helical segments of the thermostable MjCM', a protease-resistant version of the dimeric *M. jannaschii* chorismate mutase.^[37] This amounted to randomizing 37 % and 42 % of the entire protein for the H1 and H2/H3 libraries, respectively. At each randomized



Scheme 10. Generation of large binary-patterned libraries of AroQ chorismate mutase genes. A) A two-stage strategy was adopted involving separate randomization and selection of functional variants of the H1 (red) and H2/H3 (blue) helices, followed by combination of functional binary-patterned segments from the initial libraries and reselection. In the final constructs, approximately 80 % of the protein was randomized. B) The binary-patterned genes were constructed from synthetic random oligonucleotides corresponding to the segments encoding the H1 or H2/H3 helices (red or blue, respectively) and assembled in vector fragments (black) as shown. In the initial step, the randomized segments were combined with their wild-type counterparts H2/H3-WT and H1-WT (white). Selection was carried out using the system shown in Scheme 2. WT, wild-type MjCM'; ds = double stranded.

position, only four polar (asparagine, aspartate, glutamate, and lysine) or four nonpolar (isoleucine, leucine, methionine, and phenylalanine) residues were permitted. The loops and conserved catalytic residues were kept constant in the initial design.

The chorismate mutase deficient bacterial strain was transformed with the binary-patterned libraries and the ability of the partially randomized proteins to fold into ordered, catalytically active structures was assessed by complementation of the chorismate mutase deficiency. Roughly 0.02 % of the H1 library clones were active. In other words, about 1 out of 4500 binary-patterned enzymes is a viable catalyst. A somewhat smaller fraction of the H2/H3 library, about

0.006 % (1 out of 17500 variants), was found to complement the genetic deficiency. Given the apparent abundance of catalysts, it would have been possible to find active enzymes even if the three active site residues in each of the two libraries (Arg 11, Arg 28, and Lys 39 in H1, and Arg 51, Glu 52, and Gln 88 in H2/H3) had been additionally mutagenized. Assuming that only a single amino acid is tolerated at each active site position, the probability of finding a catalyst would only have decreased by a factor of 20^3 . Thus, active chorismate mutases would still have been isolable from experimentally accessible libraries containing as few as 10^8 members ($20^3 \times 10^4$). Binary patterning is clearly useful for catalyst design.

Catalysts from both the H1 and H2/H3 libraries were isolated and characterized.^[119] Despite the restricted set of building blocks used in the randomized segments, the enzymes are remarkably active, with modestly elevated K_m values and k_{cat} values in the range 0.2 to 2.3 s^{-1} , which compare favorably with the value of 5.7 s^{-1} for wild-type MjCM.^[37] While most positions in the protein are relatively tolerant to substitution, sequence analysis has identified a few sites as quite restrictive. For instance, all active clones have a strictly conserved Asn–Lys dyad at positions 84 and 85; such a preference is not evident in unselected clones. In retrospect, this finding can be rationalized by examination of the EcCM structure.^[33] The homologue of Asn 84 contributes a hydrogen bond to one of the carboxylate groups of the bound ligand, and the methylene groups of the adjacent lysine residue probably make van der Waals interactions with the ligand. Apparently, none of the other amino acids allowed at these positions would have been able to make such interactions.

The preselected H1 and H2/H3 modules were combinatorially crossed in much the same way as immunoglobulin heavy and light chains are combined in the immune system to create diverse antibody structures.^[120] In the resulting H1/H2/H3 binary-patterned libraries, about 80 % of the MjCM' residues were randomized and >90 % of the protein was constructed from only eight amino acids (Scheme 10 A). Interestingly, only about 1 out of every 10000 possible combinations yields a functional catalyst, even though each of the preselected H1 and H2/H3 components is individually functional in a wild-type context. The native MjCM' H1 and H2/H3 helices are clearly much more effective than the selected segments in templating correctly folded, active enzymes, presumably because they are better able to tolerate destabilizing changes in the varied portions of the protein.

As observed for the individual H1 and H2/H3 libraries, certain positions are highly conserved in active H1/H2/H3 variants. In addition to the Asn 84/Lys 85 dyad seen previously, the majority of complementing clones contain the residues Ile 14, Asp 15, and Asp 18. These amino acids, which are not as highly conserved in the H1 library, probably help stabilize the active site. The residue at position 14 in EcCM interacts directly with a bound ligand through van der Waals contacts, whereas residues 15 and 18 are involved in second-sphere interactions with the catalytically important residues Arg 51 and Arg 28.

Biochemical characterization of one of the selected H1/H2/H3 variants confirms that it is a helical homodimer that undergoes cooperative thermal denaturation ($T_m \approx 44^\circ\text{C}$). It

catalyzes the chorismate to prephenate rearrangement with a k_{cat} value only 15-fold lower than that of the *M. jannaschii* enzyme and a K_{m} value that is 40-fold higher.^[119] By combining binary patterning with two evolutionary cycles, we have thus created a novel helix-bundle protein from a simplified set of building blocks that has biophysical and functional properties similar to those of natural chorismate mutases. Optimization of this and related catalysts from the library through additional rounds of mutagenesis and selection will be useful for identifying key interactions that influence folding, function, and stability.

Experiments of this kind can provide valuable insights into the chemical determinants of protein structure. The results support speculations that ancient proteins might have been constructed from small numbers of amino acids.^[121–123] Nevertheless, the relatively low abundance of active enzymes in the combinatorial libraries underscores the difficulty of generating helical scaffolds capable of catalysis—even when both the position and identity of all critical active site residues are specified in advance.

We estimate^[119] that finding a catalyst among binary-patterned but fully randomized AroQ templates would have required a library containing at least 10^{24} members. Experience suggests that other folds will be substantially more difficult to engineer.^[124] Since libraries of this size are experimentally inaccessible, incremental approaches will undoubtedly be needed for the de novo design of tailored enzymes. Initially, a stable scaffold might be developed,^[228] binding and catalytic groups subsequently introduced, and the entire ensemble finally optimized for the desired activity. It is clear that having an efficient selection system will be invaluable, if not absolutely essential, for the success of such endeavors.

4. Other Applications of in vivo Selection

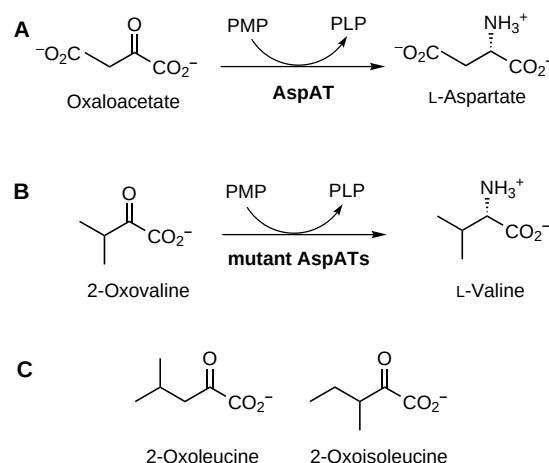
The principles of genetic selection, illustrated above with the chorismate mutase system, are broadly applicable to the study of protein structure and function. A few additional examples are presented below to highlight applications not considered in the previous sections.

4.1. Altering Selectivity

As a consequence of their exceptional efficiency, high selectivity, and mild reaction conditions, enzymes are valuable tools in organic synthesis, particularly for transformations that cannot be carried out with more conventional methods or those involving structurally complex and biologically important substances such as carbohydrates, nucleic acids, and proteins.^[125] For chemists seeking general synthetic methods, however, the narrow substrate specificity of many natural enzymes may represent a significant limitation. For this reason, considerable effort has been devoted to the rational redesign of enzyme binding sites, but, with a few notable exceptions,^[126–128] success has been meager.

Randomization plus screening or selection offers an attractive alternative to rational redesign as a means of altering substrate specificity. Conversion of a homodimeric aspartate aminotransferase (AspAT) into an efficient valine aminotransferase furnishes a striking example of what can be achieved by in vivo selection.^[129]

Wild-type AspAT catalyzes the interconversion of oxaloacetate and aspartate (Scheme 11), but has barely detectable activity for hydrophobic β -branched amino acids. The parent



Scheme 11. A) Aspartate aminotransferase (AspAT) catalyzes the transamination of oxaloacetate to L-aspartate, concomitant with conversion of the cofactor pyridoxamine phosphate (PMP) into pyridoxal phosphate (PLP). The reaction is readily reversible. B) Mutagenesis and selection in an auxotrophic *E. coli* strain yielded AspAT variants that also promote the interconversion of β -branched 2-oxovaline and L-valine.^[129] C) 2-Oxoleucine and 2-oxoisoleucine are also substrates for the mutant AspATs.

gene was mutagenized by DNA shuffling (see Scheme 8) to alter its substrate preference, and variants were selected for their ability to complement an auxotrophic *E. coli* strain lacking the gene for the branched-chain amino acid aminotransferase. This enzyme is required for the synthesis of valine, leucine, and isoleucine. Consequently, the mutant strain can only grow in the absence of valine if the activity of AspAT for the interconversion of 2-oxovaline to valine increases.

Five rounds of mutagenesis and selection were performed. Each round yielded 10^6 to 10^7 transformants, and the plasmid DNA of 90–100 of the fastest growing clones was used for the next round of shuffling. The stringency of selection for mutant AspATs able to utilize 2-oxovaline as a substrate was increased at each round: first by shortening the incubation time on selective medium, then by omitting 2-oxovaline from the growth media (thus lowering its intracellular concentration), and finally by lowering the expression level of the AspAT mutant genes.

One of the mutants that emerged from this process showed a 10^5 -fold increase in the $k_{\text{cat}}/K_{\text{m}}$ value for 2-oxovaline and a 30-fold decrease in the $k_{\text{cat}}/K_{\text{m}}$ value for acidic 2-oxo acids relative to the wild-type enzyme (Scheme 11 B). It contained 13 amino acid substitutions.^[129] The use of an AspAT triple mutant as the starting point for another evolutionary experiment yielded another, closely related variant with 17 amino

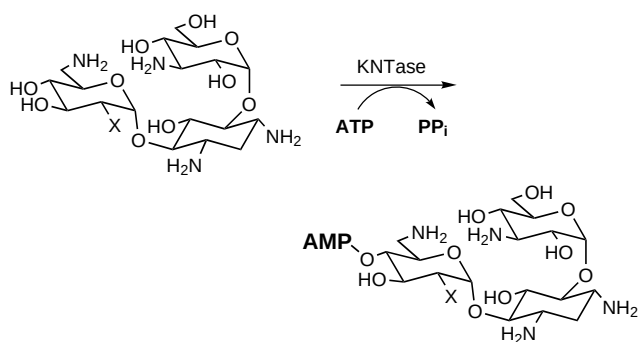
acid substitutions.^[130] Its efficiency with β -branched 2-oxo acids was increased 2×10^6 -fold. Crystallographic analysis of the latter protein complexed with isovalerate showed that only one of the 17 mutated residues contacts the substrate, and several are more than 10 Å from the active site. The resulting remodeling of the active site and subunit interface, which is ultimately responsible for the change in specificity, is thus a consequence of the subtle and cumulative effects of numerous substitutions distant from the binding pocket. Similar observations have been made in natural systems, for example, during maturation of the immune response to small haptens.^[131–133] The difficulty of predicting such second-sphere modifications a priori makes the utility of directed evolution clear.

Successful alteration of the substrate specificities of β -lactamase,^[100, 134–139] thymidine kinase,^[140] prenyltransferases,^[141] oxidosqualene cyclizing enzymes,^[142] *N*'-[5'-phosphoribosyl]formimino]-5-aminoimidazole-4-carboxamide ribonucleotide isomerase (ProFARI),^[143] and aminoacyl-tRNA synthetases,^[144, 145] to cite only a few recent examples, speaks to the versatility of the selection approach.

4.2. Increasing Thermostability

The moderate stability of enzymes isolated from mesophilic organisms may represent another limitation for many practical applications. For this reason, there has been considerable interest in isolating catalysts from organisms that grow under extreme conditions.^[146] Evolutionary approaches also lend themselves to the creation of proteins that tolerate a wider range of experimental operating conditions.

One strategy for creating thermostable enzymes entails introducing the gene for an enzyme from a mesophilic organism into a thermophile and selecting for variants that retain activity at higher growth temperatures.^[147] This approach has been used to identify mutant kanamycin nucleotidyltransferases (Scheme 12).^[148, 149]



Scheme 12. Kanamycin nucleotidyltransferase (KNTase) transfers a nucleoside monophosphate group from ATP to the 4'-hydroxyl group of the antibiotics kanamycin A (X=OH) and B (X=NH₂). This reaction provides bacterial resistance to aminoglycoside drugs.

Cells of the moderate thermophile *Bacillus stearothermophilus* containing the mesophilic wild-type enzyme are resistant to the antibiotic kanamycin at 47 °C, but not at temperatures above 55 °C. Variants that are kanamycin

resistant above 60 °C were obtained following mutagenesis and selection in the thermophile. A single mutation, Asp80Tyr, was found to be responsible for the increased resistance of the enzyme to irreversible thermal inactivation.^[148, 149] Additional selection experiments at 70 °C identified Thr130Lys as a second stabilizing mutation.^[148] The Asp80Tyr/Thr130Lys double mutant has a half-life of more than one hour at 60 °C, whereas the wild-type enzyme is denatured almost immediately under these conditions. The double mutant is also substantially more resistant to proteolytic degradation. An even more stable enzyme ($T_m = 84$ °C), which contains 19 amino acid substitutions, has been obtained by multiple rounds of DNA shuffling and selection in the extreme thermophile *Thermus thermophilus*.^[150] Interestingly, the beneficial mutations did not include the amino acids identified in the earlier studies. Moreover, most of the affected residues are located on the surface of the protein, and 5 of the 19 changes involved incorporation of proline.

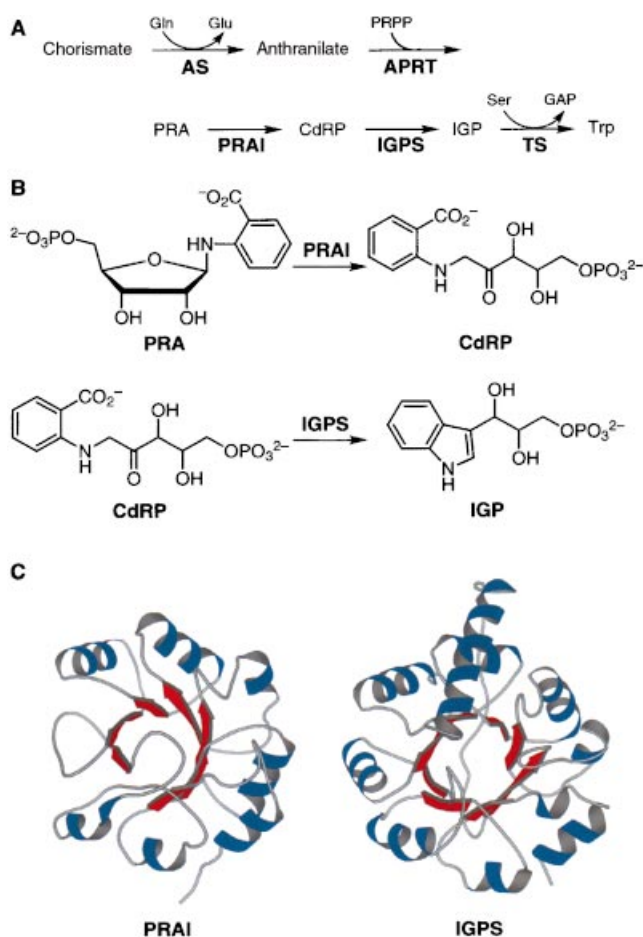
Any enzymatic activity that is selectable in thermophiles should be amenable to this approach, although in vitro strategies using various display formats (see Section 5) may ultimately prove more flexible with respect to stabilization against a wider variety of harsh conditions. In addition to the practical benefits that accrue, such experiments can greatly enhance our understanding of the interactions that contribute to protein stability.^[151]

4.3. Altering Function

A powerful strategy for conferring new functions to existing proteins is to mimic the process of divergent evolution. Nature exploits a limited number of protein folds^[152] and puts them to many and diverse uses. The generic immunoglobulin structure^[153] is a good example since it is exploited, with minor variation, to recognize the universe of foreign antigens. In the same way that chemists have exploited antibody molecules (and the microevolutionary processes inherent to the mammalian immune system) to create a wide range of tailored catalysts,^[154–156] many existing protein scaffolds might serve as starting points for the development of new active sites capable of diverse functions. The α/β -barrel fold^[157, 158] that constitutes roughly 10% of all structurally characterized proteins may be particularly versatile in this respect.^[161]

The conversion of indole-3-glycerol-phosphate synthase (IGPS) into a highly active phosphoribosylanthranilate isomerase (PRAI) demonstrates the feasibility of this strategy.^[159] PRAI and IGPS catalyze sequential steps in the biosynthesis of tryptophan (Scheme 13 A). The first promotes the conversion of *N*-5'-phosphoribosylanthranilate (PRA) to 1'-(2'-carboxyphenylamino)-1'-deoxyribulose 5'-phosphate (CdRP), and the second accelerates the rearrangement of CdRP to indole-3-glycerol phosphate (IGP) with concomitant release of CO₂ and H₂O (Scheme 13 B). Both enzymes are α/β -barrels (Scheme 13 C) that are covalently linked in *E. coli*. Both also bind CdRP.

The strategy to convert IGPS into PRAI involved rational redesign coupled with random mutagenesis and genetic selection. Guided by the crystal structures of the two enzymes,



Scheme 13. A) Biosynthesis of tryptophan from chorismate. AS = Anthranilate synthase; PRPP = phosphoribosylpyrophosphate; APRT = anthranilate phosphoribosyl transferase; PRA = *N*-5'-phosphoribosylanthranilate; PRAI = phosphoribosylanthranilate isomerase; CdRP = 1'-(2'-carboxyphenylamino)-1'-deoxyribulose 5'-phosphate; IGPS = indole-3-glycerol phosphate synthase; IGP = indole-3-glycerol phosphate; TS = tryptophan synthase; GAP = glyceraldehyde 3-phosphate. B) Directed evolution was used to create a novel PRAI, which catalyzes the conversion of PRA into CdRP, from IGPS, which catalyzes the cyclization of CdRP to IGP.^[159] C) Both PRAI and IGPS are α/β barrel proteins but have somewhat different loop structures.

the IGPS scaffold was initially redesigned to more closely resemble PRAI. First, a long N-terminal segment, absent in PRAI, was removed. In a second step, a 15 residue loop was replaced with a 4–7 residue loop (PRAI has a 4 residue loop). The variation in loop length and sequence provided a diverse set of variants from which active catalysts could be chosen in later selection steps. Finally, a second loop region was replaced with the PRAI consensus sequence GXGGXGQ, and an aspartate residue was introduced at position 184 to serve as a general base in the remodeled active site.

Active variants were isolated from the resulting library of IGPS mutants by genetic selection in a PRAI-deficient *E. coli* strain that cannot produce its own tryptophan. Approximately 500 out of 30 000 library members were found to grow at low tryptophan concentrations. The genes from the selected clones were then subjected to additional rounds of mutagenesis and selection until several variants were found that

could grow in the absence of tryptophan. One of these (ivePRAI) was characterized in detail. The encoded protein was soluble and properly folded. Moreover, it exhibited significant PRAI activity in vitro but no IGPS activity, despite having a sequence identity of only 28% to PRAI and a sequence identity of 90% to IGPS. In fact, the specificity constant ($k_{\text{cat}}/K_{\text{m}}$) for ivePRAI was sixfold higher than for wild-type PRAI, because of an enhanced affinity for the PRA substrate.^[159]

The successful interconversion of IGPS and PRAI in the laboratory supports the idea that tryptophan biosynthetic enzymes arose naturally by a process of divergent evolution.^[160] In particular, this experiment shows how new catalytic activities can evolve quite rapidly from an existing binding site. Although extension of this approach to reactions of substrates that have no affinity for the template protein may prove more difficult, the promising results with IGPS suggest that the commonly occurring α/β -barrel motif may indeed be a generally useful scaffold for designing new enzymes.^[161]

4.4. Optimizing Multistep Processes

As in natural Darwinian evolution, multiple components of a biosynthetic pathway—even whole organisms—can be optimized simultaneously in evolution experiments in the laboratory. A usual prerequisite for a genetic selection system is the construction of an appropriate selection strain through the mutation or elimination of chromosomal genes. However, another approach is to use an existing organism and apply selective conditions that require foreign genes. Optimization of the efficiency of an arsenate detoxification pathway,^[162] which has potential value as a tool for bioremediation, is an interesting example of this strategy.

The arsenate resistance operon from the bacterium *Staphylococcus aureus* consists of three genes, *arsR*, *arsB*, and *arsC*, which afford resistance to arsenate and arsenite ions. The entire operon was mutagenized by DNA shuffling (see Scheme 8) and introduced into a standard *E. coli* strain. Arsenate-resistant bacteria were then selected from the population by plating on media containing increasing concentrations of arsenate (16–400 mM). After three rounds of mutagenesis and selection, fast growing clones were isolated. One of these was even able to grow in liquid media containing 500 mM arsenate.

Analysis of the evolved operon region revealed a total of 13 mutations. Ten (seven of which were silent) occurred in *arsB*, which encodes a membrane-associated pump that actively exports arsenite from the cell; these mutations collectively yielded a four- to sixfold increase in arsenite resistance, possibly by increasing the production levels while simultaneously reducing the toxicity of the ArsB protein. Two silent mutations occurred in *arsR*, which encodes the operon repressor protein, and one mutation occurred immediately downstream of the operon. Interestingly, the arsenate reductase gene *arsC* contained no mutations at all. Nevertheless, the overall rate of arsenate reduction was improved 12-fold,

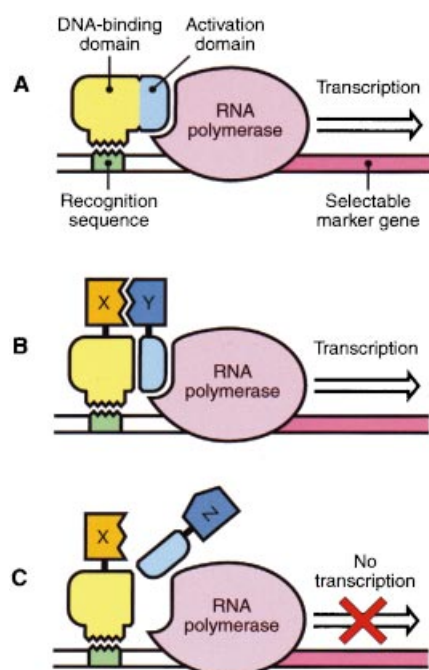
which was explained by an increased level of gene expression as a result of the mutations in the upstream gene *arsB*.^[162]

These experiments underscore again the fact that many changes other than those leading to higher kinetic efficiency of a particular enzyme can help an organism survive under selection conditions. It is irrelevant to the selection process whether enhanced survival results from greater catalytic ability, more efficient gene expression, or increased protein stability. In fact, fine-tuning noncatalytic properties is likely to be far easier, in general, than optimizing catalytic activity. The corresponding mutations are presumably more frequent and more easily tolerated in almost all systems.

4.5. Finding Ligands and Inhibitors

Although enzyme structure and function is the primary focus of this review, it is worth mentioning that genetic selection has also emerged as a powerful tool for identifying novel peptide ligands for biological receptors and for characterizing ligand–receptor interactions in vivo. Yeast two-hybrid systems^[163–167] are particularly useful in this regard.

The yeast two-hybrid system is depicted schematically in Scheme 14. Gene transcription in yeast relies on the interaction of RNA polymerase and an activator domain of a transcriptional activator. The activator domain is positioned in the DNA promoter region through the activator's DNA binding domain. In the yeast two-hybrid system, the covalent interaction between the two domains of the transcriptional



Scheme 14. Yeast two-hybrid systems.^[163–167] A) Gene expression in yeast involves interaction of RNA polymerase with a DNA-binding transcriptional activator. B) Two different proteins or protein libraries are fused to the DNA-binding and activator domains of a yeast transcriptional activator, respectively. An interaction between two cognate proteins (X and Y) reconstitutes the activator, which leads to transcription of the reporter gene. C) Noncognate library members cannot assemble a functional activator.

activator is disrupted. Individual libraries of hybrids are formed by linking one or both of the separated domains to ensembles of other molecules (DNA, RNA, proteins, and small compounds are all conceivable candidates). If any two of the linked molecules interact, then the activator and DNA-binding domains will come together and transcription can occur from the promotor proximal to the DNA binding site. This transcription leads to the expression of a reporter gene and, hence, to a selectable or screenable phenotype.

Two-hybrid screening is most common,^[164–166] where the reporter gene product provides a nonessential function to the cell that is easy to detect. However, two-hybrid genetic selection systems that utilize essential reporter genes are also used. For example, selection based on transcriptional activation has been used to identify peptides that bind to cyclin-dependent kinase 2 (Cdk2) in vivo.^[168] The peptides were displayed as a 20-residue insert in the active-site loop of *E. coli* thioredoxin; the best variants had nanomolar affinity for their target. A yeast three-hybrid system for detecting ligand–receptor interactions has also been described.^[169] The relatively low transformation efficiencies of yeast limit the size of the libraries that can be exhaustively studied, but *E. coli*-based two-hybrid systems have been developed^[170, 171] that could extend the capabilities of such systems.

Genetic selection in yeast has also been used directly to identify peptides that inhibit biological pathways, without recourse to two-hybrid formats.^[172] For example, cleverly engineered yeast strains have been used to select inhibitors of the spindle checkpoint and mating pheromone signaling pathways from libraries of peptides displayed on a surface loop of an inactive variant of staphylococcal nuclease. The putative intracellular targets were subsequently identified by a combination of two-hybrid analysis and genetic dissection of the target pathways.^[172] A similar genetic selection approach, albeit in *E. coli*, was recently used to identify nine-residue peptides that inhibit intracellular dimerization of HIV-1 protease.^[173]

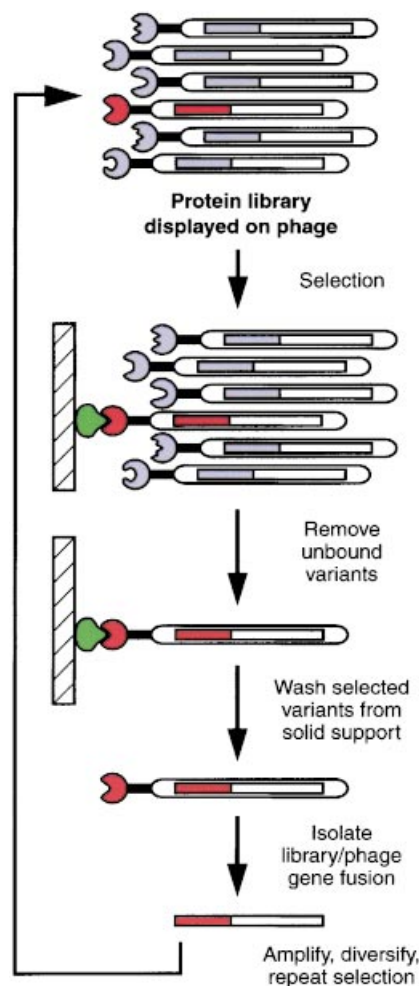
Such studies are advantageous to drug discovery since they provide novel leads and a wealth of information about structure–function relationships in the biological receptor. This information can help guide the design of potent non-peptidic inhibitors. Characterization of the site of action of the selected peptides can also reveal new components of the investigated pathway which may, in turn, represent formidable targets for inhibition by small organic molecules.

5. In vitro Selection Systems

The examples cited in this review show that a wide range of different protein functions can be linked to a genetically selectable phenotype. Nonetheless, establishing such a connection can be nontrivial for many interesting applications and may be impossible in some cases (for example, if nonphysiological conditions are required). In such instances, in vitro selection can often be a practical alternative. Selection in vitro, as in vivo, necessitates linking genetic information with protein function in some fashion. Some of the ways this task can be realized are outlined below.

5.1. Phage and Cell-Surface Display

Display of proteins (and peptides) on the surface of cells^[174, 175] or bacteriophage^[176, 177] is a particularly effective method for coupling phenotype and genotype. The repertoire of proteins to be sampled is fused to a membrane protein or to one of the outer capsid proteins of filamentous phage (Scheme 15). By exposing the resulting library to a ligand that



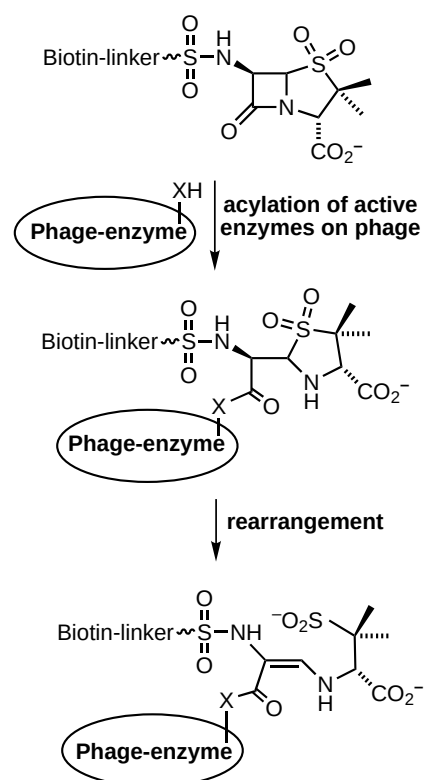
Scheme 15. Selection by phage display.^[176, 177] Fusion of the gene encoding an antibody or another protein to the gene for one of the coat proteins of the bacteriophage allows the display of the protein and its variants on the surface of the virus particle. Affinity selection with an immobilized ligand (green) can be used to isolate proteins with desirable binding properties from large populations and, concomitantly, their topologically linked encoding genes (red).

is immobilized (or immobilizable) on a solid support, specific receptors can be isolated by affinity selection *in vitro*. The gene encoding the functional polypeptide is selected simultaneously since it is housed within the cell or bacteriophage bearing the receptor. It can be recovered from the solid support and amplified *in vivo*. It can also be diversified and subjected to additional rounds of selection to fine-tune the properties of the encoded protein.

Display technologies have been broadly used to identify peptide ligands for diverse receptors and to select proteins

with novel binding activities. Optimization of DNA-binding proteins for the specific recognition of any desired DNA sequence,^[178–185] minimization of the immunoglobulin Z-domain of protein A,^[186] and evolution of antibody fragments with femtomolar antigen-binding affinity^[187] are notable in this regard. The selection of proteins directly on the basis of their catalytic activity has proved more difficult to achieve, but several promising strategies have been reported.

In one approach, reactive ligands^[188] and mechanism-based inhibitors^[189, 190] have been used to enrich enzymes and catalytic antibodies from phage display libraries. Phage particles with appropriate chemical functionality react covalently with the agent, either directly or after activating it chemically. As a consequence of this chemical event, active phage are captured and immobilized on a solid support (Scheme 16). The same protein group(s) responsible for

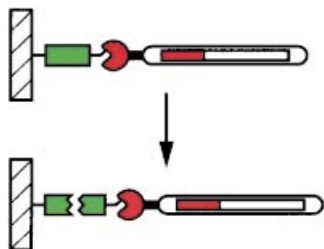


Scheme 16. Trapping phage enzymes with mechanism-based inhibitors. Penicillanic acid sulfones connected through a spacer to biotin can be used to trap enzymes displayed on filamentous phage that hydrolyze β -lactams through a nucleophilic mechanism.^[189] Enzymes lacking the active site nucleophile (X) are not covalently modified. Biotin binding to immobilized streptavidin allows isolation of the trapped phage.

activating the inhibitor can be subsequently exploited for the catalytic conversion of a substrate. This approach can be useful for the discovery of new catalysts,^[189, 190] but it is necessarily indirect. Selection requires irreversible modification of the receptor—a single step—rather than efficient substrate turnover, which may require stabilization of multiple transition states and avoidance of product inhibition. The need to design and synthesize appropriately reactive derivatives and inefficient trapping of the reactive intermediate

generated by the catalyst may represent additional practical limitations to this approach.

Another viable strategy for selecting catalysts takes advantage of co-localization of the catalyst and substrate or product. For example, immobilized substrates can be cross-linked to phage in the vicinity of a displayed enzyme (Scheme 17).^[191] If the enzyme is active, intramolecular



Scheme 17. Intramolecular conversion of substrate to product provides a means of selecting phage-bearing active enzymes if the reaction results in cleavage of the phage from a solid support.^[191]

cleavage of the substrate selectively releases the corresponding phage particle from the solid support, which allows isolation and amplification of the encoding gene. Analogous schemes have been devised for synthetic reactions that result in the attachment of a tag to the phage or that produce a tagged product that can subsequently react with the phage particle before diffusing away.^[192] The peptide-ligating activity of a modified protease has been optimized by such a scheme: enzyme variants that successfully attached a biotinylated peptide to their own extended N-termini were selectively captured by streptavidin.^[193] In another variation of this general approach, antibodies against the product have been used to isolate catalytically active enzymes displayed on phage particles to which the reaction products were anchored.^[194]

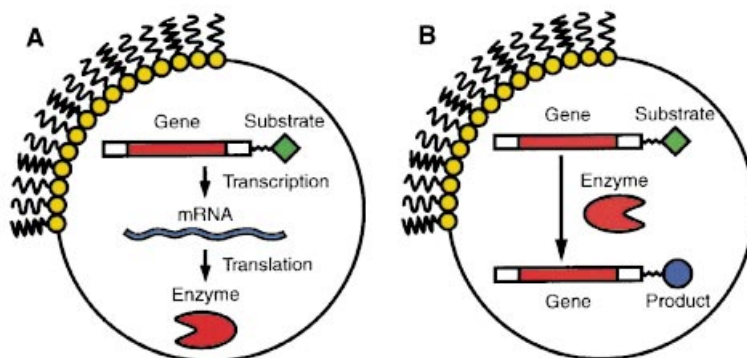
A very different scheme was recently reported for identifying functional proteases in protein libraries displayed on the surface of a bacterial cell.^[195] It relies on electrostatic interactions to retain positively charged peptide substrates and products on the negatively charged cell surface. Cleavage of the substrate, which is covalently linked to fluorescent FRET (fluorescence resonance energy transfer) groups, releases the quenching component, and gives rise to a fluorescent signal. Active catalyst variants can then be isolated by fluorescence-activated cell sorting (FACS). Mutants of the serine protease OmpT with 60-fold improved efficiencies for cleavage of Arg–Val bonds were isolated in this way from libraries of 6×10^5 random variants. This method, which is technically a high-throughput screening procedure rather than genuine Darwinian selection, should be applicable to a variety of proteins displayed on the surface of bacteria and yeast.

5.2. Compartmentalization

Cell-free transcription/translation systems can constitute an entirely *in vitro* alternative to phage display. Such systems

have all the RNA molecules, proteins, and small molecules needed to transcribe a gene and translate it into protein.^[196, 197] Genotypes and phenotypes can be topologically linked by encapsulating these components in an artificial cell-like compartment. For example, the ability of a translated enzyme to modify its co-localized encoding gene can be exploited as a means of selection (Scheme 18). In compartments in which an inactive protein is produced, the corresponding genes remain unmodified and thus not amplifiable.

The feasibility of this approach has been demonstrated by using mixtures of genes encoding *Hae*III methyltransferase and another, unrelated enzyme. The methyltransferase methylates DNA and renders it resistant to cleavage by *Hae*III endonuclease. The mixture was encapsulated in water/oil emulsions, which have aqueous compartments with volumes close to those of bacteria.^[198] After transcription/translation in the aqueous compartments, genes encoding the methyltransferase were protected by methylation and amplifiable by PCR, whereas those encoding the other protein were



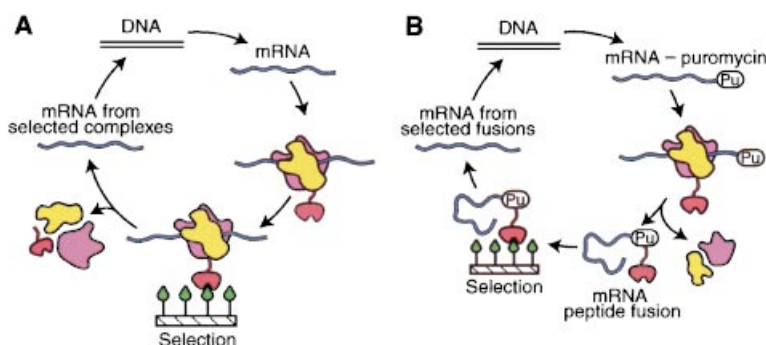
Scheme 18. Gene selection by compartmentalization is based on transcription/translation of single genes in aqueous compartments of a water/oil emulsion (A).^[198] Subsequent modification of the gene by the encoded and co-localized enzyme (B) affords a means of selection. Genes encoding an inactive protein will not become modified and can be eliminated in the selection process.

degraded upon treatment with the endonuclease *Hae*III. In this way methyltransferase genes were isolated from a 10^7 -fold excess of genes encoding the other enzyme.^[198]

If a general strategy for selection can be developed, compartmentalization could prove broadly applicable for molecular evolution experiments since passage through cells is unnecessary. As a consequence, library sizes are potentially much larger than for *in vivo* experiments and difficulties associated with inefficient microbial production of some proteins can be circumvented.

5.3. Linking mRNA and Protein during Synthesis

Ribosome (polysome) display^[199–203] and generation of mRNA–protein fusions^[204–206] are two additional methods that can be exploited for the evolution of proteins *in vitro*. Both rely on cell-free translation of mRNA templates to produce proteins on ribosomes (Scheme 19). To forestall the uncoupling of message (protein) and messenger (mRNA) that would normally occur during protein synthesis in



Scheme 19. Selection by ribosome display or mRNA-peptide/protein fusions.^[199–203] A) In vitro translation of mRNA on ribosomes can be halted by the addition of Mg^{2+} ions and incubation at low temperature. Ribosome/mRNA/nascent peptide complexes containing a desired receptor (red) can be selected by binding to an immobilized ligand (green). Recovery and reverse transcription of the retained mRNA, followed by PCR amplification, yields DNA for characterization or the next round of selection. B) An mRNA-protein fusion is formed upon ribosome-catalyzed translation of an mRNA-puromycin conjugate. Upon completion of the protein synthesis, the protein (red) is transferred from tRNA to puromycin (Pu) at the peptidyl transferase center of the ribosome. The folded protein and its encoding mRNA are subsequently amenable to in vitro selection.

the absence of compartmentalization, the system can be rigged so as to prevent dissociation of the translated protein from the ribosome.^[199–203] Alternatively, the protein can be joined to its own mRNA through a stable covalent linkage.^[204–206]

Complexes of ribosomes, mRNA, and protein can be stabilized against dissociation by low temperatures and addition of magnesium acetate to the buffer.^[201] More importantly, eliminating the stop codon that normally signals binding of polypeptide release factors and termination of protein synthesis greatly slows the hydrolysis of the ribosome-bound peptidyl-tRNA.^[201] If the displayed proteins fold properly, affinity selection protocols can be used to isolate variants that recognize small molecules, oligonucleotides, or other proteins (Scheme 19A). For example, this technology has been used successfully to increase the stability and ligand affinity of antibody molecules.^[201, 207] Addition of ribonuclease inhibitors to the reaction mixture and incorporation of stem-loop structures into the mRNAs to prevent degradation of the messenger have helped to optimize the efficiency of such experiments.^[201] Providing molecular chaperones to promote proper folding of the displayed proteins in vitro can also be beneficial.^[201]

The generation of covalent fusions between an mRNA and the protein it encodes may ultimately prove to be a more robust method of linking genotype and phenotype.^[204–206] Such constructs are formed by in vitro translation of mRNA templates containing the antibiotic puromycin at their 3'-end (Scheme 19B). After the RNA is translated, puromycin can enter the peptidyl transferase site of the ribosome and attach itself to the C-terminus of the nascent protein to form a covalent mRNA-protein conjugate. The translated protein can then be selected as described for the other formats. In contrast to the strategy of Scheme 19A, selection can be performed after removal of the ribosome and other components of the in vitro translation system because the protein is covalently linked to its own mRNA.

In vitro transcription/translation systems are attractive for directed evolution for a number of reasons. First, an expanded range of conditions can be exploited for the selection step itself. Second, unnatural amino acids can be incorporated directly into the proteins through suppressor codons.^[208] Finally, libraries of much greater complexity and size can be generated in vitro than in vivo because these systems are not limited by the transformation efficiency of *E. coli*. Libraries containing 10^{11} to 10^{13} different members can be constructed with current protocols, and modest scale-up and improved construction protocols may make ensembles of 10^{15} members accessible.^[205, 209]

As with phage and cell surface display technologies, these systems are ideally suited for selection schemes based on receptor-ligand interactions. For example, four ATP binders were recently selected using the mRNA-peptide fusion approach starting from a library of 6×10^{12} proteins in which 80 contiguous amino acids were randomized.^[210] It has been suggested that libraries constructed from

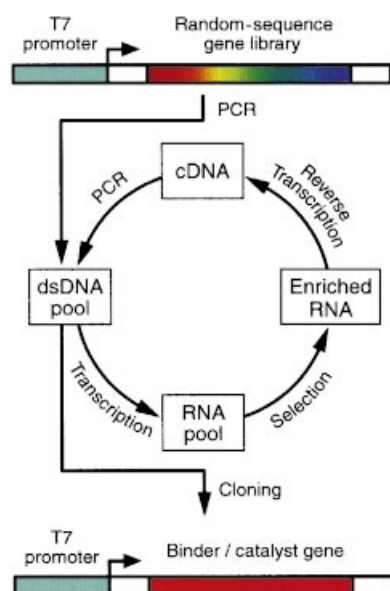
natural sources of genomic or cDNA will also be useful for rapidly finding interacting proteins in organisms where genetic analysis is difficult.^[205] The combination of some of the in vitro transcription/translation strategies discussed herein with methods for selecting proteins on the basis of their catalytic activity (see Section 5.1) may also allow the directed evolution of novel enzymatic function.

5.4. RNA Evolution Experiments

The value of having large libraries has been demonstrated by the direct selection of RNA and DNA molecules that function as receptors and catalysts.^[210] Such experiments routinely involve generating and screening molecular repertoires with as many as 10^{15} independent members. Since nucleic acids combine structure, function, and genetic information within a single molecule, in vitro recovery and amplification of rare genes for a desired phenotype is straightforward and very efficient (Scheme 20). Consequently, populations of nucleic acids can be easily subjected to multiple cycles of selection and enrichment.

RNA and DNA catalysts have been prepared for a wide variety of reactions, such as the cleavage, ligation, and modification of oligonucleotides, alkylations, acyl transfer reactions, and even abiological Diels-Alder cycloadditions.^[210] Although selection of functional nucleotides appears to be easier than functional proteins (see, for example, Section 3.6), the resulting catalysts often have relatively modest efficiency relative to highly evolved enzymes, perhaps because they are constructed from a limited palette of four nucleotides. In some cases, however, extraordinary activities have been achieved.

The transfer of phosphoryl groups is particularly amenable to ribozyme catalysis. Direct selection of highly efficient self-ligating ribozymes from large pools of random sequence is one of the most impressive accomplishments in this regard.^[211]



Scheme 20. In vitro evolution of RNA catalysts. A combinatorial library of RNAs generated by in vitro transcription is subjected to selection for ligand binding or catalysis. Molecules possessing the desired trait are separated from inactive variants to yield a new RNA pool enriched in functional sequences. The enriched pool is reverse transcribed to cDNA and amplified by the polymerase chain reaction (PCR) to obtain material for another round of selection. The cycle is repeated until the pool is sufficiently enriched in active sequences, which can be cloned for detailed characterization. If an error-prone PCR method is used,^[3] each round represents a true Darwinian-type cycle of evolution.

An initial library was designed as a pool of approximately 10^{15} different RNAs with 220 random nucleotides. About 1 in 10^{13} random sequences proved to have the desired ligase activity. Clearly, if the starting library had contained 10^{10} sequences, the chances of finding these molecules would have been vanishingly small. Further diversification and selection was used to evolve the population of ligase ribozymes, one of which was subsequently re-engineered to function as a true catalyst that promotes an intermolecular ligation with multiple turnovers.^[212] It exhibits a rate constant of $>1 \text{ s}^{-1}$, which corresponds to a rate acceleration approaching 10^9 over the uncatalyzed reaction.

Nucleic acid ligands, receptors, and catalysts are novel analogues of their peptide/protein counterparts with many conceivable applications as drugs or diagnostic agents. The strategies used to generate them, an understanding of how they evolve from random sequence pools, and insight into their relative advantages and disadvantages compared to polypeptides are all likely to improve our ability to create and optimize novel protein catalysts.

6. Summary and Perspectives

Nature has solved the problem of protein design through the mechanism of Darwinian evolution. Every one of the proteins in our cells, from enzymes and receptors to regulatory and structural proteins, has arisen by this process. As the examples presented in this review amply demonstrate, evolutionary strategies can also be successfully exploited in

the laboratory to study the structure and function of existing proteins and to engineer new ones.

Site-directed mutagenesis, by which one amino acid in a protein is substituted for another, is an established and invaluable tool in protein chemistry. However, the size and complexity of proteins often precludes unambiguous interpretation of the results of such experiments. This arises from the non-uniform distribution of structural information within a protein sequence—the fact that some residues are tolerant to substitution, whereas others cannot be replaced without deleterious consequences—and to the many energetically similar conformational states available to any polypeptide. These factors, and the extreme sensitivity of catalytic activity to seemingly modest structural perturbation, make characterization and (re)design of enzymes in the laboratory so difficult.

In contrast to methods involving single substitutions, combinatorial methods are ideally suited for evaluating the complex network of interactions in proteins that ultimately determine form and function.^[73] As shown in the investigations of chorismate mutase enzymes (Sections 3.2 and 3.3), degenerate protein ensembles can illuminate subtle trends that might be missed in analyses of individual amino acid replacements. This information can be used to verify and refine our understanding of protein structure and function. Evolutionary strategies can also be used in conjunction with data on structure and mechanism to support design efforts,^[8] as shown by the creation of novel monomeric and binary patterned mutases (Sections 3.4–3.6) and by the alteration of aspartate aminotransferase specificity (Section 4.1) and IGPS function (Section 4.3). The iterative nature of evolutionary approaches is particularly advantageous in this regard, since it allows the rapid optimization of the population of molecules under study toward some desirable goal. Even in the absence of detailed structural information or a clear understanding of how a system works, directed evolution can be used to tailor the properties of proteins for specific practical applications (Section 4.4).

Of course, successful application of evolutionary methods requires an efficient method for sifting through the many library members to identify those that have desirable properties, be they recognition of a particular ligand or catalysis of an interesting chemical transformation. For small libraries, finding the useful variants is readily accomplished by screening. Even with large libraries, depending on the difficulty of the task at hand—and the distance the starting structure is from the desired endpoint in sequence space^[213]—screening can often be exploited with great effectiveness. For example, enantioselective esterases and lipases,^[214, 215] thermostable hydrolases,^[216, 217] and metal-dependent enzymes with novel specificities^[218, 219] have been identified through random mutagenesis and screening. Moreover, advances in automation and miniaturization can be expected to extend the utility of screening methods for high-throughput analysis of reasonably large molecular ensembles.^[10, 11, 195, 198]

Genetic selection is likely to be the method of choice for problems where statistically meaningful correlations are sought or solutions are extraordinarily rare. In vivo selection schemes allow exhaustive searches of libraries of 10^{10} protein

variants, and significantly larger libraries can be handled with in vitro methods (Section 5.2–5.4). The principal challenge in developing an effective selection protocol is the linkage of the macromolecular property of interest to a biological readout, thereby allowing facile recovery of the responsible encoding gene. In principle, any metabolic process can be exploited for this purpose, as can transformations involving the creation or destruction of vital nutrients or toxins (Sections 3 and 4). Clever in vitro schemes further expand the range of possibilities for selecting molecules with interesting binding and catalytic properties (Section 5).

Although laboratory evolution experiments are now well established, improvements in several areas could significantly enhance the utility of such approaches for diverse practical applications. Improved protocols for mutagenesis^[134, 220, 221] and for transforming microorganisms,^[23, 222] for example, might allow the creation of better-designed and still-larger protein libraries. Selection schemes that are more versatile and general than those currently available, and which would permit selection for any desired function, would also clearly be of great value. Furthermore, we can dream of extending evolutionary principles to a wider range of molecules than nucleic acids and proteins. Realization of such an ambitious goal would require developing novel encoded combinatorial libraries as well as strategies for extracting desirable molecules and their blueprints from such ensembles. To that end, new methods for “autocatalytic” detection and selective amplification of the “best” molecules after each evolutionary cycle will be needed.

Evolutionary approaches will never supplant “rational” approaches for the study of molecules. Rather, they powerfully augment the classical strategies of dissection and design. In fact, directed evolution is likely to be most effective in combination with detailed structural and mechanistic information for exploring and engineering the properties of proteins, nucleic acids, and other encoded molecules. In the post-genomic era, these methods are likely to become increasingly valuable in analyzing and integrating the flood of data that is emerging for so many biological systems. In the future we can expect that intelligent application of evolutionary methods will help to clarify the links between sequence and structure, structure and function, and between interacting macromolecules in the cell. Novel applications in medicine and industry will not be far behind.

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Not a Library**

Roald Hoffmann*

I

Combinatorial chemistry evokes conflicting responses—it is a superbly efficient way to discovery, it is unthinking to unsporting, it is the ultimate biomimetic process. All these perspectives capture some aspect of a very hot field. I find especially enlightening Albert Eschenmoser's view:^[1]

"The essential step in combinatorial chemistry is not the synthesis; rather it is finding a target, based on specific properties. Combinatorial synthetic research is an adaptation to what nature has been doing all the time: synthesis followed by selection, as opposed to synthesis by design. The aim of combinatorial chemistry is primarily discovering a molecule, whereas that of chemical synthesis is constructing a molecule."

Central to modern combinatorial practice is the generation at some intermediate stage of a large set of molecules. The ways *in* to this set are many and ingenious—the compounds may be pooled all in one vessel, produced separately (and readied to signal their identity) by a set of efficient reactions. The molecules may be as globally diverse as chemistry can make them, or scattered round a structural feature of proven utility. The ways *out* of that set are equally rich—one may sample for activity with respect to what one's heart (or employer) wishes, one may let a biological molecule seek out a partner among the many, to inhibit or accelerate. But whatever one does, if the process is to work, one has to have many.^[2]

I admire the ingenuity of combinatorial chemistry, love the way it demystifies normal exploratory synthesis, and ponder the philosophical questions it raises. But there is one aspect of its nomenclature that just rubs me the wrong way. This is the common denomination of the large combinatorial set as a "library".

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[**] I am grateful to Albert Eschenmoser, Sylvie Coyaud, and Henning Hopf for their comments.

It's about as far from a library as one can get in this world! To a secular refugee of World War II who did not own a book until age 13, a library was about as close to a holy place as I could imagine. I remember most of mine—the small refugee camp library in Bindermichl, Austria; the city library in Munich where I put my name on a long waiting list for the next installment of Karl May's adventures of Kara Ben Nemsi or Old Shatterhand; the beautiful public library in Woodside, Queens, decorated with murals by superb artists set to work during the Great Depression.

Now these were *libraries* (Figure 1). They were filled with organized knowledge, with wisdom, with the salacious (to a boy), hidden under the librarian's desk. These true libraries were the work of men and women shaping laughter and tears, defeating entropy. The set of all possible molecules or a finite subset thereof, even if we can "read" it chemically, is no more a library than the set of all books made of random letter arrangements.

II

Neither the lure of combinatorics, nor the critical appreciation of the lack of human meaning in the outcome of the combinatorial process is new. One of the oldest of kabbalistic texts is the *Sefer Yetzirah*, probably originating in the 3rd or 4th century of our era. It is a brief, mysterious, and poetic work, perhaps a guide to Jewish mystical practice. The gate to enlightenment is through permutations of the letters of the Hebrew alphabet:^[3]

*"Twenty-two foundation letters:
He engraved them. He carved them.
He permuted them. He weighed them.
He transformed them.
And with them, He depicted all that was formed
And all that would be formed."
Sefer Yetzirah does some math:^[4]
"Two stones build two houses
Three stones build six houses
Four stones build twenty four houses
Five stones build one hundred and twenty houses
Six stones build seven hundred and twenty houses
Seven stones build five thousand and forty houses
From here on go out and calculate
That which the mouth cannot speak
And the ear cannot hear."*

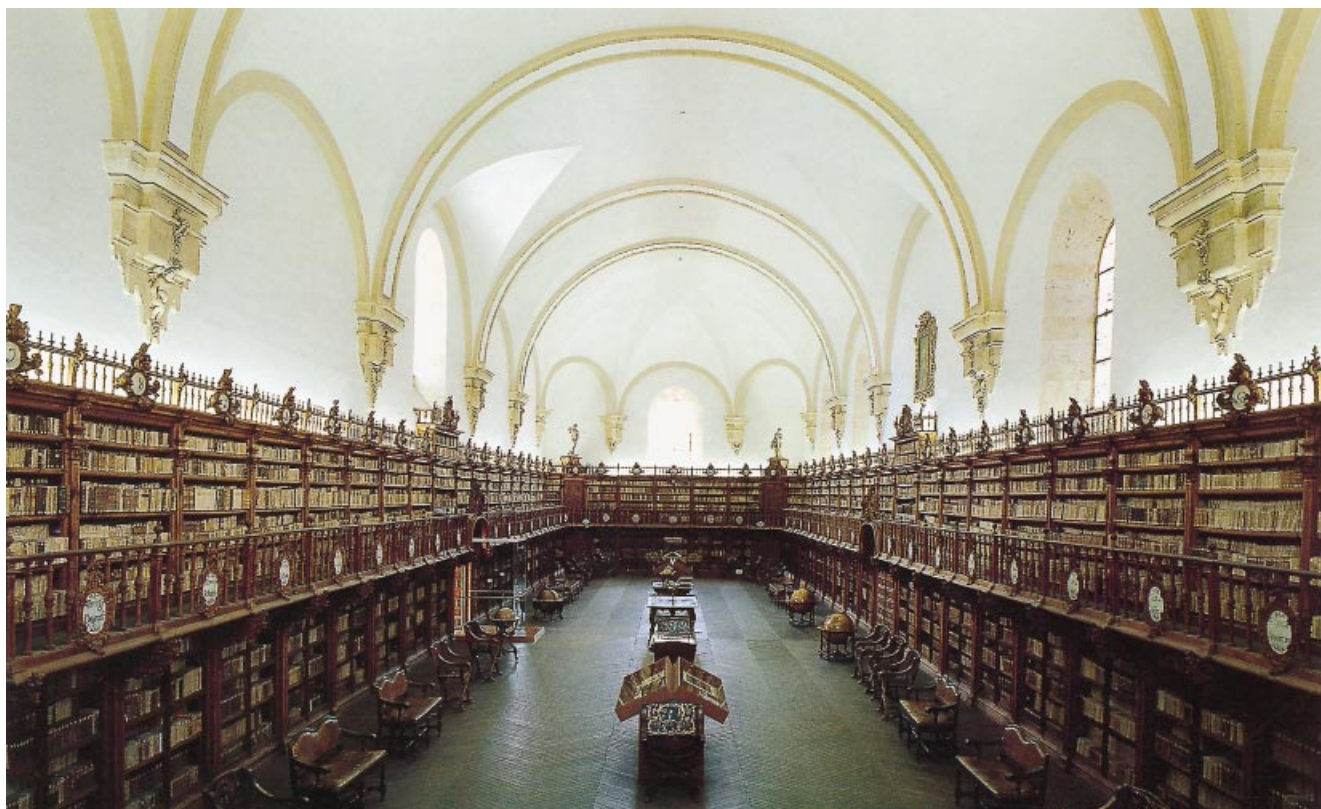


Figure 1. A view of the library of the University of Salamanca, as an example of a “proper” library. (From *Salamanca—Ciudad patrimonio de la humanidad de España*, Artec Impresiones, Segovia, 1998.)

As for the intelligibility of what emerges, notwithstanding the entry to the garden of philosophy it provides for the mystic, this is what Cicero has to say (speaking through the stoic Lucilius Balbus) in *De Natura Deorum*, in the context of arguing with the Epicureans:^[5]

“Hic ego non mirer esse quemquam, qui sibi persuadeat corpora quaedam solida atque individua vi et gravitate ferri mundumque effici ornatissimum et pulcherrimum ex eorum corporum concursione fortuita? Hoc qui existimat fieri potuisse, non intellego, cur non idem putet, si innumerabiles unius et viginti formae litterarum vel aureae vel qualeslibet aliquo coiciantur, posse ex iis in terram excussis annales Enni, ut deinceps legi possint, effici; quod nescio an ne in uno quidem versu possit tantum valere fortuna.”^[*]

Despite this logic, the irrational yet deeply human search for meaning has persisted in seeking inspiration from the combinatorial. The kabbalistic path is a distinguished one. Along it ambles the remarkable Catalan mystic and writer, Ramon Llull (1232–1316), who placed around one wheel nine

absolute principles, around another nine questions (whether? what? of what? why? and so on), nine subjects, nine relative principles, nine virtues, nine subjects, nine vices. Imagine these arranged as concentric wheels, spun. Llull reasoned that all the elements of a rational universe were contained in this, his *Ars Combinatoria*.^[6]

Four hundred years later, two very different people were seduced by the Llull’s vision. One was the remarkable polymath (and alchemist) and Jesuit priest Athanasius Kircher (1601–1680), the other the great philosopher and coinventor of the calculus Gottfried Wilhelm Leibniz (1646–1716). Leibniz wrote a *Dissertatio de Arte Combinatoria*, and in a letter from 1679 he describes the value of his combinatorics:^[7]

“Car mon invention contient l’usage de la raison tout entier, un juge des controverses, un interprete des notions, une balance pour les probabilités, une boussole qvi nous gvidera sur l’ocean des experiences, un inventaire des choses, un tableau des pensées, un microscope pour eplucher les choses presentes, un telescope pour deviner les eloignées, un Calcul general, ...”^[**]

[*] Is it possible for any man to behold these things, and yet imagine that certain solid and individual bodies move by their natural force and gravitation, and that a world so beautifully adorned was made by their fortuitous concurrence? He who believes this, may as well believe, that if a great quantity of the one-and-twenty letters, composed either of gold, or any other matter, were thrown upon the ground, they would fall into such order as legibly to form the Annals of Ennius.

[**] My invention contains the application of all reason, a judgment in each controversy, an analysis of all notions, a valuation of probability, a compass for navigating over the ocean of our experiences, an inventory of all things, a table of all thoughts, a microscope with which to prove the phenomena of the present, and a telescope with which to preview those of the future, a general possibility to calculate everything.

Sounds like one of my theoretical chemistry colleagues... But Leibniz was extravagant only in his claims; he actually made something of them, to our benefit.

Combinatorics was in the air in the 17th century. In 1622 Paul Guldin actually calculated the size of a library that would hold the printed sequences of 23 letters, regardless as to whether they make sense or could be spoken, and in 1636 Marin Mersenne did an analogous computation for musical sequences running over three octaves.^[8]

And it is so easy to make fun of the *Ars combinatoria*. So, Jonathan Swift (1667–1745) does the Ciceronian turn; Captain Gulliver comes to the Grand Academy of Lagado, where he spots:^[9]

“...a frame... twenty-foot square, placed in the middle of a room. The superficies were composed of several bits of wood, about the bigness of a dye, but some larger than others. They were all linked together by slender wires. These bits of wood were covered on every square with paper pasted on them, and on these papers were written all the words of their language... but without any order. The pupils... took each of them hold of an iron handle... and giving them a sudden turn the whole disposition of the words was entirely changed... The professor shewed me several volumes in large folio already collected, of broken sentences, which he intended to piece together, and out of these rich materials to give the world a compleat body of all arts and sciences; which however might be still improved, and much expedited, if the publick would raise a fund for making and employing five hundred such frames in Lagado.”

I cannot pass by the ancient English art of change-ringing of church bells. A complete set of permutations is called a peal; to ring one on twelve bells would take forty years. No claims to deep knowledge in this delightful musical ritual, only a way in to the sublime, in the original sense of the word. Change-ringing has led to an idiom in English, and is central to Dorothy Sayers’ entertaining 1934 novel, *The Nine Tailors*.^[10]

The line continues into the twentieth century; combinatorics continue to fascinate, and not only chemists. In one of Arthur C. Clarke’s striking short stories, *The Nine Billion Names of God*, a Tibetan monastery make a very early use of a computer to list the permuted nine-letter names of God. A small combinatorial set, by today’s standard. When it’s done... well, you’ll have to read the story.^[11]

One of Raymond Queneau’s inventions is a sonnet where each line may be chosen from among ten variants. Meaning remarkably emerges, like function in a tetradecapeptide.^[12]

III

To me there are two possible cultural rationalizations for the word “library” in the context we are looking at. The first is not one combinatorial chemists may like. It is to be found in one of the most remarkable short stories of the great Jorge Luis Borges, *The Library of Babel*. Borges’ protagonist is a man who lives in a phantasmagoric setting.^[13]

“The universe (which others call the Library) is composed of an indefinite and perhaps infinite number of hexagonal galleries... There are five shelves for each of the hexagon’s walls; each shelf contains thirty-five books of uniform format; each book is of four hundred and ten pages; each page, of forty lines, each line, of some eighty letters which are black in color.”

In elegant prose Borges explores the deeply melancholy and delusional side of the optimistic longing in *Sefer Yetzira*. In the ultimately meaningless world of all letter combinations, human beings, destined to seek order, put all hope into a random phrase. It’s the bleakest of libraries.

The second justification is more positive. The DNA sequence of humans, of any organism, now that’s a library, as Eschenmoser reminded me.^[14] The genome is a historical record of evolutionary experiments. Of blind alleys and superb improvisations. The greatest biological tragedy of our time—massive species extinction as a result of human actions—can be seen as a betrayal of our intellect, as a violation of what the second Adam in *Genesis 2:15* was instructed to do, as ... the ultimate book burning. Species extinction is like going into an ancient library (the DNA of a species) and instead of opening the book, or even shelving it, just consigning it to the flames of our greed.

I have digressed, in anger, but I think with cause. The “library” locution for the trial sets of combinatorial chemistry probably originated in the context of DNA sequences.^[15] Not libraries, I would still say.^[16] But bearing a hint of legitimacy by association with the genome.

IV

Why the word “library” to describe a cleverly made assortment of molecules about to be interrogated in some way? Why not “warehouse”, or “supermarket”, or even “lottery”? Could it be for the same reasons scientists gave and still give Greek-origin and Latinate names to concepts, long past the time when Greek and Latin were parts of their education—a reaching for cachet mixed with a soupçon of scholarly pretentiousness? The beauty of human design, wedded to the aleatory wonder of directed free energy, suffices. We don’t need this seeming sophistication.

But we do, did need a word. And for some reason just plain “pool”, a nice neutral word of much currency in the early combinatorial literature, would not do. Never having to struggle with recalcitrant beads or robots, reactions that should go but don’t, I have no right to criticize the choice that was made in the heat of the experimental moment by the protagonists. But it’s still fun to think about what might have been. If Anglo-Saxon linguistic dominance is to be combated, in a friendly way, a friend suggests that we might have co-opted “pot-pourri”. Or “méli-mélo”. Or “olla-podrida”. Or, I would add, “Vivoli”, to celebrate the best selection of ice cream in this world. You could try the pseudopithy modernese of “combiset”, or “diverset”, if only the latter were not already a trademark. If you want to hint at the wealth that’s a

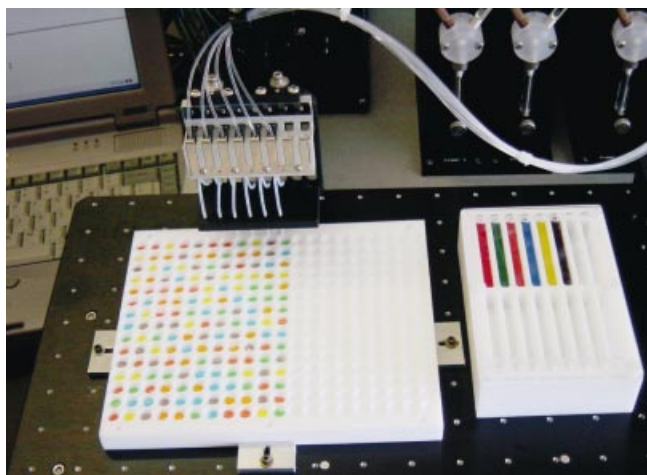


Figure 2. A combinatorial library (with thanks to Professor Selim Senkan, University of California at Los Angeles, for this picture that we found in his review^[17]) has certain similarities to a praline assortment (the picture was supplied by the Confiserie Sprüngli, Switzerland).

twinkle in the eye of some (maybe too many) of those entrepreneurial combinatorial chemists, why not “portfolio”?

I’ve saved my favorites for last. First the simple and lovely international word we owe the French language, and suggested to me by Sylvie Coyaud, “bouquet”. It mixes flowers with a hint of books (bouquins). And finally, the German “Sortiment”, derived from the Italian “sortimento”, and made

English by decapitalizing it. A plain word, assortment, which the Duden dictionary actually defines by a reference to the choice of books in a bookstore! To me the word is hardly prosaic—I think of what is offered to all my chemical senses when I walk into the Confiserie Sprüngli on the Paradeplatz in Zürich. The most luscious of sweet worlds, made by ingenious human beings. A sortiment—waiting to be sampled (Figure 2).

- [1] A. Eschenmoser, in an interview with I. Hargittai, *Chem. Intell.* **2000**, 6(3), 6.
- [2] See, among others: P. Seneci, *Solid-Phase Synthesis and Combinatorial Technologies*, Wiley-Interscience, New York, NY, **2000**; *Molecular Diversity and Combinatorial Chemistry* (Eds.: I. M. Chaiken, K. D. Janda), American Chemical Society, Washington, DC, **1996**; *Combinatorial Chemistry* (Eds.: S. R. Wilson, A. W. Czarnik), Wiley, New York, NY, **1997**.
- [3] *Sefer Yetzirah* (Tr.: A. Kaplan), Samuel Weiser, York Beach, ME, **1997**, 2:2, p. 100.
- [4] *Sefer Yetzirah* (Tr.: A. Kaplan), Samuel Weiser, York Beach, ME, **1997**, 4:16, p. 190.
- [5] M. T. Cicero in *De Natura Deorum/Of the Nature of Gods* (Tr.: C. D. Yonge), Prometheus, Amherst, NY, **1997**, II:37, p. 78.
- [6] See for example: U. Eco, *The Search for a Perfect Language*, Blackwell, Oxford, **1995**, pp. 140–141; R. Llull in *Selected Works of Ramon Llull* (Ed. and Tr.: A. Bonner), Princeton University Press, Princeton, NJ, **1985**. My source here is a paper by Werner Künzel, *The Birth of the Machine: Raymundus Lullus and His Invention*, www.c3.hu/scca/butterfly/Kunzel/synopsis.html.
- [7] G. W. Leibniz, April 1679 letter to Johann Friedrich, the Duke of Hanover, in *Sämtliche Schriften und Briefe*, Akademie-Verlag, Berlin, **1970**, 1st collection (general political and historical exchanges), 2nd vol. (1676–1679), p. 168.
- [8] My source here is secondary, from a remarkable book: U. Eco, *The Search for a Perfect Language*, Blackwell, Oxford, **1995**, pp. 140–141.
- [9] J. Swift, *Gulliver’s Travels into Several Remote Nations of the World, Part III. A Voyage to Laputa, Balnibarbi, Luggnagg, Glubbdubdrib and Japan*, Benjamin Motte, London, **1726**.
- [10] D. L. Sayers, *The Nine Tailors*, Harcourt Brace, New York, NY, **1934**.
- [11] A. C. Clarke, *The Nine Billion Names of God: The Best Short Stories of Arthur C. Clarke*, Harcourt Brace & World, New York, NY, **1967**, pp. 3–11.
- [12] R. Queneau, *Cent mille milliards de poèmes*, Gallimard, Paris, **1963**. A search of websites devoted to this poem reveals some interesting commentary on intellectual property rights—are all of Queneau’s sonnets copyright?
- [13] “The Library of Babel”: J. L. Borges in *Labyrinths: Selected Stories and Other Writing* (Tr.: J. B. Irby), New Directions, New York, NY, **1964**, p. 52.
- [14] A. Eschenmoser, personal communication.
- [15] I would appreciate the readers’ direction to the earliest appearance of the word “library” in the current chemical or biological context.
- [16] This is not the place to trace the roots of the word “library” or “Bibliothek”. A full account would surely fill one, and certainly feature the definitive novel of bibliomania, which happens to be written by a Chemistry Ph.D.: E. Canetti, *Auto-da-Fe* (Tr.: C. V. Wedgwood), Seabury, New York, NY, **1979**; *Die Blendung*, Hauser, München, **1963**.
- [17] S. Senkan, *Angew. Chem.* **2001**, 113, 322–341; *Angew. Chem. Int. Ed.* **2001**, 40, 312–329.

Medicinal Chemistry: Challenges and Opportunities

Günther Wess,* Matthias Urmann, and Birgitt Sickenberger

1. Introduction

There is no doubt that interest in chemistry is declining throughout the world.^[1] It was not without reason that Stephen Lippard felt the need to examine the current status of chemistry and, with the collaboration of numerous colleagues, to formulate 22 objectives of chemistry under the heading "New Frontiers in Basic Chemistry."^[1c] The papers entitled "New Voices in Chemistry," which were published in *Chemical & Engineering News* to mark the 125th anniversary of the American Chemical Society, also describe the search for new areas of activity and challenges.^[2]

In the particular case of medicinal chemistry in the pharmaceutical industry, the developments that have taken place in recent decades are no more encouraging. Driven by the increase in knowledge and in the number of new methods, the role of natural sciences in drug research has been continually changing.^[3, 4] Thus the first era was dominated by organic chemistry, whereas the second was characterized by a more rational approach, in which knowledge about enzymes and receptors developed, and the dialogue between chemists and biologists became more significant. This dialogue seemed to Arthur Kornberg to be so important that he wrote a remarkable article on the subject entitled "The Two Cultures: Chemistry and Biology."^[5] Finally, at the end of the 1980s and in the 1990s, high-throughput technologies, such as high-throughput screening (HTS) and combinatorial chemistry, became established and, together with the rational design of active substances, are now regarded as the major driving forces behind medicinal chemistry.

At present, it seems that biotechnology will be dominating the future of the pharmaceutical industry. It is no longer chemistry based, but is driven instead by knowledge gained in human biology and genetic information. Chemistry apparently no longer has any particular significance.^[6] Most strikingly, it is not even mentioned in many accounts. The papers published in the journal supplement *The Pharmaceutical Century*^[7] and others^[6, 8] confirm this impression, one that is also shared by many outsiders, including consultants, investors, and politicians. They document major advances in biology and engineering, and acknowledge that chemistry has contributed to these developments, but do not regard it as

exerting any major driving force as an independent scientific discipline.

The authors of this paper can understand this view but believe that the pharmaceutical industry cannot look forward to a bright future, unless chemistry develops into a dynamic and innovative scientific discipline again. This is because a lack of new chemical entities (NCEs) is being lamented worldwide, despite the intensive use of high-throughput technologies in drug research, despite new insight in genomic research and, in parallel, despite the progress made in data processing and the opportunities provided by the Internet (digital revolution). Analysts and opinion-leaders speak of an innovation deficit, which has actually worsened in recent years.^[9] We are convinced that chemistry, in particular, has the potential to overcome the innovation deficit.

We substantiate our thesis herein by discussing the critical significance of chemistry in terms of time and success, identifying the current place of chemistry, formulating future challenges, and finally presenting trends and possible solutions. In doing so, we move along the value chain of drug research (Figure 1). There are numerous publications on the topic that we have chosen, but they are seldom comprehensive and often they merely analyze the problem without providing any solutions. We look at solutions. In this context, our views are based on the everyday practice of drug discovery. The focus is on scientific aspects, without claiming to be objective or aiming to establish a benchmark for the pharmaceutical industry.

1.1. Tasks and Bottlenecks in Medicinal Chemistry

The main contributions and tasks of chemistry in pharmaceutical research now consist of:

- the identification of new leads,
- their optimization to clinical candidates, and
- the provision of sufficient amounts of these substances for further studies and for development.

Furthermore, chemistry has an increasing number of valuable contributions to make in other areas, such as in the elucidation of biological mechanisms and in target validation. The rapid progress that has been made in biology, culminating in the sequencing of the human genome,^[10] entails new challenges for chemistry: the aim is to use the newly acquired knowledge about the genome and proteome to develop new forms of treatment. Information about the structures and functions of biological macromolecules and about complex biological processes needs to be transformed to allow molecules to be found that intervene in this complex process (although in this paper we refer solely to small molecules and not to therapeutic proteins, for which the situation is different).

There is currently a bottleneck in the innovation of low-molecular substances: biological research reveals potential

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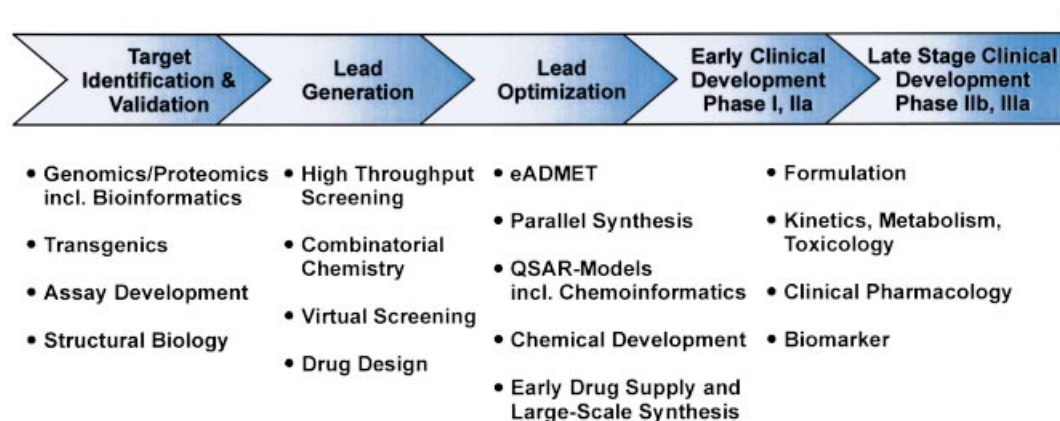


Figure 1. Value chain of drug discovery and selected key technologies/activities. There is an overlap of their activities along the value chain, some might be needed along the entire value chain, for example, Genomics/Proteomics or Biomarker (eADMET = early absorption/distribution/metabolism/excretion/toxicity; QSAR = quantitative SAR).

new approaches for active substances much more quickly than “suitable” molecules or optimized leads can be provided. Even high-throughput technologies such as HTS and combinatorial chemistry have not yet changed this situation. Their use has not yet led to the expected abundance of candidate drugs,^[11] whereby “suitable” also—or rather primarily—means that the substances fulfill a drug profile defined from a medical point of view. It is only by finding such specific molecules or optimized leads that drug research can create real value. In short, chemistry is one of the disciplines at the center of this bottleneck in drug research.

2. Lead Generation

2.1. Biological Mechanisms

The aspects discussed in this section will be considered from the point of view of medicinal chemistry, that is, with regard to the need to find new biological approaches for drugs. The number of these potential biological targets for new drugs, often referred to as “drugable targets,” has until now been based on a rough assumption.^[3] According to Jürgen Drews, there are about 100 different diseases that pose a significant medical problem in industrial nations. Each of these diseases is thought to be influenced by about ten genes. We thus arrive at approximately 1000 disease genes. However, not every disease gene corresponds exactly to one target. Instead, each of these genes leads to a network of protein–protein interactions and is therefore associated with the function of five to ten proteins. This gives a total of between 5000 and 10000 potential drug targets.

The sequencing of the human genome has provided a rough idea of the number of human genes, but has by no means removed all doubts about these estimates. The classification of the genes into families of targets (Figure 2) has not yet provided any major insights either. Functional genome analysis, combined with proteomics, is needed to acquire further knowledge.^[12] However, it is becoming increasingly apparent that the complexity of biological systems lies at the level of the proteins to a much greater extent than was

previously assumed. A new biological target validated in a complex disease process is therefore of very great interest and differs considerably in qualitative terms from unvalidated putative approaches.^[13] There is a great deal of competitive pressure to find substances with which these validated targets can be modulated. However, even for targets that have not yet been well validated, it would be useful to have molecules with which we learn more about the biological function of these targets, that is, which are suitable for their validation. In any case, it is a challenge for chemistry to provide suitable substances.

An example of target identification is illustrated in Figure 3. In an experimental model on endothelial dysfunction, we studied the differential gene expression of endothelial cells from the aortas of rats. In the course of the study, we found approximately 400 differently expressed genes, and these can be assigned to different gene families by using bioinformatic methods. A range of gene products of these new genes were identified as potential targets. We developed corresponding assay systems for these targets to quickly find suitable substances that interact with the proteins expressed by these genes. This in turn provides further information on the significance of these proteins in the pathological process of endothelial dysfunction.

However, a series of examples also shows that chemical compounds and hence potential drugs can activate or suppress a number of genes. It was recently reported that various glitazones, a new class of peroxisome proliferator-activated receptor (PPAR) gamma agonists, which are used as anti-diabetic agents, lead to very different gene expression patterns in some cases, although the observed pharmacological effect on carbohydrate and lipid metabolism is similar.^[14] A detailed analysis of the affected genes reveals the differences between various active substances and hence provides a “fingerprint” of a pharmacon and can explain effects or side effects (often in hindsight for modern pharmacons).

We know from animal experiments that certain substances can influence the expression of several hundred genes. Our ideas about the mechanisms of action of established drugs, such as HMG-CoA reductase inhibitors, are also becoming more specific. Results have now accumulated which indicate

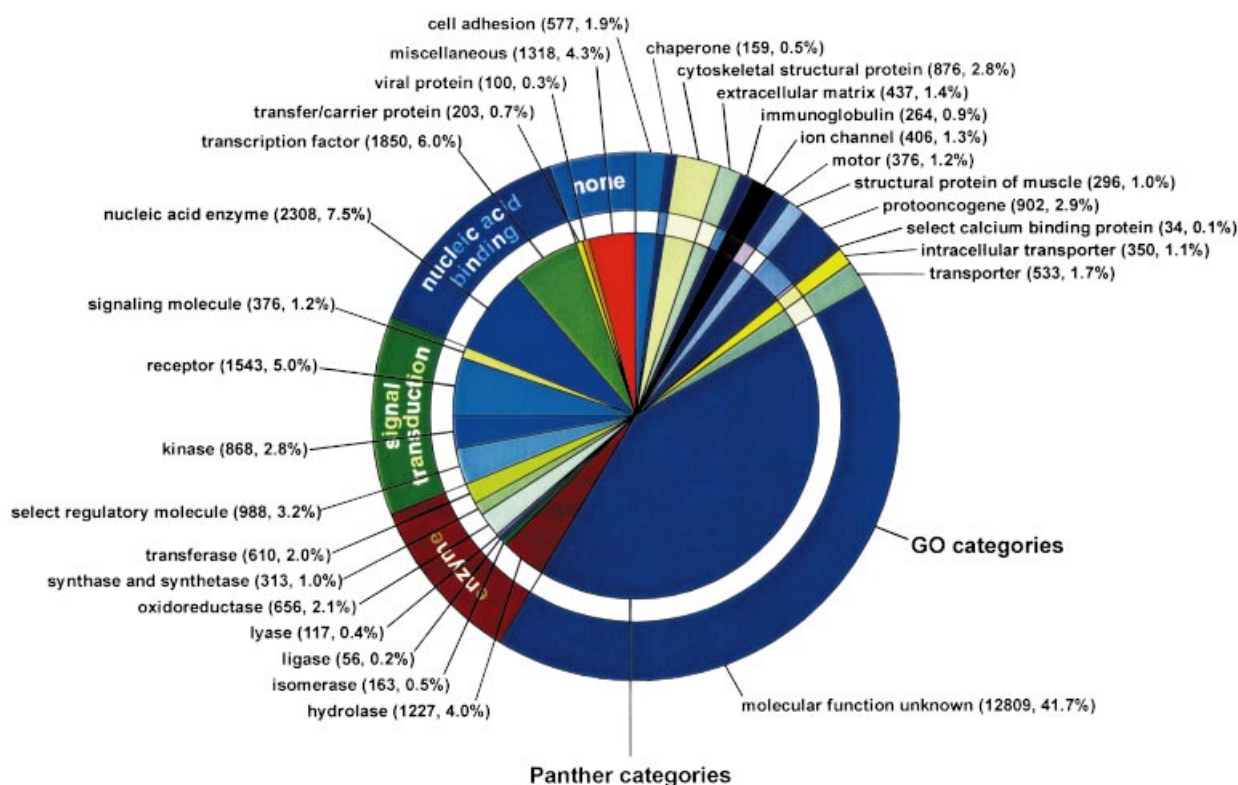


Figure 2. Classification of human genes into target families (reproduced with kind permission from ref. [10b]).

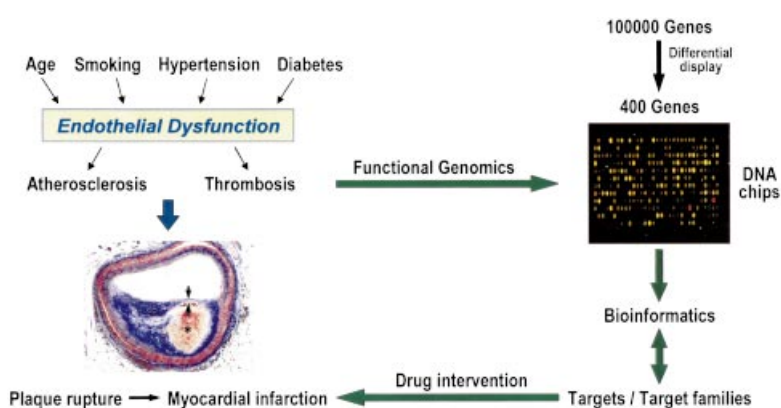


Figure 3. Identification of new targets by differential gene expression analysis.

that the anti-atherosclerotic effect of the statins might be caused by mechanisms that are independent of the cholesterol-lowering effect.^[15]

Our understanding of mechanisms of action is changing. Up to now there has been a great tendency towards monocausal correlations such as “one target, one disease”. How do these ideas need to be modified in the context of complex biological processes? The focus is already shifting from isolated biological systems to a holistic form of systems biology, including the interaction between highly complex networks.^[16] In the search for new substances, new questions arise which chemistry is not currently able to answer. For example: What will future drug profiles need to look like in this context? What requirements will new biological targets need to fulfill to be regarded as validated? What kind of targets will be found by using the new approaches? Could aspirin or β -blockers have

been discovered by using genomic approaches? It can be seen from these examples and questions that reductionist approaches have reached their limits from the systems biology point of view. We need to broaden our approach. In this context, it is vital that chemists and biologists identify and work on joint problems.

Validation involves demonstrating the relevance of a target in a disease process, that is, the question of whether the desired effect on the disease can be achieved by influencing a biological system. Definitive proof of a causal relationship such as this can only be provided by clinical studies on patients. Clearly, it is vital that target validation should already be performed in early phases and repeated in the further course of drug research and development.

Target validation in early phases is based on several criteria, for example, different gene expression in healthy and diseased tissue, functional studies at the protein level, and studies in animal models on the disease-related phenotypes. Gene chips, antisense technologies, ribozymes, neutralizing antibodies, and knockout or transgenic mice are some of the methods and instruments used in this context.

Pharmacogenomic studies can help us to answer these questions.^[17] In conjunction with proteomics, they will provide further clues about the complexity of biological mechanisms

and drug effects. These experiments and their interpretation are highly significant for medicinal chemistry, because they modify the understanding of the previously simplified causal chain and can thus lead to a completely different approach.

2.2. Biological and Chemical Structure Spaces

If the protein binding sites for potential ligands are defined as a biological structure space, the task is to find compounds that are “complementary” to this structure space, that is, to correlate the biological structure space with a chemical structure space (Figure 4). In the early stages of combinatorial chemistry, it was widely held that this could be achieved by using an optimally diverse chemical structure space. Diversity

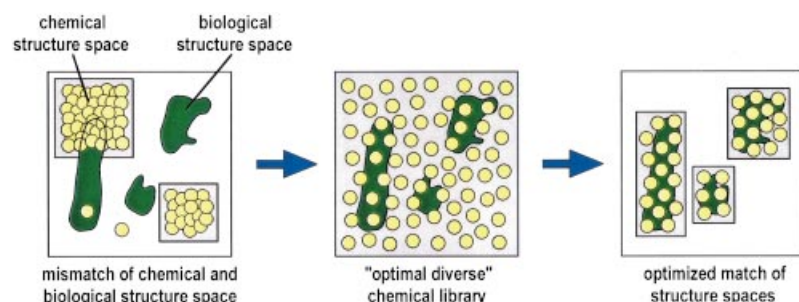


Figure 4. Matching of biological and chemical structure spaces.

was the dominant theme. In the process, the real question was neglected: how biologically relevant is the diversity created in chemistry?^[18] Even the most beautiful molecules synthesized using the most elegant methods are useless if they do not reach a biological target. Even experts fail to agree on how diversity should be defined.^[19] We use the term “diversity” in the sense of structural variety on the basis of molecular scaffolds and substitution patterns.

Despite the sequencing of the human genome, the biological structure space is largely unknown. However, it is clear that it cannot be filled by using the substances that exist today. In theory, it is conceivable that the entire chemical structure space of all the possible drug molecules could be synthesized and hence filled. HTS would then automatically identify the suitable molecules. In practice, this approach is not feasible: theoretical considerations have shown that the universe does not contain enough atoms to synthesize even one copy of every conceivable molecule.^[20]

The only solution is therefore to gather as much knowledge as possible about the biological targets and to use this knowledge to fill the limited structure spaces, for example, of a subclass of a target family, in a targeted manner by using high-throughput technologies or molecular design. This knowledge, which should preferably be based on information of structures and functions, needs to be processed such that molecular design is possible. Not only bioinformatics, cheminformatics and virtual screening technologies, but also molecular design will be the instruments used to generate this knowledge. However, the basic data need to be available first for the aforementioned technologies to have any prospect

of success. It is therefore not surprising that considerable efforts are being undertaken in the field of structural genomics, the systematic investigation of the three-dimensional structure of the gene products.

It can be seen that the chemical structures of new compounds are becoming more complex, larger, and more diverse in the pharmaceutical industry. The requirements for new active substances are now considerably higher. This is true both of efficacy and of absorption, distribution, metabolism, excretion, and toxicity (ADMET). For a long time these aspects were not taken into account until later phases of drug development, but in the meantime they have become increasingly important, even in the lead generation phase, and they too may increase the complexity and size of the compounds. In this context, combinatorial chemistry (see Section 4) is also called upon to generate new chemical scaffolds, in which the provision of enantiomerically pure compounds with one or more stereogenic centers is still underdeveloped. The requirements for the selective design of stereogenic centers are thus growing. All this is happening in the context of growing competition, in which the time factor plays an increasingly important role and the pressure to achieve a higher hit rate is also growing.

In summary, we would like to present the following few theses:

- ◆ the bottleneck in lead generation lies in the provision of new, biologically relevant substances and hence largely in the field of chemistry,
- ◆ the high-throughput technologies have not led to the desired innovation and productivity, and
- ◆ it is only by means of new, knowledge-based approaches in joint efforts undertaken by chemistry and biology that we will be able to “synthesize into” the complexity of biological structure spaces.

3. Lead Optimization

At first glance, the optimization of a lead to form a clinical candidate is not particularly spectacular. However, the fact that little is published on the subject (and that such publications are always very formal) does not mean that this task is trivial. On the contrary, lead structure optimization is the most challenging part of drug research.^[18, 21] Those who master the art are hardly keen to reveal their know-how. This is because in this phase, the object is ultimately to turn a chemical that has attracted attention in an efficacy test into an effective and safe drug, that is, to use structural variations to improve a molecule such that its profile of characteristics fulfills defined criteria for a therapeutic application (Figure 5). Lead optimization thus contributes a great deal towards value creation and offers the best opportunity to set oneself apart from the competition. The parameters that are focused on in this process include potency, selectivity, oral

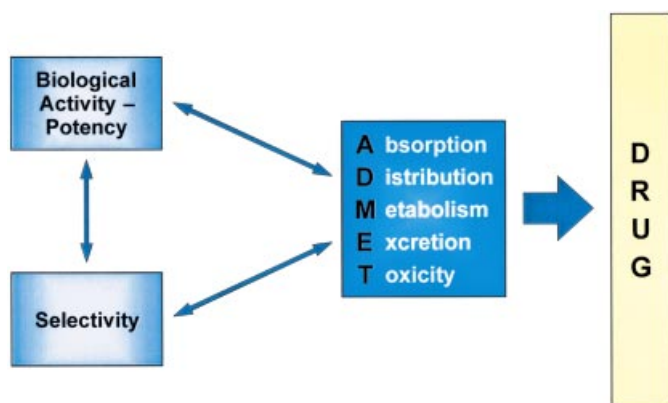


Figure 5. Requirements for the property profile of a drug.

absorption, distribution, metabolism, excretion, toxicity, and certain physical substance characteristics. The more of these that have to be optimized, the more complex the task becomes. The time that is usually required for this process constitutes a further, if not *the* bottleneck.

This is one of the reasons why it is vital to optimize several parameters simultaneously rather than proceeding sequentially. This requires meaningful assay systems and extremely close cooperation with biology to be able to estimate the overall biological profile of a substance as early as possible. On account of such highly complex optimization problems, each project needs its own strategy in which the critical factors for success are clearly defined (Figure 6). High throughput

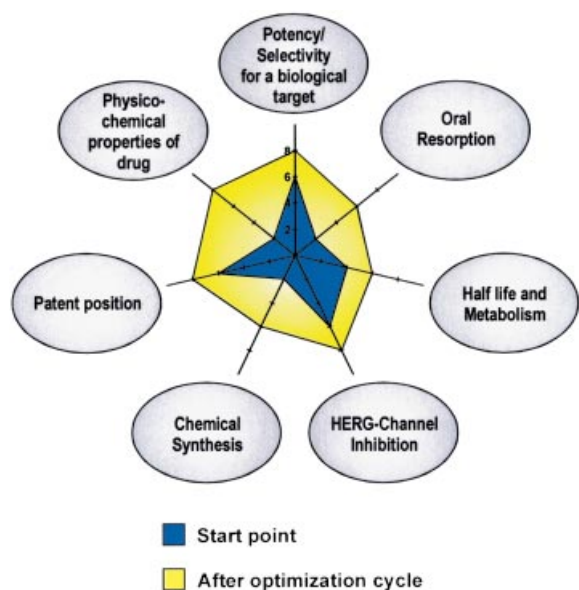


Figure 6. Example of a multiple-parameter optimization of a lead structure.

alone is not enough here. Knowledge about the critical factors for success is decisive. Only knowledge can help to turn high throughput into high output. Fundamental issues in lead optimization are:

- ◆ What biological testing systems are required to select the clinical candidate?

- ◆ What question do we want to answer by using a particular compound and what can we learn from the answer?
- ◆ How many and which compounds need to be synthesized to recognize the potential of a lead?
- ◆ Which structural elements (substructures) determine the profile of biological characteristics?
- ◆ Can the profiles of a class of substances be predicted or extrapolated to other leads?
- ◆ Which synthetic methods yield the highest throughput?

However, intimately linked with these issues is also the question of how to synthesize the substances, particularly in terms of efficiency and timely supply. Only by taking the accessibility of the compounds into account in the design can a lead optimization cycle be successfully completed. In the interaction with lead generation, the creativity and a potential for value creation lie in the discovery of new, biologically relevant structures and new substitution patterns of scaffolds that can solve previously unsolved problems of lead optimization.

Optimization strategies require knowledge management and the combination of a knowledge-based and technology-integrated approach. These variables will be decisive for success in the future. Only by using knowledge-based approaches can skills be developed to predict substance characteristics.

3.1. ADMET Challenges

Many promising projects fail in the lead optimization phase. Compared with other parameters, potency is relatively easy to optimize, and most computer methods are designed to optimize potency. However, potency is just one aspect. Taken as a whole, the crucial aspect is something quite different, namely the identification of the profile of biological characteristics. The greatest hurdles in this context are the ADMET characteristics, particularly absorption, metabolism, and toxicity (AMT).^[18] It is in these areas that many programs show deficits. The predictability of AMT characteristics is not very well developed.^[22] This is all the more surprising because the organism's transporters and metabolizing enzymes "are always the same," whereas the biological targets are constantly changing. The dilemma is highlighted in the field of peptide mimetics, in which suitable substances only became available after years of laborious efforts.

Long before Lipinski et al. 1997 published their "rule of five",^[23] the potential that such principles hold for comparable systems could have become clear. On the basis of experimental and computer methods, Lipinski et al. laid down a set of rules for the absorption of an active substance. According to these rules, poor absorption is likely if

- the active substance contains more than five hydrogen-bond donors,
- the molecular weight is higher than 500,
- the distribution coefficient LogP is more than 5, and
- there are more than ten hydrogen-bond acceptors.

These rules explicitly do not apply to substrates for biological transporters. The "rule of five" is so named because all the classification parameters are multiples of five. Although there are exceptions, the rule has proved to be very

useful, because it provides a very easy way of estimating the absorption of an active substance. In addition, the Lipinski rules addressed a problem that pharmaceutical research had been working on intensively at the beginning of the 1990s: as a result of the transition from *in vivo* to *in vitro* experiments, it was no longer possible to determine the pharmacokinetic profile of an active substance at the same time. Attempts to do so were made by using new approaches, but new predictive models were necessary to steer lead optimization. The “rule of five” was one of the first such models. For example, a fibrinogen-receptor antagonist illustrates this point well (Figure 7). Since then, many other, more sophisticated models have been published, and computer-supported optimization of ADMET parameters has now become a standard part of pharmaceutical research. The challenge is now to extend these rules and to develop further models, for example, for metabolism and toxicity.

The most recent example is the problem of QT lengthening in connection with HERG channel activity:^[24] long QT syndrome is a cardiac muscle repolarization abnormality. Those affected are particularly susceptible to ventricular arrhythmia, which may lead to sudden cardiac death. The molecular cause involves a functional disorder of the potassium channel protein HERG that is based on genetic defects or caused by treatment with medication. The design of active substances that do not or only slightly inhibit the HERG channel is therefore an important criterion in lead optimization. In a very thorough study conducted at Merck, Mitcheson et al. tested the HERG activity of more than 4000 compounds and from the data identified structural motifs that have a negative impact on HERG inhibition.^[24a] Their model aimed to facilitate the design of active substances without this unwanted profile.

A basic issue is the predictive power of the models. This in turn leads to the question of how to make better use of the available information. The demands to be made on chemists and the tasks that lie ahead become very clear here:

- ◆ the lead must be rapidly optimized,
- ◆ the improvement of several parameters must be worked on simultaneously,
- ◆ ecologically and economically acceptable syntheses must be developed, and
- ◆ the ADMET parameters must be optimized and their efficacy increased.

In addition to structure–activity relationships (SAR), structure–absorption–metabolism–toxicity relationships (SAMTR) also have to be determined. Therefore it is clearly a good idea to work with a basis of leads from different structural classes. The simultaneous optimization of several parameters and the discovery of rules on how molecular characteristics can be retained and modified are the most important challenges. The added value is created in a network of parallel activities that is knowledge-based and technology-integrated and that extends right up to clinical studies. Conversely, the results and experience gathered with drugs during therapy are incorporated into the approach. Team work and knowledge management are additional decisive skills.

4. Combinatorial Chemistry

What contribution can combinatorial chemistry make to lead generation and optimization? Where can it help to overcome the bottlenecks? Combinatorial chemistry has now come to the end of its first decade, and there are widely

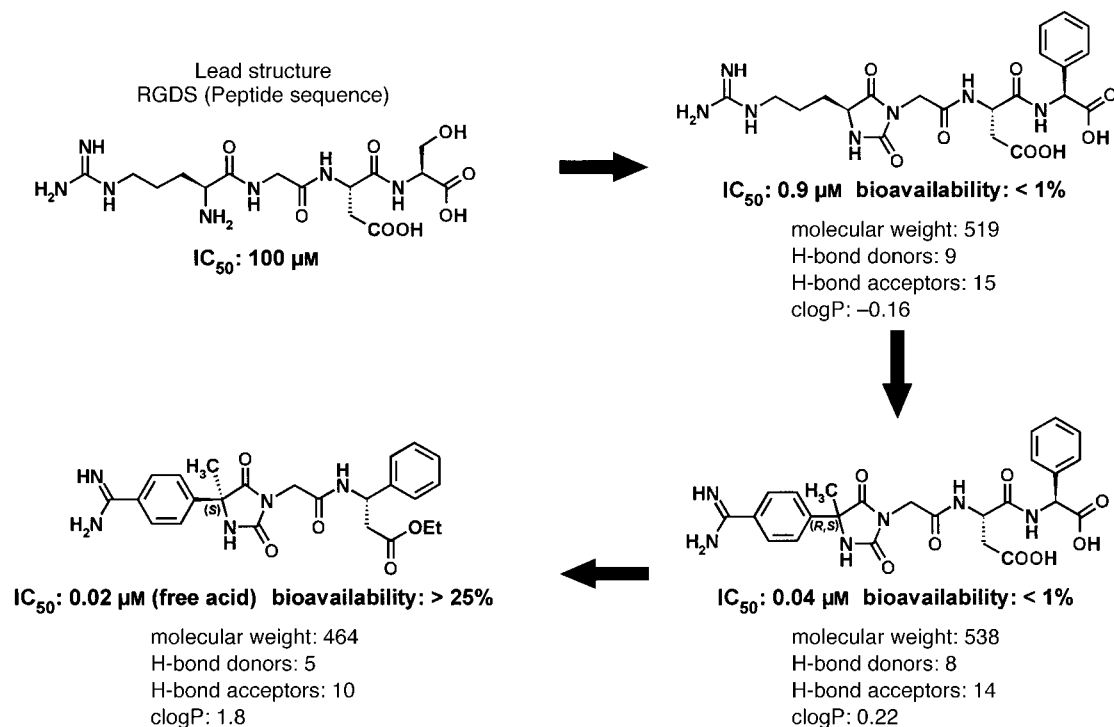


Figure 7. Optimization of bioavailability by using the “rule of five”.

differing views regarding its success.^[25] In the mid-1990s, people were still euphoric. Combinatorial chemistry was seen as a “wonder technology” that would lead to a wealth of new drugs in no time at all and with very little effort. A certain sobriety or even skepticism has widely established itself in the meantime. Many combinatorial chemistry startup firms are in the process of modifying their strategies towards integrated drug discovery and offer services that cover lead generation and optimization.

The term combinatorial chemistry is still used in a very broad sense and covers a range of technologies. The following definition is therefore useful: “Combinatorial Chemistry is the art and science of synthesizing and testing compounds for bioactivity en masse, instead of one by one, the aim being to discover drugs and materials more quickly and inexpensively than was formerly possible”.^[26]

How successful combinatorial chemistry has been thus depends on one's point of view. From a technological point of view, it was a complete success. Firms have introduced parallel syntheses and automated syntheses on a broad basis. Associated with this, productivity increased considerably in terms of the output of compounds. In addition, working with combinatorial chemistry has encouraged ways of thinking towards diversity: considerations on diversity were introduced, and we have broadened our horizon from individual compounds to substance libraries and structure spaces.

It should also be mentioned that *in silico* methods have been developed that can be classified under the heading of “virtual combichem” and that represent virtual screening.

The traditional approach taken by chemistry in drug research was and still is largely dominated by the principle of trial and error. Chemists develop an hypothesis about the structure of a potential drug, synthesize this substance, and have biological tests conducted to determine its efficacy: the hypothesis is confirmed or falsified. In the latter case, the chemist then proposes a new structural hypothesis and synthesizes a new molecule. Statistically, this cycle has to be repeated an average of 10000 times before a new drug is found. A characteristic feature of this approach is that the structure is known before the test. Eschenmoser defines this as “pre-synthesis design”.^[27]

The fascinating thing about the combinatorial principle is that the traditional trial-and-error approach is replaced by the principle of trial and selection. By means of combination (permutation) of the individual components (scaffolds and building blocks), all possible molecules in a substance family (chemotypes) are synthesized simultaneously/in parallel. The active representatives are subsequently selected from this library of compounds by using an assay and their structure is determined. Eschenmoser terms this approach as “post-synthesis discovery.” It follows the principle of evolution, in which the most active representative is selected from a number of compounds (survival of the fittest). The combinatorial selection process thus differs considerably from the traditional optimization cycles.

There is still a great deal of unexploited potential in the optimization of the principle of trial and selection. This is because biological aspects have not been sufficiently integrated in the process up to now. People concentrated on chemical

diversity and failed to take into account the fact that combinatorial libraries are not very diverse, not to mention their biological relevance. However, they do fill the structure space very densely. In addition, the performance capability of the assays was overestimated in terms of the screening of substance mixtures. Ultimately, the characteristics of the assay systems have to determine almost the entire approach in combinatorial chemistry. Only then can the selection process work. The screening of combinatorial libraries has not yet led to the desired success, so why is the basic principle still so fascinating?

Combinatorial processes are at work in all of us. Antibody genes are “combined” by the somatic recombination of the building blocks of the immunoglobulin genes. A limited number of genetic building blocks gives rise to the huge diversity in the humoral immune response. If an antigen comes into contact with an immature B-lymphocyte, the latter begins to mature and proliferate and antibodies are secreted. This is the decisive difference between nature and combinatorial chemistry: antigen contact not only leads to selection, but also to amplification. There are as yet no suitable systems for amplification in the chemistry laboratory. However, it is certainly conceivable that evolutionary systems will exist in the future^[28] that will function in the same way that the immune system does with antibodies. After a selection process, the component that demonstrates the most favorable profile of characteristics will be reproduced.

What, then, of the much-cited change of paradigm that combinatorial chemistry is supposed to have brought about? The authors of this paper believe that the basic principle of combinatorial chemistry has not lost any of its fascination, but that the change of paradigm has yet to take place.

5. Organic Synthesis

The significance of organic synthesis (we use the term “technology of organic synthesis”) is currently completely underestimated. Synthesis is a bottleneck, and the situation is going to get worse. The challenges facing synthesis will increase considerably in the future to meet the requirements of new biological targets and ensure rapid upscaling. The structures are becoming more complex and stereochemically more demanding. New carbogens will be needed,^[29] and the call for diverse, biologically relevant scaffolds has already been mentioned. In international competition, increasingly complex synthetic problems will need to be solved in research, development, and production in shorter and shorter spaces of time.

The increasing use of parallel synthesis and combinatorial synthesis will help to considerably increase productivity, but it will also entail a series of methodological challenges. The latest synthetic methods, for example, catalytic reactions, have to be reproduced on an industrial scale more quickly. Mastering the repertoire of synthetic methods and retrosynthetic analysis to creatively develop new carbogens will basically be a competitive advantage in view of more complex structures and greater diversity. A rapid upscaling ability also constitutes a strategic competitive advantage. The opportuni-

ties offered by outsourcing and the choice of partners will need to take these criteria into account to a much greater extent in future.

The synthetic aspects need to be taken into account right from the beginning of a project. Pharmaceutical science, toxicology, and experiments on chronic effects require multigram amounts of material of outstanding quality. The technology of synthesis, which encompasses the earliest research phases, including combinatorial synthetic methods, is a competitive advantage in terms of time, especially if it is integrated in an overlapping, non-consecutive value chain. Knowledge management, know-how, and efficient partnerships clearly play an important role in this context.

6. Chemical Biology

If medicinal chemistry can apparently no longer meet the requirements of the post-genomic era in the traditional paradigm of drug research, namely those associated with delivering new structures with unique profiles of action within short periods of time, what kind of strategies do we need to address this problem in a sustainable way in the long term? In view of the complex situation at the outset, short-term patent remedies are not possible. The only solution is to use and implement the rapidly developing knowledge about biological principles in a strategic manner. However, this can only be done by using an interdisciplinary approach between chemistry and biology. In the world described by Arthur Kornberg in "The Two Cultures: Chemistry and Biology," the project remains hopeless.

We refer to our strategic approach to solving the problem by the term also used by Schreiber and Nicolaou, "chemical biology,"^[30, 31] which we understand in a very broad sense as the creation of biological response profiles by "small molecules," selected on the basis of what we know about the structures and functions of biological targets. Therefore, biologists and chemists jointly generate knowledge about the structure and function of biological targets, for example, about the requirements of the biological structure spaces of target families, and turn this knowledge into new molecules, which then create the relevant biological responses. The cultural barriers that Kornberg spoke of have disappeared. Working sequences overlap and take place within networks of multiple partnerships. However, several changes in the way that the relevant disciplines see themselves will still be necessary.

Chemistry was traditionally primarily concerned with structure and synthesis, and biology with function. Research into structure–activity relationships was always an interdisciplinary affair and was therefore fairly underdeveloped in view of the actual opportunities. Many of the molecules that are synthesized still depend on the synthetic skills and particular preferences of the chemists or on the historic areas of activity of the firms, and this is reflected in the results of HTS. In

view of the progress made in the field of biology, however, the future lies in a much greater concentration on research into structural and functional relationships. Patent remedies to address this problem are not yet available. Rules need to be found for the design of profiles and a technology-integrated and information-based approach needs to be followed. If all the disciplines work together in a targeted way, a steep learning curve can be achieved.

To avoid misunderstandings, it should be emphasized once again that excellent organic chemists are vital to put the concept of chemical biology into practice. People who can find the best synthetic paths for the necessary molecules within a short period of time, specialists in organic synthesis technology. Something that has long since become common practice in biology will also take place in chemistry: more technology platforms will be set up, for example, organic synthesis or high-throughput structural analysis. In addition, there will also be specialists in chemical biology.

In the picture of chemical biology, the actual added value of drug discovery is created by the identification of structure–function profiles. Here, molecules or even libraries that have to be produced in highly specialized synthetic groups are defined. It might be better to say that the structure spaces that need to be filled are defined. However, we have a long way to go before we can predict the biological response profile of individual molecules. Even in this knowledge-based approach, we cannot do without HTS. The difference is that we use it to work in pre-selected structure spaces.

Two examples will be presented to give a general idea of how such strategies can be applied in practice. In the first case, new ligands had to be found for ion channels as quickly as possible. With the help of these ligands, the biological relevance of new channels was to be further investigated—a type of chemical target validation. However, practically no structural data are available for ion channels. On the basis of the knowledge about the ligands of ion channels identified so far, compound libraries were therefore compiled or newly synthesized. The hit rate in these knowledge-based libraries is many times higher than in HTS. These libraries have already proved to be very valuable for the biological validation of new ion channels (Figure 8).

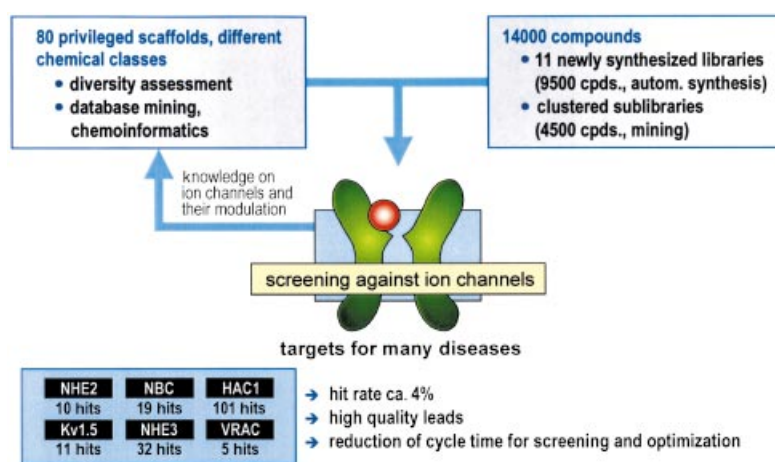


Figure 8. Knowledge-based design of compound libraries for the target family of ion channels.

In the second example, three-dimensional data on representatives of a target family, in this case kinases, and structural data on various inhibitors of this kinase family were available^[33]. HTS had not identified any selective substances for the I_K B-kinases. We therefore compared the structure spaces of the target family and their inhibitors. The result was a high-class selective lead for the I_K B-kinase that could be further optimized (Figure 9).

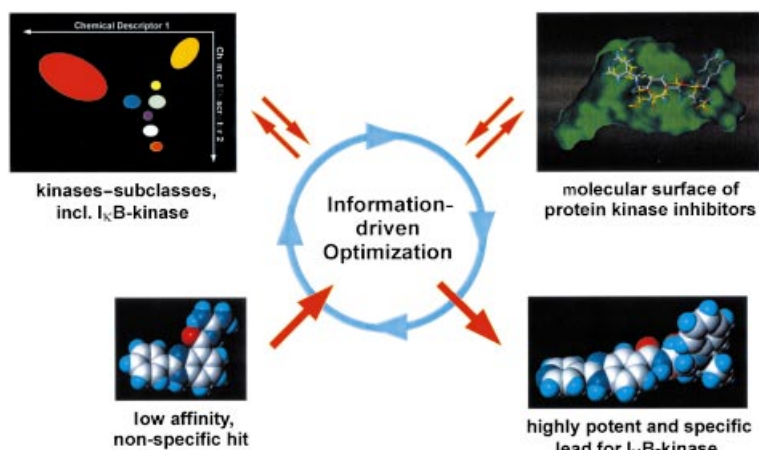


Figure 9. Knowledge based lead structure generation of I_K B-Kinase inhibitors by matching chemical and biological structure spaces.

The tasks that need to be performed by research and management in putting the concept of chemical biology into practice are immense and need to be planned on a long-term basis. Strategies need to be developed and implemented, and the way in which the disciplines involved traditionally see themselves needs to be overcome. The long-term objective is to progress from trial and error to prediction. During this transition, chemical biology will show us the way and help to generate the necessary knowledge. Only knowledge can turn high throughput into high output (Figure 10).

7. Conclusion

In the transition from trial and error to prediction, the number game of the high-throughput systems needs to be

transformed into a quality and strategy game. The transformation of knowledge and the ability to develop learning curves are becoming factors that are critical for success. The greatest demands are made on chemistry in this approach. It can once again be seen to be a decisive force for innovation and value creation. It is doubtful that in future the individual chemist (as a generalist, so to speak) will be able both to master the entire repertoire of organic synthesis and also to

cope with all the other requirements of drug discovery as it is perceived today. We therefore see a need for specialization in two directions: on the one hand, in the direction of organic synthesis technology including combinatorial synthesis techniques, classical organic synthesis to develop new structure types, and scaling up by using the latest synthetic methods, and on the other hand, in the direction of chemical biology with specialists for lead generation and optimization, including a molecular understanding of biological processes and molecular design—in short: drug discovery experts.^[5c,d, 33]

In addition to the broad range of different specialist knowledge that each expert has, there are also a range of skills that both groups require. These include, inter alia, working in teams and networks, including external partners, managing such cooperation, conceptional thinking, implementation of strategies, technology-integrated work, project organization, parallelization of processes, and last but not least, the use of information systems in the broad sense of the term, including the options opened up by the internet. This development has consequences for training and further education at colleges, universities, and in industry. With medical chemists who have this specialization and the combination of excellent specialist knowledge and the aforementioned skills, the vision of the transition from trial and error to prediction can be realized.

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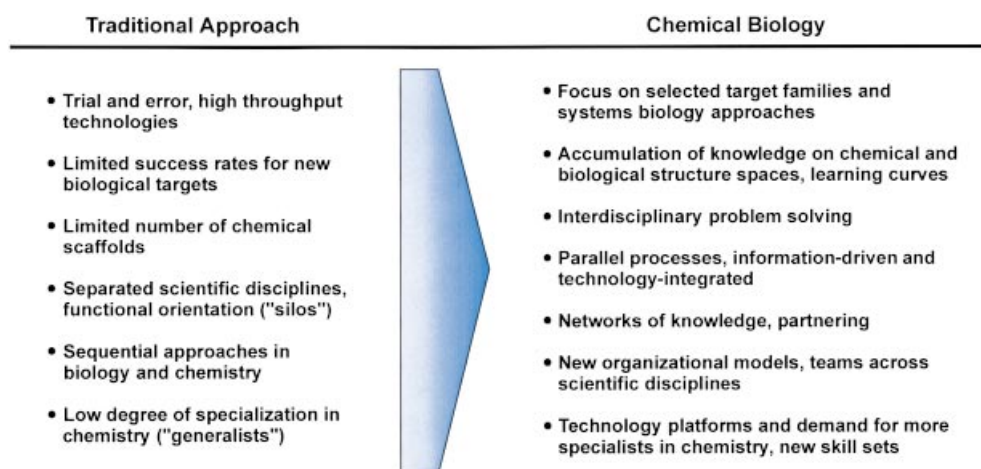


Figure 10. Characteristics of the traditional approach and chemical biology approach.

their help in producing this manuscript but also for their constant support and commitment. Last but not least, we thank all colleagues, too numerous to list, who have given us so much support.

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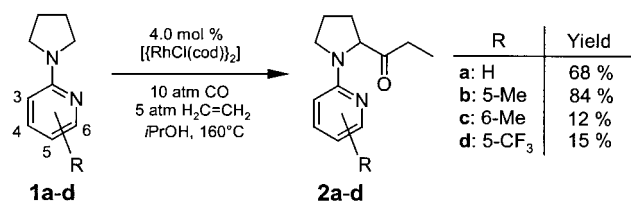
Catalytic C–H Activation of sp^3 C–H Bonds in α -Position to a Nitrogen Atom—Two New Approaches**

Sven Doye*

During the last 10 years, the activation of unreactive bonds by transition metal complexes has developed into a new and independent field of organic chemistry.^[1] Whereas initial investigations centered on stoichiometric transformations, the major interest is now focused on the development of procedures for the catalytic activation of unreactive bonds. Among such processes, C–C bond forming reactions involving selective activation of C–H bonds, which cannot be cleaved easily under classical reaction conditions, are particularly challenging. However, in contrast to corresponding reactions of sp^2 C–H bonds only a few transformations that involve the cleavage of sp^3 C–H bonds have been reported. Such transformations are especially interesting when the C–H bond that undergoes reaction is in α -position to nitrogen or oxygen atoms,^[2] because important building blocks for organic synthesis can be formed in a single step using this approach.

Recently, two new and promising catalytic procedures for the catalytic activation of sp^3 C–H bonds adjacent to a nitrogen atom have been published by Murai et al.^[3] and Ishii et al.^[4]

The procedure developed by Murai et al.^[3] offers the possibility of $[(\text{RhCl}(\text{cod}))_2]$ -catalyzed, regioselective carbonylation of several tertiary amines in the α -position to the nitrogen atom. Unfortunately, the optimized reaction conditions (4.0 mol % $[(\text{RhCl}(\text{cod}))_2]$, 2-propanol, 160 °C, 10 atm CO, 5 atm ethylene, 40–60 h) are relatively harsh (Scheme 1).



Scheme 1. Carbonylation of various pyrrolidine derivatives developed by Murai et al.^[3]

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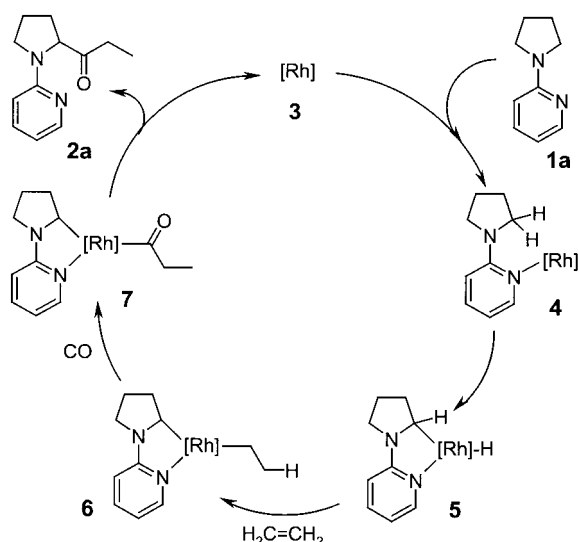
However, the fact that the formed products possess significant similarity to α -amino acids is noteworthy to mention.

In addition, it is remarkable that the “correct” rhodium complex as catalyst and the solvent (2-propanol) are important for a successful reaction. Previous studies by the same group showed that under comparable reaction conditions the carbonylation of piperazine derivatives performed in toluene in the presence of a catalytic amount of $[\text{Rh}_4(\text{CO})_{12}]$ takes place via an initial dehydrogenation to tetrahydropyrazine derivatives. Subsequently, the activation of a resulting sp^2 C–H bond leads to the carbonylated and finally isolated tetrahydropyrazine products.^[5]

As in the case of known $[\text{Ru}_3(\text{CO})_{12}]$ -catalyzed carbonylation reactions^[6] or the procedure developed in 1998 by Jun et al. for the catalytic alkylation of benzylamines^[2b] it is essential for the developed reaction to proceed that a directing group, for example a suitable pyridine substituent, is located close to the reaction center in the substrate.

A detailed investigation of the behavior of several pyrrolidine derivatives (**1a–d**) under the reaction conditions showed that the electronic and steric properties of the directing pyridine substituent, which is in this case directly bound to the amine nitrogen atom, has a significant influence on the reactivity of the substrate. The reaction of **1b**, which bears a relatively electron-rich 5-methylpyridine substituent, takes place with a much higher yield than reactions of substrates possessing a sterically more demanding 6-methylpyridine (**1c**) or an electron-deficient 5-trifluoromethylpyridine (**1d**) substituent (Scheme 1). This finding as well as the fact that the carbonylation takes place at a C–H bond located next to the pyridine substituent, with no other regioisomeric products being observed, leads to the assumption that a coordination of the catalytically active rhodium species to the nitrogen atom of the pyridine ring is an essential step in the catalytic cycle. A corresponding mechanism, which has not been verified by experimental data yet but was proposed by the authors, is shown in Scheme 2.

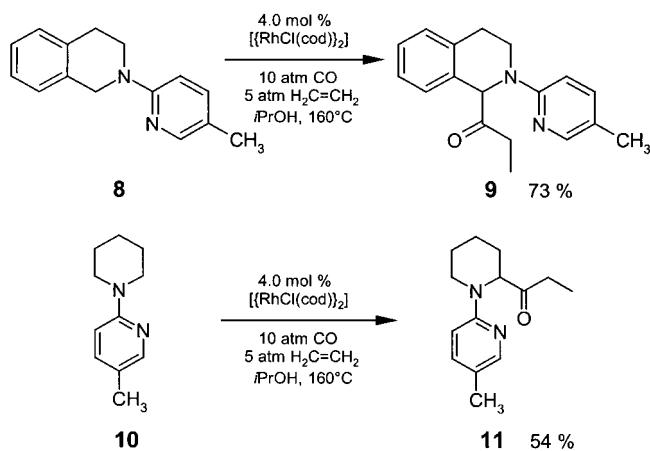
In this mechanism, the catalytically active rhodium species **3** is initially coordinated to the pyridine nitrogen atom to give complex **4**. The rhodium center in **4** can insert into the neighboring C–H bond that is located in an α -position to the amine nitrogen. The insertion of ethylene into the Rh–H bond in **5** (to give **6**) followed by CO insertion provides access to the rhodium acyl complex **7**. Reductive elimination of the



Scheme 2. Proposed mechanism for the $[[\text{RhCl}(\text{cod})]_2]$ -catalyzed carbonylation of amines.

product **2a** finally leads to the regeneration of the catalytically active species **3**.

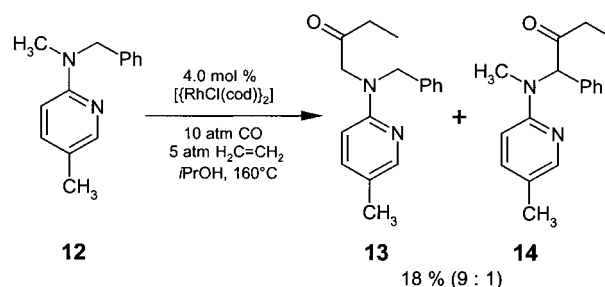
Experiments to transfer the rhodium-catalyzed carbonylation to cyclic amines other than pyrrolidine derivatives were only successful for compounds **8** and **10**, both of which bear the well-suited 5-methylpyridine substituent. Interestingly, the carbonylation reaction of the unsymmetrical amine **8** takes place at the benzylic position to give **9** exclusively (Scheme 3).



Scheme 3. $[[\text{RhCl}(\text{cod})]_2]$ -catalyzed carbonylation of cyclic amines.

Finally, the authors also present the first successful example for the carbonylation of an acyclic amine. In contrast to the mentioned reaction of **8**, the acyclic amine **12** is converted into a mixture of two regioisomers (Scheme 4), of which that carbonylated at the benzylic position is the minor product (**14**). Furthermore, the obtained yield (18%) is lower than that for reactions of cyclic amines.

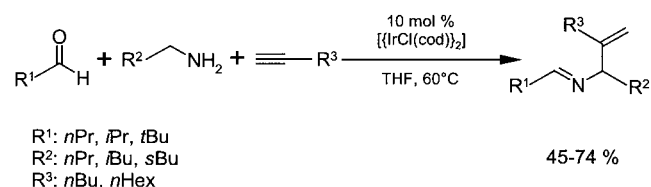
Given the scope and limitations of the described carbonylation procedure, Murai's newly developed process still requires significant work before it can be considered as a widely applicable synthetic method. Nevertheless, the results



Scheme 4. $[[\text{RhCl}(\text{cod})]_2]$ -catalyzed carbonylation of an acyclic amine.

presented will most probably be the starting point for extensive optimization studies which may finally result in the successful development of a highly elegant and maybe enantioselective method for the synthesis of α -functionalized amines.

The second study published later by Ishii et al.^[4] describes a three-component coupling reaction of aldehydes, primary amines, and terminal alkynes in the presence of a catalytic amount of $[[\text{IrCl}(\text{cod})]_2]$ (10 mol%) to give allylic imines under mild reaction conditions (THF, 60°C) (Scheme 5).



Scheme 5. Three-component coupling reaction of amines, aldehydes, and alkynes developed by Ishii et al.^[4]

The authors found that the reaction is initiated by the reaction of the aldehyde with the amine to give the corresponding imine, followed by coordination of the catalytically active Ir^{I} species to the imine-nitrogen atom. Then, the Ir^{I} species inserts into the C–H bond adjacent to the nitrogen atom to give an Ir^{III} –H complex, which can subsequently insert the terminal alkyne and reductively eliminate the product.

Impressively, sterically less demanding aldehydes (1-butanal) as well as sterically demanding aldehydes (2-methylpropanal, 2,2-dimethylpropanal) can be coupled with good yields. Furthermore, *n*-alkyl- as well as *sec*-alkylamines can be employed in the reaction successfully, whereas *tert*-butylamine was found to be a poor substrate. In the case of alkynes, the reaction is limited to the use of terminal alkynes, since internal alkynes do not react.

In summary, both developed procedures clearly demonstrate that a selective, catalytic activation of sp^3 C–H bonds in α -position to a nitrogen atom is also possible by the use of rhodium and iridium catalysts. Therefore, the presented results impressively expand this relatively new and elegant synthetic approach towards the synthesis of complex amines. However, we will have to wait for the results of future optimization studies to find out whether the presented new procedures can be sufficiently optimized for a broad application in research and the industrial production of amine derivatives.

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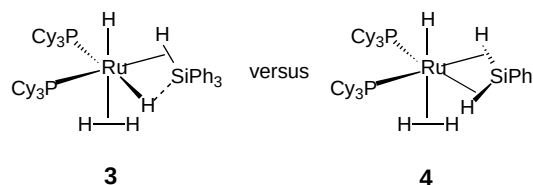
Going Beyond σ Complexation: Nonclassical Interligand Interactions of Silyl Groups with Two and More Hydrides**

Georgii I. Nikonov*

Transition metal silane σ complexes (**1**) are a well-documented class of nonclassical compounds in which the Si–H bond is considered to be at an intermediate stage in the oxidative addition to a metal center and a direct interaction between the silyl and hydride ligands is present.^[1] The question as to whether the silyl ligand can interact simultaneously with more than one hydride has recently received a positive answer.

In 1990 the possibility that a silyl group could interact simultaneously with two hydride ligands was proposed for the first time by Crabtree and colleagues to describe the structure of the compound $[(PPh_3)_2ReH_6(SiPh_3)]$.^[2] The presence of a $[H_2SiPh_3]^-$ ligand was postulated but the experimental evidence was scarce. Further progress in the identification of multicenter Si–H interactions came from the studies of other polyhydride diphosphane systems. The ruthenium fragment $[(PCy_3)_2RuH_2]$ (Cy = cyclohexyl) was shown to stabilize a variety of dihydrogen and silane σ complexes,^[3] a useful entry into this chemistry being Chaudret's bis(dihydrogen) complex $[(PCy_3)_2Ru(H_2)_2H_2]$ (**2**).^[4] The research group of Sabo-Etienne and Chaudret showed that a reaction of **2** with $HSiPh_3$ affords the compound $[(\eta^2-H_2)(PCy_3)_2RuH_2(\eta^2-HSiPh_3)]$ (**3**)^[5] which was originally formulated as a mixed dihydrogen and silane σ complex. However, its surprising structural features suggest an unusual type of interligand interaction. Firstly, in this complex the very bulky phosphane ligands unexpectedly occupy *cis* rather than the anticipated *trans* positions. Secondly, although the phosphanes lie *trans* to two supposedly different ligands, namely $\eta^2-HSiPh_3$ and hydride, the observed Ru–P bond lengths do not differ by

very much (2.392(2) and 2.406(2) Å). Moreover, density functional theory (DFT) calculations on a model complex with much less sterically demanding phosphanes PH_3 ($[(\eta^2-H_2)(PH_3)_2RuH_2(\eta^2-HSiPh_3)]$) show that the Ru–P bond lengths are also almost identical (2.370 and 2.367 Å), which suggests that an electronic factor is in operation.^[5] Finally, the X-ray and DFT-calculated structures of **3** show that the silicon



atom is bound almost equivalently to two hydride units (1.72(3) and 1.83(3) Å observed versus 1.946 and 2.071 Å calculated values) rather than one hydride as the silane σ -complexation model would imply; the distance to the third hydride is much longer (X-ray: 2.40(3) Å, DFT: 2.116 Å). Therefore, **3** contains no more than one classical hydride, the other two are involved in nonclassical bonding to the silyl.

The unusual structural features of **3** were originally attributed to weak bonding between the hydride and silicon ligands which was regarded as “attractive nonbonded interactions”.^[5] While the nature of these interactions was not clearly defined, several bonding schemes were possible. For example, this unusual bonding between the silyl and hydride groups can be described in terms of an interaction between the fragment $[(PCy_3)_2RuH_2]^+$ and the dihydrosilyl ion $[H_2SiPh_3]^-$ (Figure 1), which leads to the nonclassical complex $[(\eta^2-H_2)(PCy_3)_2RuH(\eta^3-H_2SiPh_3)]$ (**4**), rather than the bonding model originally proposed in **3**. The molecular levels of $[H_2SiPh_3]^-$, analogous to the familiar $[H_3]^-$ ion, are shown on the right of Figure 1. Complexation of the $[H_2SiPh_3]^-$ ion to a metal center occurs by electron-density transfer from the orbitals ψ_1 and ψ_2 to metal-centered nonbonding orbitals of symmetry a and b, and back donation of the metal lone pair into ψ_3 . This bonding scheme strongly resembles synergic

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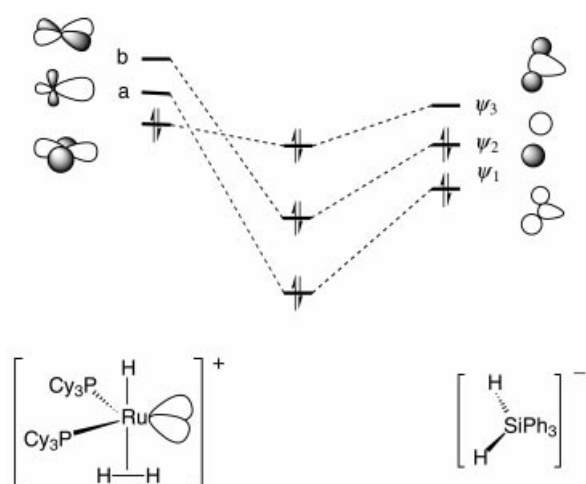
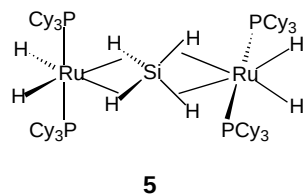


Figure 1. Qualitative fragment MO interaction diagram for the complexation of $[\text{H}_2\text{SiR}_3]^-$ to $[(\eta^2\text{-H}_2)(\text{PCy}_3)_2\text{RuH}]^+$ to give **4**.

donation/back-donation interactions responsible for the complexation of σ bonds to metals and clearly implies bonding between the silyl and hydrides. Formation of the $[\text{H}_2\text{SiPh}_3]^-$ ligand within the ruthenium coordination sphere allows **4** to avoid an unfavorable situation when ligands with a strong *trans* influence are in mutually *trans* positions. In **4** the hydride, the ligand with the strongest *trans* influence, is *trans* to the weakest σ donor—dihydrogen—whereas other ligands with strong *trans* influence—the phosphanes—lie *trans* to the $[\text{H}_2\text{SiPh}_3]^-$ ion. The absence of the Si–H interaction and any other arrangement of the ligands would bring two or more ligands with strong *trans* influence into positions *trans* to each other. In addition, formation of the Si–H bond compensates for the weakening of the Ru–H bond. The driving force for the formation of nonclassical complex **4** is the poor back donation from the metal d level into the $(\text{H-Si-H})^*$ antibonding orbital (it is this effect which is responsible for the whole range of silane and dihydrogen σ complexes formed with this system) and optimization of the ligand-to-metal bonding. Similar “secondary” Si–H interactions were identified in the chelate bis(silane) complexes $[(\eta^4\text{-(H-SiR}_2)_2\text{X})\text{Ru}(\text{PCy}_3)_2\text{H}_2]$ (X = bridging ligand) reported by the same group.^[3c]

Another type of multicenter H–Si–H interaction was found in a dimetallic ruthenium complex $[(\text{PCy}_3)_2\text{RuH}_2]_2(\eta^3, \eta^3, \mu\text{-SiH}_4)$ (**5**) obtained serendipitously by the reaction of **2** with H_2SiMePh .^[6] This compound has a tetra- $(\sigma\text{-Si-H})$ -complexed SiH_4 group, which bridges two ruthenium moieties, and is the first example of a complex in which two H–Si

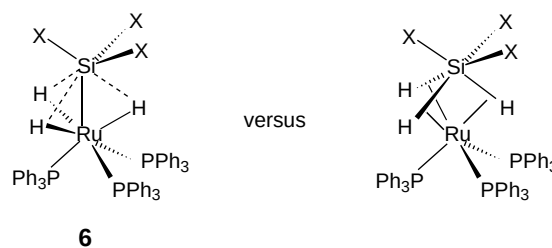


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bonds originating from the same silane are coordinated to one metal center, two pairs of the silane Si–H bonds are coordinated to each of the two ruthenium atoms. Two Si–H bonds and an Ru center lie in the same plane as observed in **4**. However, the essential difference between **5** and **4** is that the $\eta^3\text{-SiH}_4$ ligand is neutral and, therefore, in going from **4** to **5** the $\eta^2\text{-H}_2$ ligand must be replaced with a hydride. In **5**, each

Ru center is pseudo-octahedral, with the $\eta^3\text{-SiH}_4$ ligand occupying one of the equatorial edges, analogous to the situation in **4**. The weakest *trans*-influence ligands to be positioned *trans* to the hydride groups are now the Si–H bonds of the $\eta^3\text{-SiH}_4$ ligand. Phosphanes, which have a weaker *trans* influence than hydrides, occupy the apical sites. Again, as in **4**, the metal–ligand bonding is perfectly optimized. Neglecting the ruthenium centers, the silicon atom in **5** has only four substituents in contrast to the five groups in **4**. Therefore the Si–H bonding in **4** and **5** is different: in **5** it does not differ much from that in the bis(silane) σ complexes, whereas in **4** the bonding of the silicon center to the hydrides is in a sense hypervalent.

A unique case of interligand interaction between one silyl and three hydride groups was discovered by Hübner and Roper and co-workers for the complexes $[(\text{PPh}_3)_3\text{M}(\text{SiPyr}_3\text{H}_3)]$ (**6**; $\text{M} = \text{Ru, Os}$) bearing three electron-withdrawing pyrrolyl (Pyr) substituents on the silicon center.^[7] Very high Si,H coupling constant of 47.4 Hz was observed in



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the ^{29}Si NMR spectra of the Ru compound (29.2 Hz for Os). Low-temperature NMR experiments revealed no temperature dependence of the spectrum and the absence of fluxionality down to -85°C . It appears unlikely that a fast equilibrium between two classical hydrides and one η^2 -silane ligand, equalizing the three hydrides on the NMR time scale, could exist at such a low temperature. Natural bond order (NBO) analysis suggested that Si–H interactions in **6** stem from electron-density donation from the M–H bonding orbitals into the $(\text{Si-M})^*$ antibonding orbital.^[7] The main disadvantage of this bonding scheme is that it cannot explain the strong dependence of the molecular parameters of **6** on the value of the H–Si–Pyr angle. DFT calculations show that rotation of the silyl group around the Si–M axis, which breaks the optimal *trans* position of the hydride and the pyrrol with regard to the silicon atom, is a very unfavorable process.^[7] In contrast, the proposed M–H $\rightarrow (\text{Si-M})^*$ donation must be unaffected to rotation because of the cylindrical symmetry of the M–Si bond.

NBO analysis of **6** also identified another type of nonclassical Si–H interaction which arose from electron-density transfer from the M–H bonding orbital into the $(\text{Si-N})^*$ antibonding orbital.^[7] This bonding is analogous to the interligand hypervalent interaction (IHI), $\text{H}\cdots\text{Si-X}$ (where X = halide), first discovered in basic transition metal hydrides having halosilyl groups SiR_2X in a position *cis* to the M–H bond.^[8] Noteworthy is that a sharp rise in energy upon rotation of the SiPyr_3 group in **6** is accompanied by significant lengthening of the M–Si bond, consistent with the loss the IHI stabilization. The *trans* positions of the H and X ($\text{X} = \text{N}$,

halogen etc) groups relative to the Si atom is a prerequisite for IHI and, therefore, the occurrence of IHI in this system can, in principle, explain the dependence of the energy on the rotation of the silyl group. However, the presence of IHI requires the ruthenium center in **6** to be in the less favorable oxidation state (+IV).

An alternative approach to rationalize the Si–H bonding in **6** is to consider the interaction of the complex $[(R_3P)_3M]^{2+}$ with the fragment $\{H_3SiR_3\}^{2-}$. The qualitative molecular orbitals (MOs) of the latter can be easily obtained by mixing MOs of the closed form of trihydrogen ion $[H_3]^-$ with the MO of the silyl ion $[SiR_3]^-$ (Figure 2). Since the silyl hybrid orbital

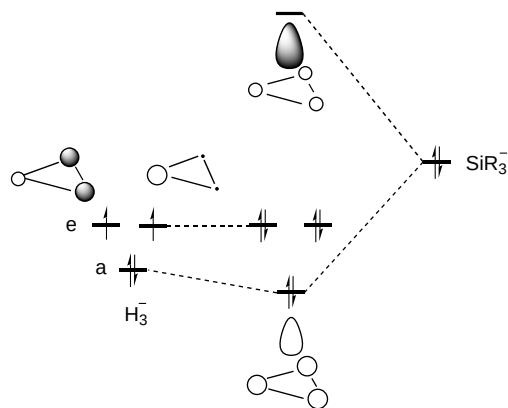


Figure 2. Formation of $\{H_3SiR_3\}^{2-}$ upon interaction of $[H_3]^-$ with $[SiR_3]^-$.

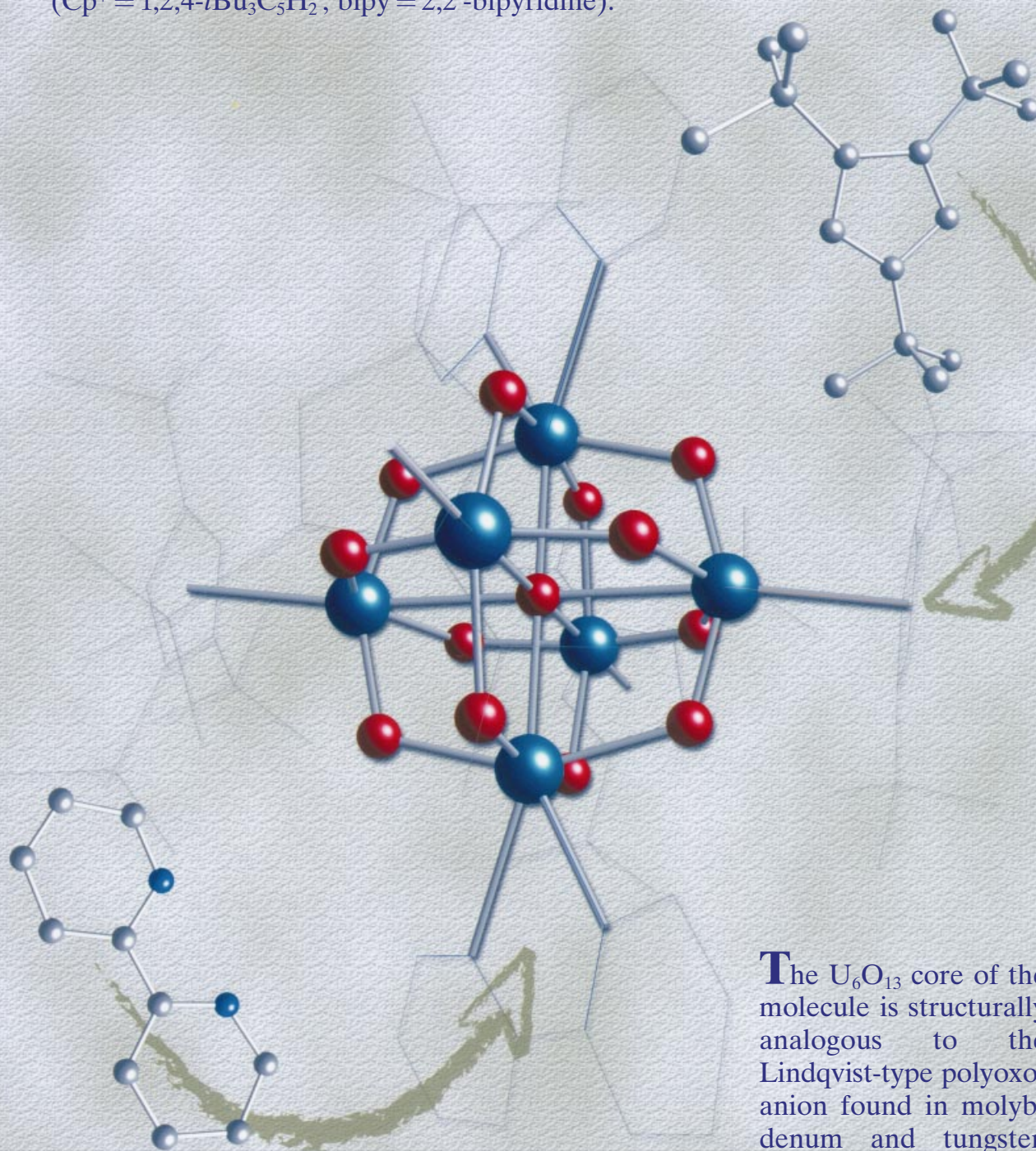
lies at higher energy than the combination of s orbitals of hydrogens (at large H–H separations three levels of $[H_3]^-$ do not differ much in energy from the 1s orbital of hydrogen atom), orbital stabilization can be seen. Further stabilization of this doubly charged unit occurs upon coordination to the cationic osmium (ruthenium) complex, where the a and e levels find perfect matches. The Si–H interaction in **6** will be retained provided the back donation from the metal to the $(Si-H_3)^*$ antibonding orbital is not complete. This descrip-

tion implies that in the resultant neutral compound the oxidation state of the metal is intermediate between II and IV (a classical trihydridosilyl structure would imply Os^{IV}) and the proposed $\{\eta^4-H_3SiR_3\}^{2-}$ ligand is a relative of the η^2-HSiR_3 and $[\eta^3-H_2SiR_3]^-$ ligands discussed above. Since the silicon atom in $\{H_3SiR_3\}^{2-}$ is hypervalent the reason for the *trans* disposition of the substituents at Si becomes clear.

These recent examples of $(H)_2-Si$ and $(H)_3-Si$ interactions show that studying nonclassical Si–H interactions now goes beyond the more usual silane Si–H σ complexes. Other types of multicenter Si–H interactions such as Si_3-H are feasible and offer a challenge for both the synthetic and theoretical chemists.

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Oxidation of low-valent uranium precursors bearing bulky cyclopentadienyl ancillary ligands leads to the isolation of the complex $[\text{Cp}^{\pm}_4\text{U}_6\text{O}_{13}(\text{bipy})_2]$ ($\text{Cp}^{\pm} = 1,2,4\text{-}t\text{Bu}_3\text{C}_5\text{H}_2$; $\text{bipy} = 2,2'\text{-bipyridine}$).

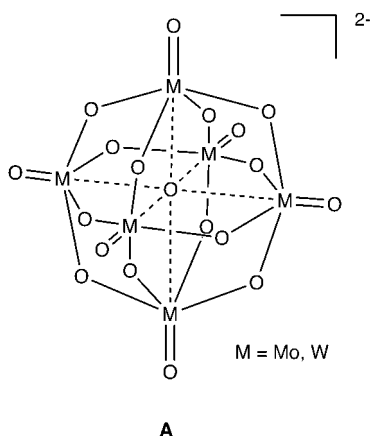


The U_6O_{13} core of the molecule is structurally analogous to the Lindqvist-type polyoxoanion found in molybdenum and tungsten chemistry, and provides a long-missing link between the structural chemistry of uranium and that of its Group 6 brethren. For more information see the following pages.

Synthesis and Structural Characterization of the First Uranium Cluster Containing an Isopolyoxometalate Core**

Paul B. Duval, Carol J. Burns,* David L. Clark,*
David E. Morris, Brian L. Scott, Joe D. Thompson,
Evan L. Werkema, Li Jia, and Richard A. Andersen

Polyoxometalate ions of the general formula $[M_xO_y]^{z-}$ ($M = Mo, W$; less commonly V, Nb, Ta) form a large and diverse class of inorganic clusters that are of broad interest due to the unique bonding and structures associated with the cluster framework, the electrochemical and magnetic properties of the reduced heteropoly blue ions, and because of the resemblance of these species to metal oxide surfaces utilized in heterogeneous catalysis.^[1–3] Applications of these clusters have been directed at oxidation catalysts, medical sensors, and solid-state devices. Although cluster topologies may vary, each member within this class exhibits uniform structural features in which MO_6 octahedra are linked by shared vertices or edges (μ_2-O), but not by faces (μ_3-O).^[4] One of the prototypes is the octahedral $[M_6O_{19}]^{2-}$ unit (**A**), or Lindqvist structure, which contains an $[M_6O_{13}]$ cluster core with each metal also coordinated by a terminal oxo ligand.



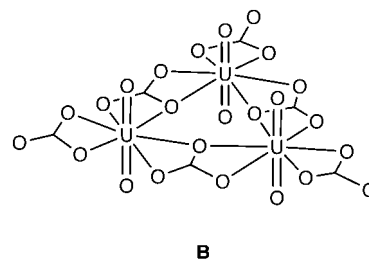
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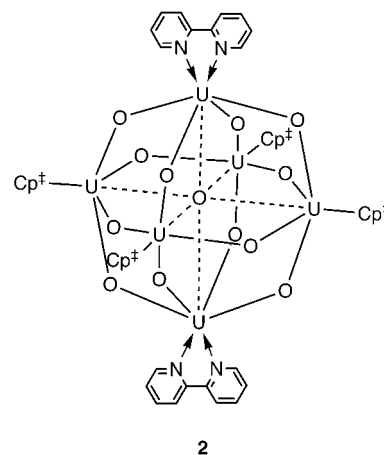
Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

Although a varied assortment of isopolyoxometalate clusters have been synthesized since the 19th century, all examples have been restricted to the Group 5 and 6 metals listed above, with Mo and W comprising the vast majority of derivatives. For potential extension of this chemistry to other elements, uranium may initially seem a logical candidate based on comparable chemical properties to Mo and W, and has been incorporated into heteropolyanions as either U^{IV} ions^[5] or uranyl (UO_2^{2+}) ions,^[6] but the structural requirements for cluster formation preclude a traditional *aqueous* synthesis of a homoleptic isopolyoxouranate species. Uranium(vi) does in fact share with Mo^{VI} and W^{VI} the strong $M=O$ π bonding necessary to terminate a structure, but as shown in **B**^[7] the



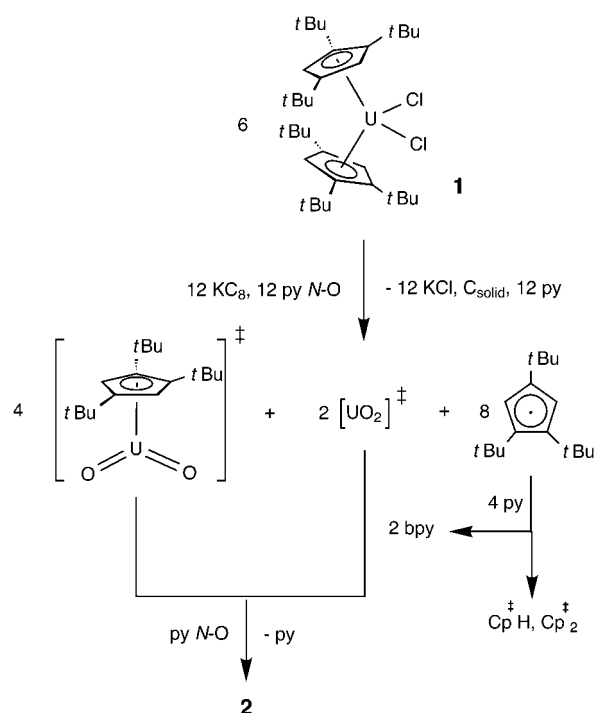
dominance of the *trans*-dioxouranyl unit in aqueous media still permits oligomerization along the equatorial plane, whereas discrete cluster topologies (as in **A**) require stable *cis*-dioxo geometries met by d^0 metals such as Mo^{VI} and W^{VI} .

We report herein how the above problems have been obviated under nonaqueous conditions, such that the genre of isopolyoxometalate derivatives is now extended to include an unprecedented uranium structural analogue **2**, which contains a core unit similar to that in **A**. Although obtained serendipitously in an attempted synthesis of an organometallic



dioxouranium(vi) complex, the results delineate some unique attributes of the actinides, as well as the combination of fortuitous circumstances, that led to the isolation and stabilization of this uranium cluster. The structural and electronic properties of **2** are assessed in the context of the exhaustive studies conducted previously on transition metal polyoxometalates.

We have previously reported an efficient synthesis of bis(imido)uranium(vi) complexes of the general formula $[(C_5Me_5)_2U(=NR)_2]$ by the direct reduction of two azide molecules RN_3 at the uranium center.^[8] This method could not be extended, however, to the preparation of analogous bis(oxo) derivatives $[(C_5Me_5)_2U(=O)_2]$ by using pyridine *N*-oxide; all attempts thus far have generated decomposition products, one of which is the pentamethylcyclopentadienyl dimer $(C_5Me_5)_2$. Considering that steric protection might inhibit potential decomposition pathways, we employed similar reaction conditions using the bulkier cyclopentadienyl derivative $[Cp^*UCl_2]$ (**1**; $Cp^* = 1,2,4-tBu_3C_5H_2$) as the uranium(vi) starting complex. Reduction of **1** with two equivalents of KC_8 in THF, followed by an addition of two equivalents of pyridine *N*-oxide (Scheme 1), was conducted in



Scheme 1. Intermediate species and by-products in the proposed mechanism for the formation of **2**.

an attempt to produce the organometallic dioxo species $[Cp_2^*UO_2]$ (**3**). However, the cluster compound $[Cp_4^*(bpy)_2][U_6O_{13}]$ (**2**) was isolated instead as the main product (54% yield) from this reaction. The complex was not readily characterized in solution (no ^1H NMR is observed).

Single crystals of **2** suitable for X-ray diffraction were obtained by slow evaporation of a solution of **2** in toluene.^[9] The X-ray crystal structure for **2** (Figure 1) reveals six uranium atoms arranged in approximate octahedral symmetry, with an interstitial μ_6 -oxo group O(7) situated in the center of the cluster. Tetragonal compression of the uranium octahedron is observed along the U(1)-O(7)-U(1A) axis. Twelve other oxo ligands form μ_2 -O bridging interactions to uranium centers around the cluster framework to furnish the $[U_6O_{13}]$ core that mimics the isopolyoxometalate Lindqvist structure. In the Lindqvist structure, however, a terminal oxo ligand on each metal center would normally complete the

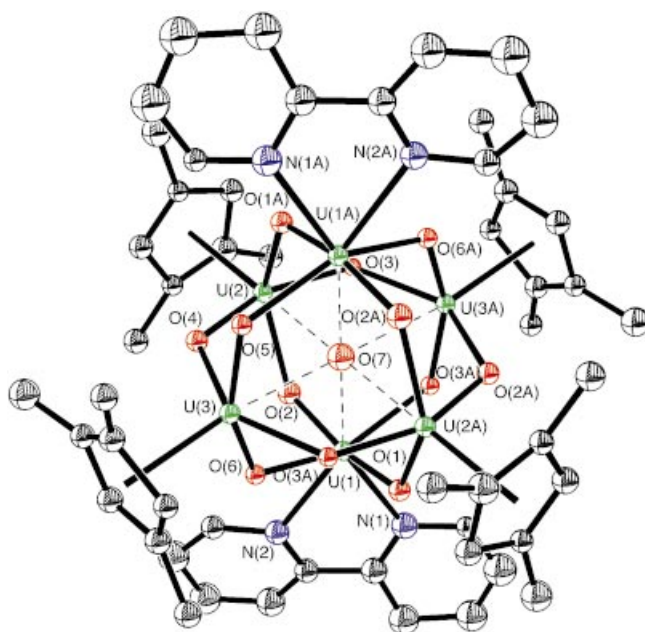
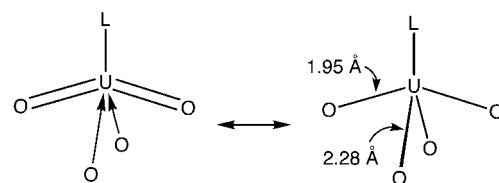


Figure 1. Molecular structure of **2** (methyl carbon atoms of *tert*-butyl groups are omitted for clarity). Bond lengths [$\text{\AA}$]: U(1)-O(1) 1.924(14), U(1)-O(2) 1.905(14), U(1)-O(5) 2.301(11), U(1)-O(6) 2.231(12), U(1)-O(7) 2.4865(8), U(2)-O(7) 2.6353(8), U(3)-O(7) 2.6671(7).

coordination sphere, whereas in **2** these terminal sites are occupied instead by either an η^5 - Cp^* ligand (on the “equatorial” uranium atoms U(2), U(2A), U(3) and U(3A)), or by a chelating bipyridine (bpy) ligand (on the “axial” uranium atoms U(1) and U(1A)). The presence of terminal ligands other than oxo groups has been noted in isopolyoxometalates ions,^[10] but with the notable exception of organoimido functionalities,^[11] only one terminal oxo ligand is typically substituted. In this example, all six are missing. Furthermore, in the transition metal analogues all of the bridging interactions are approximately equivalent, as reflected in similar $M-O_{\text{bridging}}$ bond lengths throughout the M_6 cluster. In marked contrast, in the U_6 cluster there is a distinct pattern between short and long $U-O_{\text{bridging}}$ distances, such that the LUO_4 ($L = Cp^*$ or bpy) coordination sphere at each uranium center features an approximately *trans* O-U-O unit that is elongated, and another *trans* O-U-O unit that is shortened. As illustrated in the resonance structure in Scheme 2, this bond length alternation is suggestive of the *trans*-dioxouranyl unit that pervades aqueous uranium coordination chemistry. However, a modest bending of the $O=U=O$ angles in **2** (between 20° and 30° away from the near linear geometry found in most uranyl complexes) arises from angular bridging interactions to the other uranium centers that encompass the cluster.



Scheme 2. Resonance structures for the LUO_4 ($L = Cp^*$, bpy) units in **2**.

In classical isopolyoxometalate cluster compounds the metals are invariably in their maximum oxidation state, save for $[\text{V}_{18}\text{O}_{42}]^{12-}$, in which each vanadium atom is a $d^1 \text{V}^{\text{IV}}$ system.^[12] In comparison, 13 oxo ligands and four Cp^+ ligands add up to an overall charge of 30^- in neutral **2**, which distributed over six uranium centers gives an average oxidation state of U^{V} (f^1) for each metal. This assessment is supported by the magnetic susceptibility measurements obtained for **2**; a plot of $1/\chi_{\text{M}}$ versus T is shown in Figure 2.

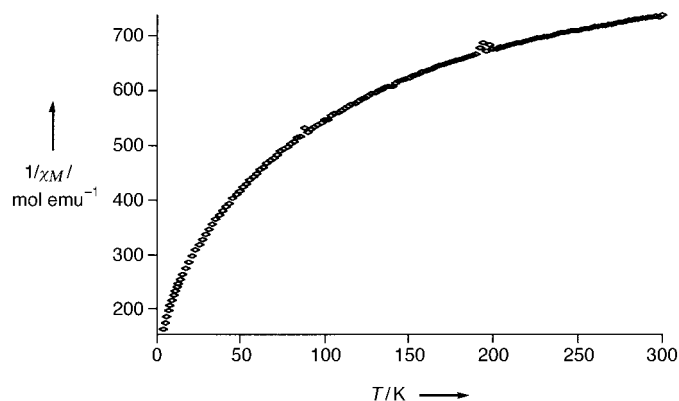


Figure 2. Magnetic susceptibility plot of $1/\chi_{\text{M}}$ versus T (χ_{M} = molar magnetic susceptibility, T = temperature) between 5–300 K.

The curve is consistent with six U^{V} centers behaving as independent paramagnets, although very weak antiferromagnetic exchange (anticipated for highly asymmetric U–O–U bridges) may be suggested by the low magnetic moment ($\mu_{\text{eff}} = 1.8 \mu_{\text{B}}$ per uranium center at 300 K). In contrast, analogous $d^1 \text{V}^{\text{IV}}$ polyoxometalates show much stronger antiferromagnetic coupling across the oxo bridges.^[13]

An important feature of transition metal polyoxometalates is that many derivatives undergo multielectron reduction. The fully oxidized Lindqvist structures are rather limited in this regard, however, as only two electrons can be reversibly added to these species. This behavior differs significantly from that of the uranium cluster **2**, which already possesses reduced U^{V} centers (based on the magnetic susceptibility data), yet is evidently capable of assimilating still more electrons. The cyclic voltammetric behavior (see Supporting Information) shows two distinct regions of irreversible electroactivity. One region at -2.0 V versus the ferrocenium/ferrocene couple is likely attributable to the direct reduction of U^{V} centers, while the second region at -3.0 V is probably associated with electron addition into the coordinated bipyridine rings. Irreversible oxidation is observed at $+1.5 \text{ V}$, suggesting the loss of electrons from the U^{V} centers. Consistent with the observation that oxidation is irreversible, attempts to chemically oxidize the species have so far only resulted in degradation of the cluster.

Regarding the origin of the components that make up **2**, it is plausible that the intended product $[\text{Cp}_2^+ \text{UO}_2]^-$ (**3**) is initially formed as a reactive intermediate that decomposes by reductive homolytic $\text{Cp}^+ \text{--} \text{U}$ cleavage to give $[\text{Cp}^+ \text{UO}_2]$ and a Cp^+ radical (Scheme 1). $[\text{Cp}^+ \text{UO}_2]$ is the fragment that occupies the four equatorial uranium sites, while the other two

sites are occupied by UO_2 units resulting from the loss of a second Cp^+ radical. The fate of the Cp^+ radicals that are generated include: 1) coupling with another Cp^+ radical to give Cp_2^+ , or 2) abstraction of an α -hydrogen from pyridine (released during the reaction with pyridine N -oxide) to generate $\text{Cp}^+ \text{H}$. Both of these organic by-products are observed in the ^1H NMR spectrum of the reaction mixture. The coupling of two pyridyl radicals accounts for the otherwise puzzling formation of bipyridine, which importantly acts as a trapping ligand for the axial UO_2 units. That a similar result (or any tractable product) is not observed in the corresponding synthetic attempt to form $[(\text{C}_5\text{Me}_5)_2\text{UO}_2]$ may derive either from the increased stability of the Cp^+ radical versus a (C_5Me_5) radical, or from greater kinetic stability conferred on **2** by the sterically bulky Cp^+ ligands.

Finally, we address the circumstances that may have provided for the stabilization of this uranium polyoxometalate derivative, where such an example was seemingly unattainable before. As mentioned earlier, the main obstacle within aqueous media has been the incompatibility of the *trans*-uranyl species with the *cis*-dioxo geometry necessary for cluster formation. However, this problem has apparently been eliminated simply by the fortuitous replacement of the terminal oxo groups in **A** with organic functionalities in **2**. In addition to providing steric protection, this substitution removes the *cis*-dioxo geometry that is so unfavorable for uranium; hence, based on the unique asymmetric oxo bridges present in **2**, this cluster actually comprises six *trans*-dioxo (“uranyl-like”) moieties. The extensive bridging interactions that connect these uranyl building blocks are promoted by nucleophilic oxo groups attached to the electron-rich uranium(v) centers. This behavior is reminiscent of cation–cation complexes in neptunyl(v) NpO_2^+ chemistry^[14, 15] (isolable uranyl(v) species are currently unknown), and similar nucleophilic interactions from uranyl(vi) oxo ligands have been noted in rare examples where strong donor equatorial ligands are employed.^[16] Thus, ironically, the incorporation of **2** as a structural analogue into a family of complexes traditionally associated with transition metals derives primarily from one of the attributes (uranyl ion complexation) that most distinguishes actinide chemistry.

Experimental Section

$[[1,2,4-(\text{Me}_3\text{C})_3\text{C}_3\text{H}_2]_2\text{Mg}]$: Dibutylmagnesium (88 mL, 1 M in heptane) was added to 1,2,4-(Me_3C) $_3\text{C}_3\text{H}_2$ (41.25 g, 0.175 mol).^[17] The heptane was removed under reduced pressure, and the pale yellow solution was heated to 130°C for three days. Upon cooling to room temperature, the mixture solidified. The solid was redissolved in pentane (200 mL) and filtered. The volume of the filtrate was reduced until solid began to precipitate. The mixture was warmed to redissolve the solid, then cooled to -20°C . Two crops of white crystals were collected by filtration. Recrystallization of the second crop was necessary to remove residual 1,2,4-(Me_3C) $_3\text{C}_3\text{H}_2$. Yield: 28.45 g (66%). ^1H NMR (C_6D_6 , 30°C): $\delta = 6.1$ (4H), 1.5 (36H), 1.4 (18H); elemental analysis (%) calcd for $\text{C}_{34}\text{H}_{58}\text{Mg}$: C 83.2, H 11.9; found: C 82.9, H 12.0; EI MS: m/z : 490 [M^+]; the parent ion isotopic cluster was simulated: (calcd. %, obsd. %): 490 (100, 100), 491 (52, 52), 492 (26, 26), 493 (7, 7).

1: $[[1,2,4-(\text{Me}_3\text{C})_3\text{C}_3\text{H}_2]_2\text{Mg}]$ (12.1 g, 0.025 mol) and UCl_4 (8.9 g, 0.023 mol) were suspended in toluene (250 mL) in a 500-mL Schlenk flask under an argon atmosphere. Freshly dried pyridine (50 mL) was added to the suspension, and the mixture was stirred at reflux for one day. The solution was cooled to room temperature and the solvent removed under reduced

pressure. The red solid residue was extracted with hexane (300 mL) and filtered. The volume of the filtrate was reduced to 100 mL and cooled to -20°C . Two crops of dark red crystals were collected by filtration. Yield: 11.6 g (65%). M.p. $192-194^{\circ}\text{C}$; ^1H NMR (C_6D_6 , 30°C): $\delta = 29.7$ (4H, $\nu_{1/2} = 110$ Hz), 9.5 (36H, $\nu_{1/2} = 12$ Hz), -19.4 (18H, $\nu_{1/2} = 7$ Hz); elemental analysis (%) calcd for $\text{C}_{34}\text{H}_{58}\text{Cl}_2\text{U}$: C 52.7, H 7.48; found: C 52.6, H 7.55; EI MS: m/z : 774 [M^+]; the parent ion isotopic cluster was simulated: (calcd. %, obsvd. %): 774 (100, 100), 775 (38, 39), 776 (71, 71), 777 (25, 25), 778 (14, 14), 779 (5, 4).

2: THF (30 mL) was added to a mixture of KC_8 (140 mg, 1.04 mmol) and **1** (400 mg, 0.52 mmol). A dark green color was generated immediately in the solution upon the addition of solvent. The mixture was stirred for 2 h, after which a solution of pyridine *N*-oxide (100 mg, 1.05 mmol) in THF (25 mL) was added. The dark green color of the solution immediately turned dark reddish brown. The reaction mixture was stirred for 12 h, after which the solvent was removed under vacuum and the residue extracted with toluene and filtered through Celite. Dark reddish brown crystals of **2** were obtained from a cold (-30°C) concentrated toluene solution. Yield: 275 mg (54%); IR (Nujol): $\tilde{\nu} = 690, 710\text{ cm}^{-1}$ (U–O); elemental analysis (%) calcd for $\text{C}_{109}\text{H}_{156}\text{N}_4\text{O}_{13}\text{U}_6$: C 41.45, H 4.98, N 1.77; found: C 41.91, H 5.40, N 1.53.

The magnetization of crystalline powdered samples of **2** was recorded between 5–300 K at 0.1 T with a SQUID magnetometer (Quantum Design). Values of the magnetic susceptibility were corrected for the underlying diamagnetic increment ($\chi_{\text{dia}} = -1660 \times 10^{-6}\text{ cm}^3\text{ mol}^{-1}$) by using tabulated Pascal constants and the effect of the blank sample holder (gelatine capsule/quartz wool).

Cyclic voltammetric studies were conducted by using a Perkin–Elmer Princeton Applied Research Corporation (PARC) Model 263 potentiostat under computer control using M270 software. Samples were run in 0.1 M tetrabutylammonium hexafluorophosphate solution in tetrahydrofuran at a platinum working electrode with a silver wire quasi-reference electrode in either a PARC microcell in the glove box or in a Schlenk cell. Measured potentials were calibrated by using the ferrocene/ferrocenium couple ($E_{1/2} \sim 0.55\text{ V}$ vs. NHE).

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- [9] Crystallographic details for **2**: a dark red crystal ($0.12 \times 0.08 \times 0.04\text{ mm}$) was mounted from Paratone N oil onto a glass fiber and immediately placed on a Bruker P4/CCD/PC diffractometer. A hemisphere of data was collected by using a combination of ϕ and ω scans, with 30 second frame exposures and 0.3° frame widths. A total of 14413 reflections ($-14 \leq h \leq 15$, $-20 \leq k \leq 20$, $0 \leq l \leq 28$) were collected at $T = 203(2)\text{ K}$ in the θ range of $1.49-23.32^{\circ}$ of which 14413 were unique ($R_{\text{int}} = 0.0000$); $\text{MoK}\alpha$ radiation ($\lambda = 0.71073\text{ \AA}$). The structure was solved by using direct methods and difference Fourier techniques (SMART Version 4.210, Bruker Analytical X-ray Systems, Inc., 1996). All non-hydrogen anisotropic temperature factors were restrained by using the ISOR option, and hydrogen atoms were placed in calculated ($d_{\text{C-H}} = 0.96\text{ \AA}$ for methyl, $0.93\text{ \AA}$ for methyl) positions. The residual peak and hole electron density was 1.770 and $-1.791\text{ e \AA}^{-3}$. The absorption coefficient was 7.641 mm^{-1} . The least-squares refinement converged normally with residuals of $R1 = 0.0686$ ($I > 2\sigma(I)$), $wR2 = 0.1610$, and $\text{GOF} = 1.152$ (F^2); $\text{C}_{109}\text{H}_{156}\text{N}_4\text{O}_{13}\text{U}_6$, space group $P\bar{1}$ (no. 2), triclinic $a = 13.8298(7)$, $b = 18.681(1)$, $c = 25.335(2)\text{ \AA}$, $\alpha = 80.118(1)^{\circ}$, $\beta = 76.867(1)^{\circ}$, $\gamma = 72.803(1)^{\circ}$, $V = 6050.9(6)\text{ \AA}^3$, $Z = 2$, $F(000) = 2916$, $\rho_{\text{calcd}} = 1.733\text{ g cm}^{-3}$. Crystallo-

graphic 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-160613. 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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Rational Molecular Design and EPC Synthesis of a Type VI β -Turn Inducing Peptide Mimetic**

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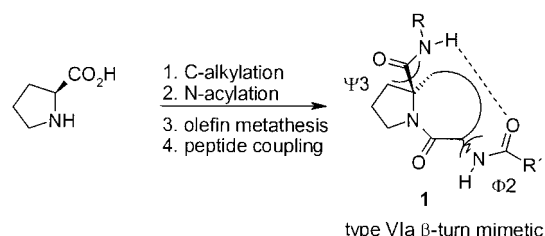
Besides the specific role of proline for the structure of proteins, experimental and simulation studies indicate that proline-containing sequence motives can act as molecular hinges, swivels, and switches and, thus, are involved in biological signaling processes.^[1] Furthermore, *cis/trans*-prolyl isomerization that can be catalyzed by rotamase enzymes is critical for the folding process.^[2] Recently, pseudoproline inducing a *cis*-peptide bond were introduced into the V3 loop of GP120 of HIV-1, which suggests that a *trans/cis* isomerization resulting in formation of a type VI β -turn conformation plays a crucial role in the infection process.^[3] Thus, the creation of type VI β -turn model systems is of interest for the discovery of molecular probes to gain detailed insight into the intermolecular interaction processes. Using appropriate con-

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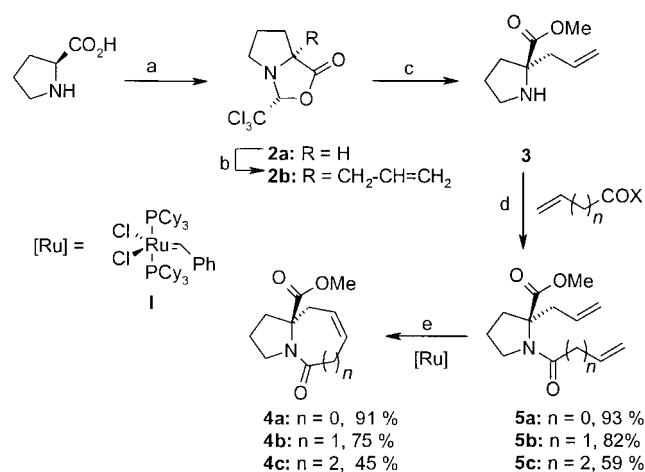
Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

formational restraints, bicyclic *cis*-peptidyl proline surrogates were investigated with low yielding and nonselective multi-step reaction sequences.^[4, 5] Stimulated by a recently described Molecular Dynamic (MD) study,^[6] we herein present the design of a lactam-bridged type VIa β -turn mimetic (**1**). NMR spectroscopy based conformational analysis and a practical and efficient EPC synthesis of a model peptide surrogate by the self-reproduction of chirality methodology of Seebach et al.^[7] and Grubbs' ring-closing olefin metathesis (RCM)^[8] are also reported (Scheme 1).



Scheme 1.

Following the above strategy, we approached to the α -alkylproline derivative **3** by employing the concept of self-reproduction of chirality^[7] which was recently re-investigated by Wang and Germanas^[9] (Scheme 2). Thus, diastereocon-

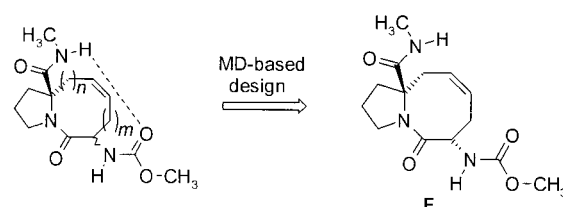


Scheme 2. a) $\text{CCl}_3\text{CH}(\text{OH})_2$, C_6H_6 , 80°C ; b) lithium diisopropylamide (LDA), allyl bromide, THF, -78°C ; c) HCl, MeOH, NaHCO_3 ; d) **5a**: $\text{X} = \text{Cl}$, $n = 0$, CH_2Cl_2 , NEt_3 , $0^\circ\text{C} \rightarrow \text{RT}$, **5b**: $\text{X} = \text{OH}$, $n = 1$, **5c**: $\text{X} = \text{OH}$, $n = 2$, 4-methyl morpholine (NMM), ClCO_2tBu , THF; e) [Ru], CH_2Cl_2 (Cy = cyclohexyl).

trolled C-alkylation of the bicyclic proline derivative **2a** furnished the N,O-acetal **2b** which was transformed into α -allylproline ester **3** by treatment with one equivalent of HCl in methanol. To evaluate the scope of our synthetic concept, we tried to prepare differently sized bicyclic model lactams of type **4**. Thus, the RCM precursors **5a–c** were synthesized by N-acylation of the proline derivative **3**. The subsequent RCM experiments were performed using the ruthenium-based catalyst **I**^[10] in refluxing CH_2Cl_2 which proved to be the solvent of choice. The dienes **5a, b** underwent clean conversion to furnish the 5,6- and 5,7-fused unsaturated bicyclic lactams **4a, b** in 91 and 75 % yield, respectively. To minimize competing intermolecular oligomerization processes,^[11] a high

dilution technique was employed for the conversion of the diene **5c** into the bicyclic azocine **4c**. 10 mol % of the catalyst was chosen to initiate the metathesis reaction and a second portion of catalyst^[12] (2 mol %) was added after 24 h. Gratifyingly, the desired cyclization took place without any detectable oligomerization products, although complete conversion could not be achieved.

To decide which ring size and configuration is most promising in terms of possible intramolecular hydrogen-bond interactions, we performed MD simulations on the different target structures **A–F** shown in Scheme 3. All calculations



Formula	<i>n</i>	<i>m</i>	Stereochemistry
A	0	0	<i>cis</i>
B	0	0	<i>trans</i>
C	1	0	<i>cis</i>
D	1	0	<i>trans</i>
E	1	1	<i>cis</i>
F	1	1	<i>trans</i>

Scheme 3.

were performed in the gas phase with no continuum model or explicit solvent molecules present, using the AM1 Hamiltonian implemented in the semiempirical program package VAMP7.0.^[13] After heating the system to 400 K, 1200 geometries were sampled at constant temperature resulting in a total data-acquisition time of 120 ps. The integration time step was 1 fs, the coupling constant to the heat bath 40 fs. The ability to form a hydrogen bond was rated by a time-dependent plot of the two possible donor–acceptor interactions, which lead either to a type VIa, or, by reorientation of the C- and N-termini by rotation about the torsion angles Ψ_3 and Φ_2 (see Scheme 1), respectively, to a type VIb turn, which involve CO^{i+2} and NH^{i+1} . Classification of the β -turn type was done by time-dependent analysis of the dihedral angles Ψ_3 and Φ_2 , in accord with the data published by Robinson.^[14]

For the six-membered lactams, the *cis*-isomer **A** allows the formation of both possible hydrogen bonds. However, for the chosen simulation conditions, no distinct turn pattern can be assigned. For the *trans*-isomer **B**, the formation of a hydrogen bond is geometrically not possible. Expanding the ring by one methylene unit leads to the fused azacycloheptenones **C** and **D**. According to the MD simulations, the increased flexibility resulted in a growing tendency of the *trans*-isomer **D** to form a type VIa2 interaction pattern. Nevertheless, the mean $\text{NH} \cdots \text{OC}$ distance of 4.6 Å is too large for a stable hydrogen bond. Similar to **A**, the homologue **C** is able to form both hydrogen-bonding interactions. During the simulations, a type VIa1 pattern was established several times, but, in spite of the corresponding hydrogen-bond interaction, no typical VIb motif was observed. The fused eight-membered lactams **E** and

F exhibited the most interesting behavior. For the *cis*-isomer **E** a hydrogen bond between the amide carbonyl and the carbamate NH was observed, however, the flexibility within the molecule allowed switching to a type VIa-like hydrogen-bond interaction (this occurred several times during the simulation). Moreover, the *trans*-isomer **F** formed only one hydrogen-bonding pattern, a type VIa2 subtype ($\Psi_3=0^\circ$ and $\Phi_2=-120^\circ$) of the β -VI turn family (Figure 1a). As a

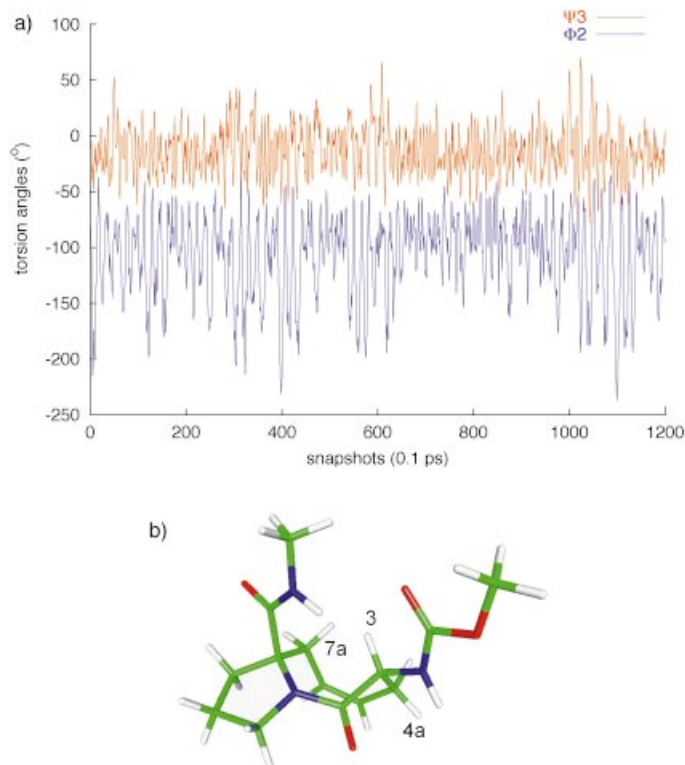


Figure 1. a) Plot of the dihedral angles Ψ_3 and Φ_2 calculated from the 120 ps semiempirical MD simulation (AM1, 400 K) of compound **F**. Note that the mean values come close to the ideal angles of a type VIa2 turn ($\Psi_3=0^\circ$ and $\Phi_2=-120^\circ$).^[14] No conformational interchange can be observed. b) Representative example of a conformation extracted from the trajectory. The dihedral angles $\Psi_3=23^\circ$ and $\Phi_2=-102^\circ$ result in a $\text{NH}\cdots\text{OC}$ distance of 2.3 Å for this non-equilibrium conformation.

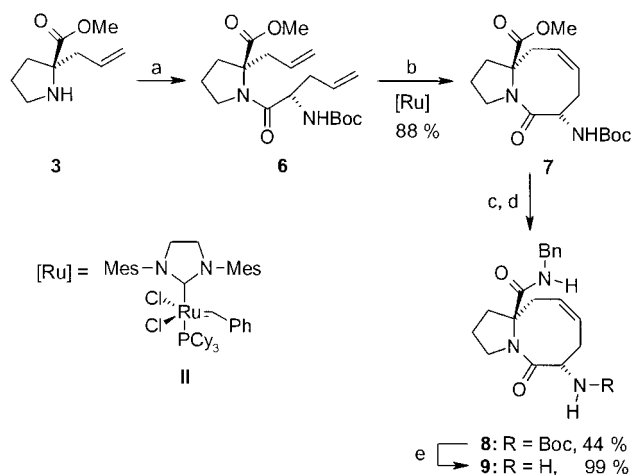
representative geometrical example, a snapshot taken from the MD simulation of compound **F** is shown (Figure 1b). The conformational behavior is independent from the starting geometry of the simulation. Beginning with a geometry-optimized type VIb turn, the conformation of the molecule switches to a stable VIa2 motif after a few picoseconds of data acquisition.

Comparison of all six simulations strongly supports the assumption that the lactam-bridged peptide surrogate **F** should be most suitable for the formation of a hydrogen-bonding pattern leading to a successful type VIa β -turn mimetic.

To account for solvent effects which may influence the conformational preferences of peptide-like structures, we repeated the calculations applying the COSMO approach of Klamt and Schüürmann.^[15] This technique describes quantum mechanically the macroscopic environment by polarization

charges at the boundary between the solute and the continuum (water, dielectric constant = 78.4) and therefore represents the dielectric properties of the bulk molecules. Both series of simulations show the same principal behavior. As a general effect, structural fluctuations are damped by the environment, which leads to a more meaningful interpretation of the data.

To experimentally verify our MD simulation prediction of type VIa β -turn inducing properties for the bicyclic peptidomimetic **F**, the RCM precursor **6** was prepared starting from *tert*-butoxycarbonyl (Boc) protected (*S*)-allylglycine and the α -allylproline **3** (Scheme 4). Subsequently, Ru-catalyzed ring-closure of the protected dipeptide **6** led to formation of the



Scheme 4. a) (*S*)-*N*-Boc-2-amino-4-pentenoic acid, NMM, ClCO_2tBu , THF; b) 7 mol % [Ru], CH_2Cl_2 , 40°C ; c) LiOH, dioxane/ H_2O ; d) BnNH_2 (Bn = benzyl), *N,N'*-dicyclohexyl carbodiimide (DCC), CH_2Cl_2 , 1-hydroxy-1*H*-benzotriazole (HOBt); e) HCl/diethyl ether.

eight-membered azacycle **7** in 57% yield. To improve the efficiency of the reaction we tried the highly active N-heterocyclic carbene-coordinated catalyst **II**.^[16] Actually, the metathesis reaction proceeded smoothly with only 7 mol % of catalyst to yield the bicyclic olefin **7** in 88% (12 h, reflux). Finally, hydrolysis of the ester function and DCC coupling to benzylamine as a representative NH-providing unit gave the target model peptide **8** that could be readily transformed into the primary amine **9**.

Conformational analysis of the lactam-bridged peptidomimetic **8** is mainly based on NMR spectroscopy, particularly on coupling constants, NOEs, and variable temperature (VT) measurements. A strong NOE was detected between H-7a and H-3 indicating the transannular proximity of these two protons in a boat conformation of the eight-membered lactam ring. In combination with a detailed analysis of the proton couplings further strong NOEs between the amide N-H and H-3 and the carbamate N-H and H-4a, respectively, were diagnostic for a type VIa β -turn conformation adopted by the lactam **8**. Clearly the fused ring system is fixed in a stable conformation which forces the substituents at C-3 and C-8 into an orientation able to form a hydrogen bond between the amide NH and the carbamate C=O as predicted by the MD calculations. The presence of an intramolecular hydrogen

bond, indicated by the comparatively downfield shift ($\delta = 8.11$) of the amide N–H resonance was unambiguously demonstrated by comparison of the amide N–H shifts in the spectra (in CDCl_3) of the carbamate **8** and the amine **9** when the signal of the amide N–H appeared significantly shifted upfield ($\Delta\delta = 1.61$) after removal of the Boc unit. Besides the main conformational population, VT NMR experiments which showed a significant chemical-shift change ($\Delta\delta/\Delta T = -5.2 \text{ ppb K}^{-1}$) gave evidence for a temperature dependant coexistence of non-hydrogen bonded conformations,^[17] corroborated by NOEs between H-3 and the carbamate N–H at elevated temperature (330 K, CDCl_3). The FT-infrared (IR) spectrum displayed an extensive absorption at 3350 cm^{-1} characteristic for a hydrogen bonded amide and an absorption at 3405 cm^{-1} also indicating the coexistence of a solvent-exposed amide.

In conclusion, a highly efficient proline-based type VI β -turn mimetic was designed employing the results of molecular dynamics simulation. EPC synthesis involving Seebach's self-reproduction of chirality methodology and Grubbs' ring-closing olefin metathesis gave access to the novel molecular scaffold.

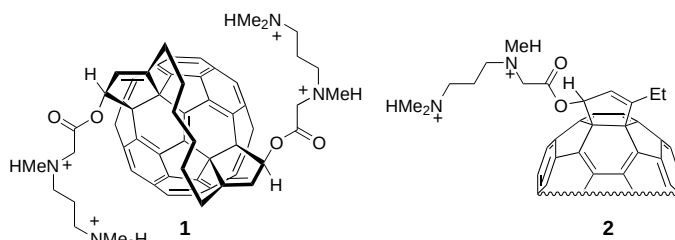
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Atomic Force Microscope Studies on Condensation of Plasmid DNA with Functionalized Fullerenes**

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A recent paper reported the first example of the use of a carbon cluster as a vector to deliver DNA into mammalian cells.^[1, 2] Thus, mixed with the tailor-made tetraminofullerene **1**, a plasmid DNA forms micrometer-sized fullerene–DNA particles as observed by optical microscope, which, after



incubation with the target cells, became located inside the cytoplasm as phagocytes. Expression of the encoded gene took place over several days, indicating that the plasmid DNA was released into the cytoplasm without being damaged by the fullerene complexation. Although these biological experiments demonstrated the ability of the fullerene **1** to condense and release DNA, the molecular nature of such reversible DNA condensation remained unclear. We report herein the results of atomic force microscope (AFM) studies^[3] that provided molecular-level information on the DNA condensation/release processes induced by fullerene vesicles.^[4] When used in a small quantity, the fullerene **1** condenses a single plasmid DNA into a **1**–DNA complex. Upon further addition of **1**, many single-molecule DNA condensates gather together to form the micrometer-sized fullerene–DNA particles observed previously by optical microscopy. Release of the DNA molecules from these large particles was achieved experimentally by removal of the fullerene through CHCl_3 extraction.

The interactions between the fullerene **1** and DNA were probed for 4 kbp supercoiled plasmid DNA (pBR322). Thus, we added an increasing amount of **1** to a pH 7.6 buffer solution of pBR322 and plotted the amount of the DNA mobile on the gel against the reagent/base pair ratio (R). As shown in Figure 1 (solid line), there was observed precipitous decrease

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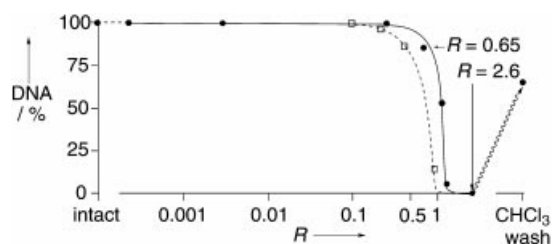


Figure 1. Amount of pBR322 plasmid DNA remaining in solution as determined by gel electrophoresis (integrated optical density of fluorescence, solid line) and by CD spectra (molar ellipticity spectra at 260 nm, broken line) in the presence of **1** at various R values. The y-axis (DNA) is relative to the value obtained for $R=0$.

of the DNA mobile on the gel for R values between 0.5 and 1.8. With this latter ratio, no mobile DNA was detected on the gel. Note that $R=0.5$ is the theoretical point of neutralization of the DNA phosphate charges with the tetraminofullerene **1** at pH 7. Although no further change was observed upon addition (and removal) of EtOH (in which **1** does not dissolve), we found that extraction of the mixture with CHCl_3 results in the reappearance of about 70% of the supercoiled DNA on the gel. No DNA nicking was observed in the recovered DNA. Since CHCl_3 freely dissolves the fullerene **1**, we conclude that DNA was released from the fullerene–DNA precipitate as the fullerene was washed away. Thus, the fullerene–DNA binding is not covalent. Note that the one-handed analog **2**, which binds to DNA very weakly,^[1] did not cause DNA precipitation even at $R=2.6$.^[5]

Measurement of the CD spectra of the fullerene–DNA mixture (Figure 2) afforded further information on the complexation process. Thus, the molar ellipticity $[\theta]$ at 260 nm starts to decrease at $R \sim 0.4$ and becomes nearly zero

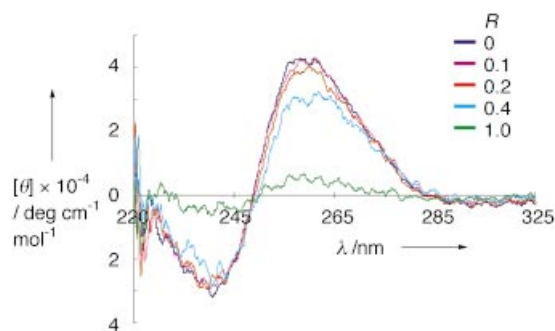


Figure 2. CD spectra of plasmid pBR322 DNA in the presence of **1** at R values between 0–1. CD spectra were measured in a THF/Mg-HEPES buffer (20% v/v, $[\text{bp}] = 100 \mu\text{M}$, pH 7.6). See Supporting Information for details of the experimental procedure.

at $R=1.0$ (namely, loss of the DNA from solution). The $[\theta]/R$ plot (broken line) in Figure 1 is parallel to the result of the gel experiment. The systematic difference between the two lines can be ascribed to the electric field applied in the gel electrophoresis, which will strip the positively charged fullerene and the negatively charged DNA off from the fullerene–DNA complex, thereby increasing the amount of mobile DNA on the gel. More importantly, the plasmid DNA in the soluble fraction, for which the AFM images in

Figures 3c and 4d were obtained, exhibited CD spectra characteristic of a B-helix (Figure 2).

Whereas the above experiments revealed only one phase transition event for the R range between 2.5×10^{-4} and 2.6, AFM analysis of the same mixture revealed that more complex events are taking place in the fullerene complexation process (Figures 3 and 4). Upon dissolution in the buffer solution, the fullerene **1** forms vesicles, which were observed as numerous humps in a thin aqueous films on mica surface (Figure 3a) as recently reported for an analogous water-soluble fullerene.^[4a] Each vesicle may contain several thousand fullerene molecules.^[4b] The solution of the intact DNA used for the gel experiments revealed a large number of overlapping molecules (Figure 3b), and a highly diluted solution of the same DNA showed separated molecules (Figure 4a).

A noteworthy AFM observation was made in the analysis of the soluble fraction at $R=0.65$ (Figure 3c), where neither gel nor CD data suggested any change of the DNA structure. When the mixture was spotted on a mica surface, the surface was covered by numerous flat disks of rather narrow size distribution. The smallest disks, which were the most abundant, measured about 50 nm in diameter and 3 nm in thickness with a calculated volume of $5 \times 10^3 \text{ nm}^3$, which is only 25% larger than the volume of pBR322 DNA ($4 \times 10^3 \text{ nm}^3$ when approximated as a rod). Unlike the fullerene vesicles, which are soft and dome-shaped (Figure 3a and ref. [4a]), the disks are rather flat and their top surface shows a fine structure (Figure 4d). Beside the small disks, we also found larger disks, which have the same thickness but are two or three times larger in diameter than the smallest disks. Though the large area of the mica surface was covered by the disks, we found in some areas partially folded objects (Figures 3d, 4b, and 4c).^[6] In Figures 4b and 4c, we can identify intact double strands between the partially condensed parts. Taken together with the volume and shape analysis of the disks (see below), comparison of these objects (Figures 4b and 4c) with the intact DNA (Figure 4a) and the disks (Figure 4d) strongly suggest that the smallest disk is a single DNA molecule condensed through interaction with the fullerene. We therefore formulate the DNA folding process as the sequence of Figures 4a $\rightarrow$ 4b $\rightarrow$ 4c $\rightarrow$ 4d. Likewise, we consider that the double- and triple-sized disks are two- and three-molecular condensates. The shape and the size distribution of the fullerene–DNA condensates did not change over 24 h for samples analyzed at several hour intervals.

When a smaller amount of **1** was used, we saw largely intact DNA rather than the disks: When a larger amount of **1** was added ($R=2.6$), we found that the fullerene–DNA disks glued together to form a single-layer domain (Figure 3e). Double and triple layers were also found. When the fullerene–DNA mixture ($R=2.6$) was washed with EtOH, we found an extremely small number of micrometer-sized aggregates with a mesh structure (Figure 3f). These objects are similar in size to the smallest phagocytes that we found inside the cells in the transfection experiments.^[1]

The above experiments indicated that the fullerene **1** condenses plasmid DNA into compactly folded single-DNA disks, and then to multi-DNA objects as the amount of the

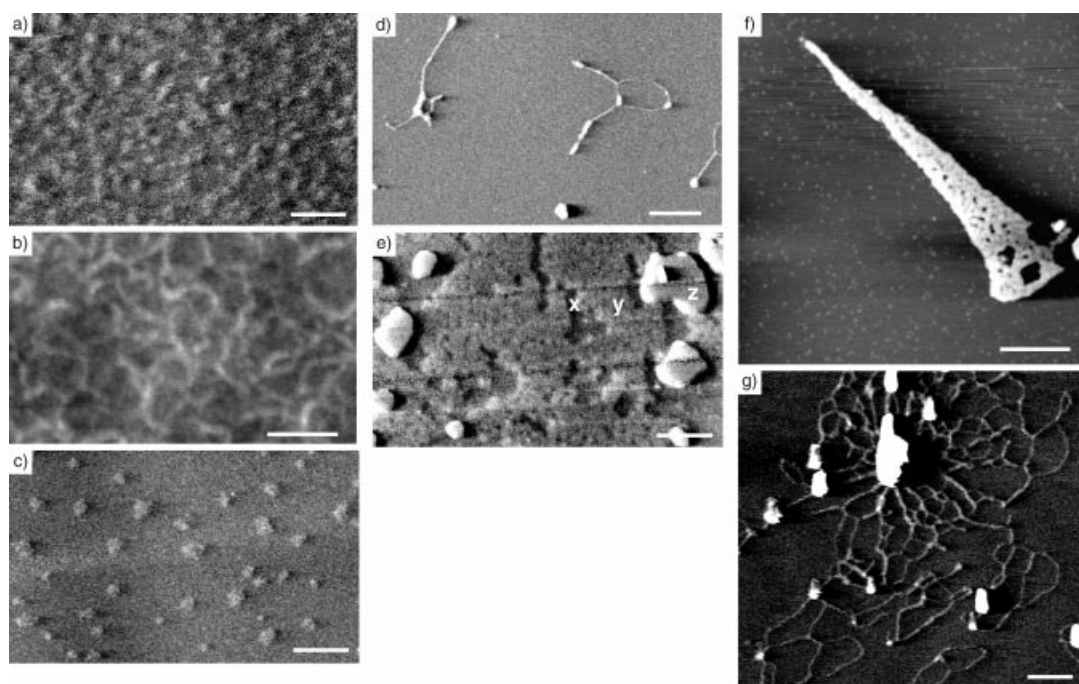


Figure 3. AFM images (micrometer-scale areas) of aggregates of fullerene **1** (a), and pBR322 DNA in the absence (b) and presence (c–g) of **1** at various R values. a) Vesicles of fullerene **1**; b) intact multiple DNA molecules, $R=0$; c) disk-shaped DNA condensates, $R=0.65$; d) partially condensed DNA molecules, $R=0.65$; e) multiple fullerene–DNA condensates, $R=2.6$ (bare mica surface is marked with x, a first layer of fullerene–DNA aggregates with y, and a second layer with z); f) an EtOH-precipitated giant aggregate; g) multiple DNA molecules after CHCl_3 extraction. All scale bars 200 nm. See Supporting Information for the detailed experimental procedure.

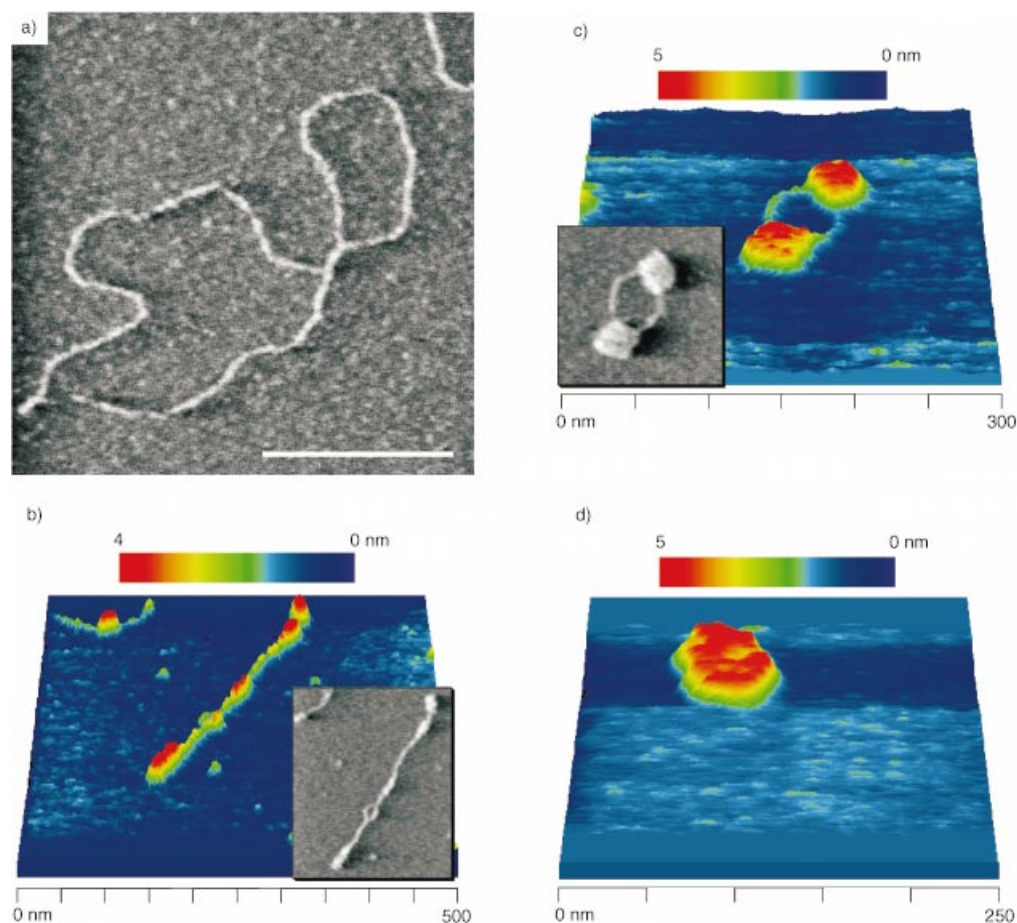


Figure 4. AFM images (submicrometer scale) of pBR322 DNA in the absence (a) and in the presence (b–d) of **1** at various R values. a) Intact DNA, $R=0$; b) partially folded single DNA molecule, $R=0.65$; c) partially condensed DNA with unfolded double strands, $R=0.65$; d) fully condensed fullerene–DNA disk, $R=0.65$. All scale bars 200 nm. Top views are given in black-and-white images. See Supporting Information for details of the experimental procedure.

fullerene in solution increases. When the fullerene was removed from the solution, we could release DNA out of the condensate. Thus, when the fullerene in the $R=2.6$ mixture was extracted with CHCl_3 , AFM analysis of the aqueous phase (the same sample used for the gel analysis in Figure 1) largely showed individual DNA molecules together with a small number of large aggregates surrounded by numerous partially folded DNA molecules (Figure 3g). The image in Figure 3g resembles the classical picture of an *E. coli* nucleus from which histone protein was washed away after surfactant treatment.^[7]

In summary, we have demonstrated that the fullerene **1** can fold a supercoiled DNA molecule into a single-molecule condensate through adhesion of DNA double strands. The rough estimate indicated that the fullerene folds the DNA with only a small increase of the volume of DNA. Such high efficiency of histone-like activity is remarkable since natural histone protein forms a chromatin structure with a volume increasing of ten to hundred times that of DNA.^[8] The formation of disklike condensates composed of a single to a few DNA molecules is unique among the cases of other DNA-condensing agents based on lipid or dendrimers, which create much larger and less structurally defined DNA aggregates.^[9] In the light of remarkably high tendency of water-soluble fullerene to form robust vesicles,^[4] the process of fullerene–DNA condensation must involve collisions between a fullerene vesicle and a DNA molecule rather than a molecule–molecule interaction. The condensation process is largely kinetically controlled rather than thermodynamically controlled, as is known generally for polyelectrolyte–surfactant complexes.^[10] We have found that the DNA condensation is dependent on the concentration of the DNA-binding fullerene. Translation of this observation into biology, we can speculate that in the cytoplasm the ester linkage in the fullerene **1** gradually yields to hydrolase activity,^[11] which will detach the tetramino DNA binding sites from the fullerene core and hence results in the release of DNA from the aggregate. We are currently studying the structure activity relationship in the fullerene-mediated transfection.

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Zero-Strain Intercalation Cathode for Rechargeable Li-Ion Cell

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The market for Li-ion batteries is undergoing rapid expansion, as portable electronic devices demand a higher energy density and a better cycle life. Even though the $\text{Li}_{1-x}\text{CoO}_2$ cathode has been widely used in commercial Li-ion batteries, electrochemical charge (Li de-intercalation) and discharge (Li intercalation) produces a phase transition accompanying nonuniform strain, which is closely related to capacity fading.^[1–3] This nonuniform dimensional change induces a shearing stress within each particle, and consequently fractures occur in most oxides.^[2–4] Hence, a zero-strain cathode material, the lattice constants of which do not change during cycling, is ideal for a long operational lifetimes.^[5–7] Here we report a zero-strain LiCoO_2 cathode material produced by thin-film coating of high-fracture-toughness

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oxides, which suppresses the phase transition during cycling. This novel method for making zero-strain compounds has potential applications in various fields of materials design.

During electrochemical charging, $\text{Li}_{1-x}\text{CoO}_2$ exhibits *c*-axis expansion of up to $\sim 2.6\%$ at $x \sim 0.5$ (corresponding to the formation of a monoclinic phase from the original hexagonal phase of LiCoO_2), with negligible variation in the *a* axis. Hence, the typical reversible limit for $\text{Li}_{1-x}\text{CoO}_2$ delithiation is $x \sim 0.5$, which corresponds to a discharge capacity of $\sim 140 \text{ mA h g}^{-1}$. This property has been mainly attributed to the mechanical failure associated with the large lattice expansion along the *c*-axis direction.^[8–10] Although many attempts have been made to stabilize the $\text{Li}_{1-x}\text{CoO}_2$ structure for $x > 0.5$ through either Al or Mg substitution into the $3a$ sites to give $\text{LiCo}_{1-y}\text{M}_y\text{O}_2$, these results failed to stabilize the hexagonal symmetry, and the materials even showed a decrease in specific capacity when compared with LiCoO_2 .^[1, 10, 11] On the other hand, LiTi_2O_4 materials are known for exhibiting little dimensional change. Still, this material can only be used as an anode material because of its low average voltage of 1.5 V in spite of a specific capacity of 150 mA h g^{-1} .^[5] However, no cathode material with zero strain has been reported to date.

We used LiCoO_2 particles with a coating consisting of various metal oxides having a wide range of fracture toughness, the included ZrO_2 , Al_2O_3 , TiO_2 , and B_2O_3 (see Table 1).

Table 1. Fracture toughness of various oxides: ZrO_2 , Al_2O_3 , TiO_2 , and B_2O_3 .^[12–14]

Oxide	Fracture toughness [MPa $\sqrt{m}$]	Reference
ZrO_2	8–12	[12]
Al_2O_3	2.7–4.2	[12]
TiO_2	2.38	[13]
B_2O_3	1.44	[14]

X-ray diffraction (XRD) patterns of the as-prepared samples were found to be consistent with a single phase ($\alpha\text{-NaFeO}_2$ structure) of the $R\bar{3}m$ space group, where the cobalt and lithium ions are located at the $3a$ and $3b$ octahedral sites, respectively, in a close-packed oxygen array. In addition, the XRD patterns of the coated samples do not show any evidence of metal oxide, and this is because of the formation of a thin-film $\text{LiCo}_{1-y}\text{M}_y\text{O}_2$ ($M = \text{Zr, Al, Ti, B}$) solid solution (tens of nm thick) on the particle surface, as confirmed by Auger electron spectroscopy. The enclosing of LiCoO_2 powders by a fracture-toughened solid-solution metal-oxide coating can effectively suppress the nonuniform lattice-constant changes during Li de-intercalation (Figure 1).

To test the cycle–life performance of each cathode material, coin-type half cells (Li/LiCoO_2) were cycled at a 0.1 C rate ($= 14 \text{ mA g}^{-1}$) for the first two cycles between 4.4 and 2.75 V, then cycling was continued at a 0.5 C rate for up to 70 cycles. The initial discharge capacities of the ZrO_2 - and Al_2O_3 -coated samples are similar to that of the uncoated sample (Figure 2), and the cycle–life performance trend is consistent with the fracture toughness of coated metal oxide (Table 1). In

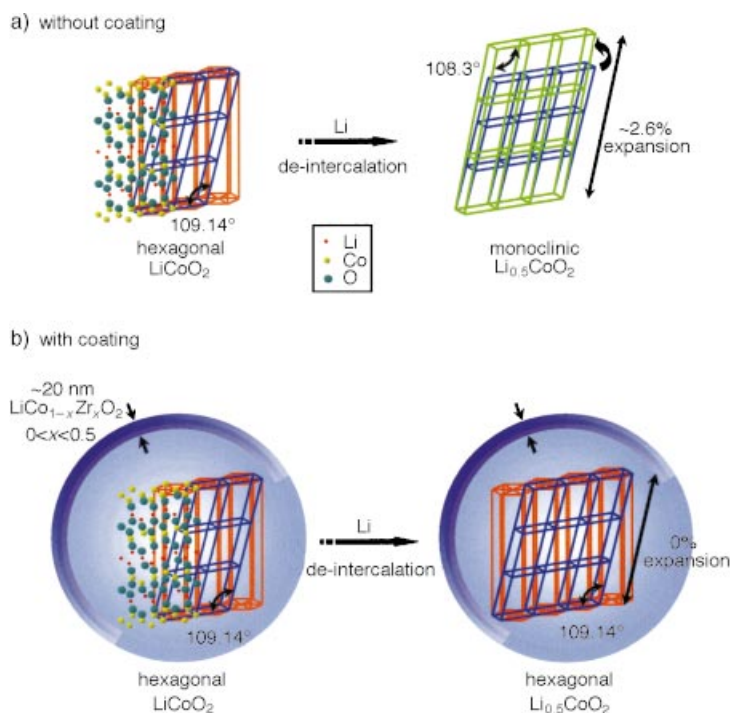


Figure 1. a) The formation of a monoclinic phase with nonuniform lattice-constant expansion ($\sim 2.6\%$) in bare LiCoO_2 during charging (Li de-intercalation), and b) the suppression of phase transition from hexagonal to monoclinic phase by a fracture-toughened thin-film metal-oxide coating.

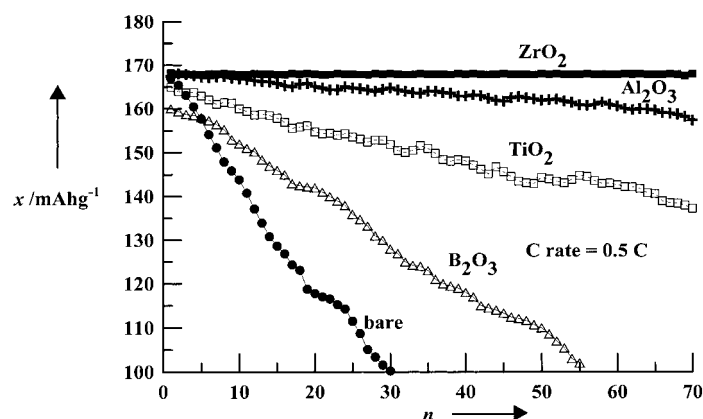


Figure 2. The cycle-life performances for ZrO_2 -, Al_2O_3 -, TiO_2 -, B_2O_3 -coated, and uncoated (bare) LiCoO_2 . The cells were initially cycled at the rate of 0.1 C, followed by 0.5 C rate between 4.4 and 2.75 V at 21°C (n : cycle number, x : discharge capacity).

addition, the ZrO_2 -coated sample does not show any noticeable capacity fading over 70 cycles, thereby retaining its original capacity. In contrast, bare LiCoO_2 has $\sim 60\%$ of its original capacity after only 30 cycles.

The lattice constant *c* as a function of *x* in hexagonal $\text{Li}_{1-x}\text{CoO}_2$ from $x = 0$ to 0.7 during the first charge is shown in Figure 3. Even though the lattice constant *a* shows a little variation during charging in both the bare and coated $\text{Li}_{1-x}\text{CoO}_2$, the lattice constant *c* shifts from $x = 0$ to 0.7, depending on the choice of metal-oxide coating. The invariability of *c*-axis dimension correlates extremely well with the fracture toughness of the coated oxides, in the order $\text{ZrO}_2 > \text{Al}_2\text{O}_3 > \text{TiO}_2 > \text{B}_2\text{O}_3$. This result confirms that suppression of

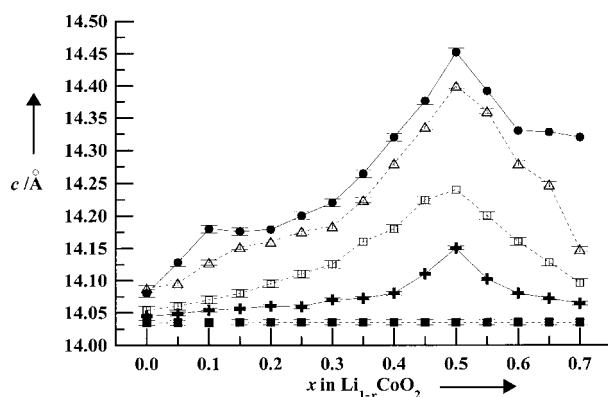


Figure 3. Lattice constants c in ZrO_2 (■), Al_2O_3 (+), TiO_2 (□), B_2O_3 (△) coated, and bare LiCoO_2 (●) as a function of x in $\text{Li}_{1-x}\text{CoO}_2$ during the first charge (c = lattice constant).

nonuniform strain during delithiation produces a new-class of cathode materials, which are extremely tolerant to electrochemical cycling. Note that the lattice constant c of the ZrO_2 -coated LiCoO_2 exhibits negligible shift in the range $0 < x < 0.7$, which indicates that the original hexagonal symmetry is unaffected by delithiation. A sol–gel coating of ZrO_2 , which has the highest fracture toughness, leads to the formation of a fracture-toughened thin-film solid solution (tens of nm thick) near the particle surface. This film significantly improves the structural stability of the cathode material by suppressing c -axis expansion (or phase transition), thereby preventing capacity fading during electrochemical cycling.

In conclusion, the encapsulation of LiCoO_2 powders by thin-film coating of high-fracture-toughness oxides effectively suppresses the lattice-constant changes during electrochemical cycling and thereby suppresses phase transitions. The order of capacity retention correlates well with the fracture toughness of the coated thin-film oxides. This innovative technology can be applied to any cathode material that has an accompanying lattice strain (or phase transition) during cycling. The commercial potential of a rechargeable Li-ion battery is enormous.

Experimental Section

Metal (or boron) ethylhexanate-diisopropoxide ($\text{M}(\text{OOC}_8\text{H}_{15})(\text{OC}_3\text{H}_7)_2$) was dissolved in 2-propanol, and stirred continuously for 20 h at 21 °C. The LiCoO_2 powder was then mixed with the coating solution, and the resulting solution was further aged at 50 °C for 24 h to give strong bonds between the metal-oxide (or boron-oxide) gel and the LiCoO_2 particle surface. After drying, each coated LiCoO_2 powder was fired at 400 °C for 10 h.

To test the cycle-life performance of each cathode material, a slurry of the desired viscosity was prepared by mixing the cathode materials, super P carbon black, and a poly(vinylidene fluoride) (PVDF) binder in a weight ratio of 92:4:4 in N -methyl-2-pyrrolidene (NMP; water content was below 0.01 wt %). The coin-type half cell (2016-size) contained a test cathode, a lithium metal counter-and-reference electrode, a 15 μm thick microporous polyethylene separator, and an electrolyte solution of 1M LiPF_6 in EC/DMC (1:1 vol % EC = ethylene carbonate, DMC = dimethyl carbonate). Each cell was aged for 24 h at room temperature prior to commencing the electrochemical tests at 21 °C. The coin-type half cells in Li/LiCoO_2 were charged with 0.1 C rate to the pre-determined voltages, then potentiostated for 10 h until the current density was equivalent to 0.01 C.

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Aromatic Mercury Clusters in Ancient Amalgams**

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Mercury and its alloys, named amalgams, were known to the Ancients. Amalgams were used for metal extraction from about 500 BC and one of the most interesting influences of

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amalgams (many of which have a golden appearance) on human history was the idea of transmutation (the conversion of base metal into gold). Efforts of transmutation flourished during the time of alchemy, when many gullible rulers of that time found themselves in embarrassing circumstances thanks to the golden appearance of the amalgams. A wide range of alkali metal amalgams is now known with different stoichiometries and structures.^[1] Low mercury-content amalgams contain isolated Hg atoms or clusters solvated by the alkali atoms, whereas high mercury-content amalgams tend to have network structures with the alkali atoms occupying interstitial positions. A substantial amount of charge transfer is believed to occur from the alkali metal atoms to mercury in the amalgams. One may think that amalgams, having such a rich and long history, should be well understood today. However, as we will show herein, that is not the case, and the chemistry, structure, and bonding of the amalgams are still not completely elucidated.

What we want to address here is a class of amalgams that contain Hg_4^{6-} square units as their building blocks, typified by that found in solid Na_3Hg_2 (Figure 1).^[1,2] Why Hg_4^{6-} is a

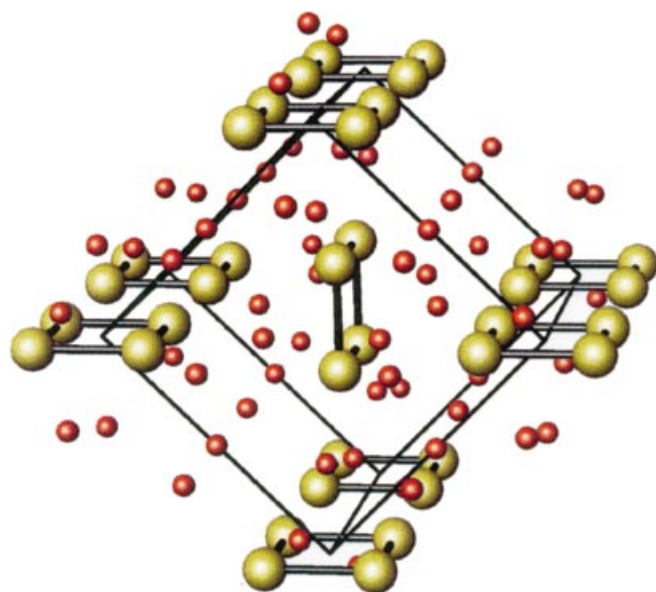


Figure 1. Crystal structure of Na_3Hg_2 , showing the Hg_4 square units (yellow) surrounded by Na (red).

particularly stable building block for the amalgams and why it assumes a perfect square structure are questions that have not been understood. To the best of our knowledge, Corbett was the first to examine these questions using molecular orbital (MO) calculations.^[3] More recently the electronic structure of mercury amalgams has been studied by Liao using relativistic density functional calculations.^[4] However, the aromatic character of the Hg_4^{6-} square units were not discussed. We have obtained a key insight into these questions through the recent discovery of aromaticity in purely metallic clusters.^[5–7] We concluded that the square-planar Hg_4^{6-} ion is directly related to this finding and its structure and stability can in fact be attributed to aromaticity.

We recently reported experimental and theoretical evidence of aromaticity in an all-metal system, the Al_4^{2-} ion in a series of bimetallic and ionic clusters, MAl_4^- ($\text{M} = \text{Li}, \text{Na}, \text{or Cu}$).^[5] The Al_4^{2-} ion, both as an isolated species and in the bimetallic clusters, was found to be square-planar and to possess two delocalized π electrons, thus conforming to the structural criterion and the $(4n+2)$ electron-counting rule for aromaticity. A simple electron counting for the Hg_4^{6-} ion immediately revealed that it is isosteric and isoelectronic with the Al_4^{2-} ion if we ignore the d electrons, which can be considered as part of the core. The analogy between Hg_4^{6-} and Al_4^{2-} suggests that the former may be aromatic as well, thus explaining its unique stability and structure.

To confirm this conjecture we performed ab initio calculations on an isolated Hg_4^{6-} ion, as well as on a model neutral Na_6Hg_4 cluster and a known aromatic hydrocarbon analogue $\text{C}_4\text{H}_4^{2+}$.^[8] While the isolated Hg_4^{6-} ion is not expected to be electronically stable as a gaseous species, it is a common practice in chemistry to divide a crystal into building blocks including highly charged anions.^[9] For example, *nido*- Ti_4^{8-} is accepted as a building block in Na_2Ti .^[10] These charges are only formal charges and do not represent the real electron transfers in the crystals. But they are very important for the proper electron counting in chemistry and for understanding the structure and bonding of the solid building blocks. To test the effects of the counterions on the MOs and bonding on the Hg_4^{6-} building block in the Na_3Hg_2 crystal, while keeping the same electron count, we further performed calculations on a model Na_6Hg_4 cluster. In our calculations for Na_6Hg_4 we used three theoretical methods: a hybrid of Hartree–Fock and density functional methods (B3LYP), a second-order Møller–Plesset perturbation method (MP2), and a complete active space multiconfigurational self-consistent field (CASSCF) calculation, including 103 740 electronic configurations.^[11] In all calculations we used the triple-zeta valence basis sets and Stevens–Basch–Krauss effective core potentials (CEP-121G).

Since the purpose of the Na_6Hg_4 calculations was not to determine its global minimum structure, but rather to examine how the presence of Na^+ ions would influence the MOs and bonding in Hg_4^{6-} , it was important to ensure that the Hg_4 unit in the Na_6Hg_4 model has the same symmetry as in the naked Hg_4^{6-} . Our geometry optimization with the D_{4h} symmetry restriction led to the square Hg_4 unit with $R(\text{Hg}–\text{Hg}) = 2.9–3.1 \text{ \AA}$, depending on the methods used. This calculated Hg–Hg bond length in the model Na_6Hg_4 cluster is actually very close to the value of $3.0 \text{ \AA}$ for the square Hg_4 cluster observed in the Na_3Hg_2 crystal.^[2] This bond length is also very close to the optimized Hg–Hg bond ($2.9–3.1$) in a linear $\text{Na}–\text{Hg}–\text{Hg}–\text{Na}$ molecule. The covalent Hg–Hg bonding in the Na_2Hg_2 molecule is undoubted and therefore the current result lends credence to the Hg–Hg covalent bonding in the model Na_6Hg_4 cluster and the Na_3Hg_2 crystal.

To examine the occupation of MOs we further performed CASSCF calculations on both Hg_4^{6-} and Na_6Hg_4 . These calculations show that the Hartree–Fock wave function is predominate in the CASSCF expansion ($C_{\text{HF}} = 0.892$), and the occupation numbers for the highest three Hartree–Fock orbitals are close to two, 1.84 e ($2a_{1g}$), 1.87 e ($1b_{2g}$), and 1.85 e

($1a_{2u}$). These results indicate that our one-electron MO picture can be used to understand the bonding of the Hg_4^{6-} cluster in the Na_3Hg_2 amalgams. There is considerable charge transfer from Na to Hg and the model Na_6Hg_4 cluster can be viewed formally as a Hg_4^{6-} cluster solvated by six Na^+ ions. Figure 2a displays the seven valence MOs of the square-planar Hg_4^{6-} and Figure 2b displays those of the Na_6Hg_4 model system. Comparing Figure 2b to 2a, we see clearly that the same set of occupied MOs is present in both cases. The counterions appear to have little effect on the occupied MOs of the Hg_4^{6-} building block; they alter slightly the MO ordering. The MOs shown in Figure 2a are identical to those of Al_4^{2-} , except for the slight difference in ordering among the top three MOs, which are bonding combinations of the valence p orbitals. The bottom four MOs are combinations of the valence s orbitals, which are essentially lone pairs in both Hg_4^{6-} and Al_4^{2-} with little s–p mixing. Based on the presence of the same two π electrons and its square-planar structure, we concluded that Hg_4^{6-} is indeed an aromatic all-metal cluster. To further confirm the aromatic nature of Hg_4^{6-} , we compare its MOs and bonding to those of an aromatic hydrocarbon $C_4H_4^{2+}$, as shown in Figure 2c. $C_4H_4^{2+}$ has four more valence electrons than Hg_4^{6-} . We observed that except for the degenerate HOMO-1 in $C_4H_4^{2+}$, the remaining seven valence MOs of $C_4H_4^{2+}$ are nearly identical to those of Hg_4^{6-} , with its characteristic aromatic π HOMO.

Mercury has a closed shell electron configuration ($6s^2$) and neutral Hg_4 is a van der Waals cluster (the bottom four MOs of Figure 2a or 2b provide no net bonding). The top three MOs of Hg_4^{6-} are completely bonding orbitals formed by perpendicular in-plane p_σ orbitals ($1b_{2g}$), out-of-plane p_π orbitals ($1a_{2u}$), and radial p_σ orbitals ($2a_{1g}$), which are filled owing to electron donation from the alkali metals. On the other hand, the eight σ MOs in $C_4H_4^{2+}$ actually describe four C–C bonds and four C–H bonds, consistent with the classical bonding picture for this saturated hydrocarbon molecule. Compared to $C_4H_4^{2+}$, the aromatic Hg_4^{6-} and Al_4^{2-} species are electron-deficient systems because we do not have four σ MOs to describe four two-electron two-center metal–metal bonds, as the square structure would suggest. Instead, in Hg_4^{6-} and Al_4^{2-} , we only have two four-center σ MOs describing the metal–metal bonding, in addition to the π MO. These multicenter σ bonds have been called σ aromaticity.^[12] Hence, the structural and electronic stability of the square-planar Hg_4^{6-} and Al_4^{2-} should be attributed not only to π aromaticity due to the presence of the two π electrons in the

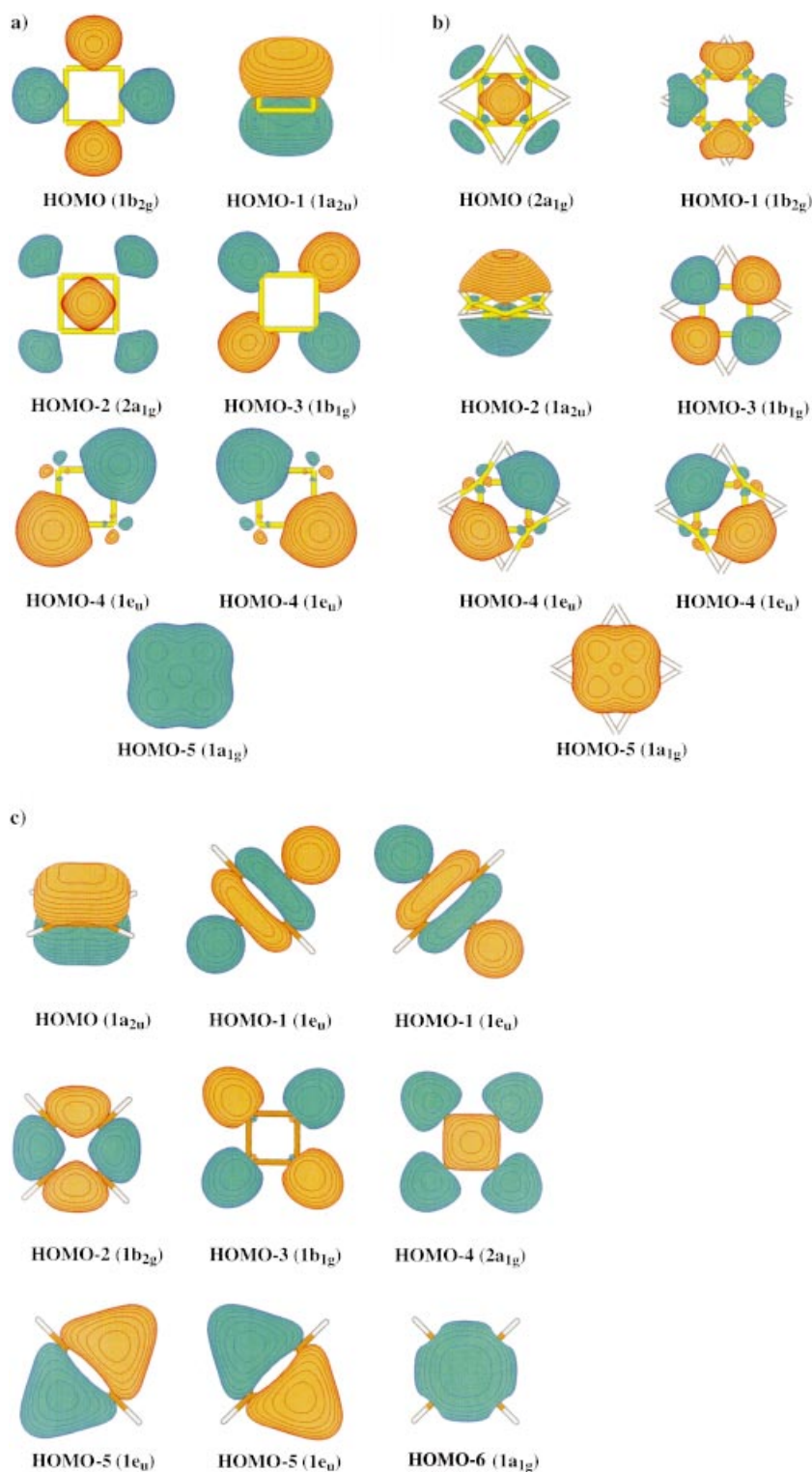


Figure 2. a) Molecular orbital pictures of square-planar Hg_4^{6-} , showing the highest occupied molecular orbital (HOMO, $1b_{2g}$) and the aromatic π orbital (HOMO-1, $1a_{2u}$) down to the fifth valence molecular orbital from the HOMO (HOMO-5, $1a_{1g}$). Note that (HOMO-4) consists of a degenerate pair ($1e_u$). b) Molecular orbital pictures of a model system, Na_6Hg_4 , containing a square-planar Hg_4^{6-} . Note the similarity between the MOs in the two systems. c) Occupied molecular orbitals of the aromatic hydrocarbon, $C_4H_4^{2+}$.

$1a_{2u}$ orbital, but also to σ aromaticity due to the occupation of the two four-center σ -bonding orbitals, $1b_{2g}$ and $2a_{1g}$.

Although an analogous aromatic Ga_4^{2-} structural unit has been recently suggested by us to exist in an organometallic

compound,^[7] bulk solid materials containing the aromatic Al_4^{2-} ion have not been synthesized. Nevertheless, the current finding of aromaticity in the isoelectronic Hg_4^{6-} ion establishes a solid bridge between our gas-phase studies of aromatic clusters and bulk materials containing such species. It is a pleasant surprise that new insight can still be obtained into such an ancient and well-known material as the amalgam. It is possible that further development in the concept of aromaticity in metallic systems, through gas-phase clusters and model investigations, will lead to discovery of new classes of materials or new insights into the electronic structures and chemical bonding of complex materials.^[13]

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Allosteric Fluoride Anion Recognition by a Doubly Strapped Porphyrin**

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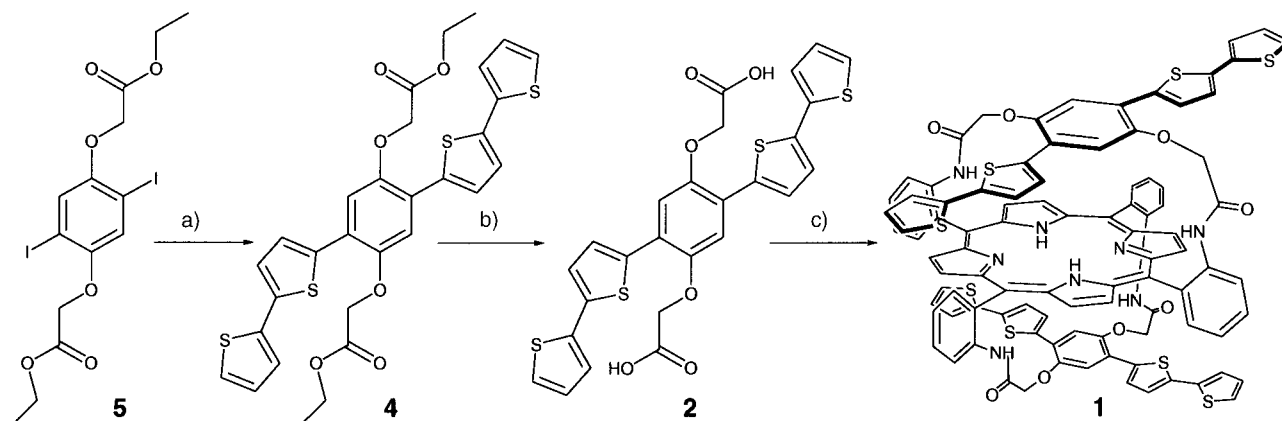
The design of anion receptors with high selectivity is challenging.^[1–6] Most systems developed use complimentary binding sites with hydrogen-bonding groups, quaternary ammonium centers, Lewis acids, and cationic metal ions.^[1–6] It is often difficult to control selectivity and sensitivity among anions because of their wide range of geometries, low charge to radii ratios, sensitivities to pH, and high solvation energies.^[1a] Receptors for the smallest anion, fluoride, are of special importance for monitoring the fluoride metabolism in nature, the analysis of drinking water,^[7] and the detection of chemical warfare agents.^[8] We report herein a highly selective allosteric fluoride recognition system^[9] by a doubly strapped porphyrin^[10] **1** that contains two small hydrogen-bonding cavities that are not able to bind larger anions. It is also demonstrated that a conducting polymer based upon **1** displays both electrochemical and conductivity responses to fluoride ions and no response to chloride ions.

Compound **1** was prepared by condensation of strap moieties **2** with $\alpha\beta\alpha\beta$ -tetrakis(2-aminophenyl)porphyrin **3** under high-dilution conditions (Scheme 1).^[11, 12] By comparison with related strapped porphyrins containing four linkers, we estimate that the cofacial distance between the phenyls in the straps and the porphyrin is between 3–4 Å.^[12] This distance creates a small pocket that is suitable for fluoride-ion binding (the radius of a fluoride anion in an octahedral environment is 1.19 Å^[1a, 13]), but should exclude larger anions. The proximate location of the strap moieties to the porphyrin in **1** is confirmed by NMR spectroscopy in CDCl_3 , which revealed significant upfield shifts of specific signals. The phenyl protons appear at $\delta = 4.24$ which is approximately 2.96 ppm upfield of the dicarboxylate strap precursor. The amide N–H protons resonate at $\delta = 7.68$, which is comparable to closely related phenyl-capped porphyrins which have an additional methylene between the amide carbonyl and the phenyl ether of the strap.^[14] These values are very different from unstrapped porphyrins where the same resonances generally appear between $\delta = 5$ and 6.5^[10, 15] and suggest that the amide N–H protons are constrained against the porphyrin core. The Soret band of **1** (5.00 μm) in dichloromethane is split into two bands of equal intensity at 404 and 423 nm, which indicate that the strap presents a significant perturbation. This

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Scheme 1. a) 5-tributylstannyl-2,2'-bithiophene, $[\text{PdCl}_2(\text{PPh}_3)_2]$, 80°C , 62 %, b) NaOH, THF/EtOH/ H_2O , RT, 92 %, c) oxalyl chloride, **3**, pyridine, THF, high-dilution conditions, RT, 37 %.

splitting is caused by π - π interactions of the bis(dithienyl)-benzene moieties with the porphyrin.^[14b, 16] The effect of solvent on the Soret bands is minimal. The spectral features were the same in THF and acetonitrile, but the Soret splitting was slightly less in DMSO (408(sh) and 424 nm).^[17] These results suggest that the solvent can not occupy the cavity defined by the straps and the porphyrin.

Upon addition of fluoride anion (as a cesium or tetrabutylammonium (TBAF) salt) to the solution of **1** (5.00 μM) in DMSO at 25°C , the split Soret band shifted to a single band at 417 nm with a simultaneous red shift of the Q band (Figure 1).^[18] The UV/Vis absorption spectra measured as a

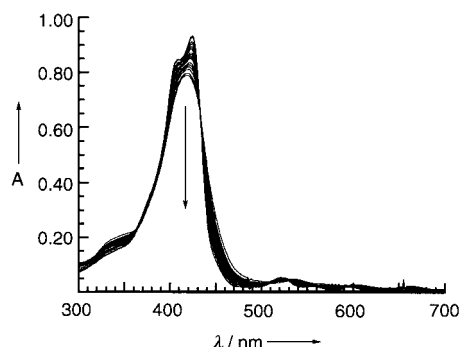


Figure 1. Absorption spectra of **1** on addition of TBAF.

function of fluoride concentration provided multiple isosbestic points visible at 364, 433, and 508 nm, which confirm that the reaction consists of only two species in equilibrium (Figure 1). No absorbance changes in either the Soret or Q band were observed upon exposure of **1** to larger anions including chloride, bromide, iodide, acetate, cyanide, and dihydrogen phosphate. Apparently only the smaller anion, fluoride, can fit in the hydrogen-bonding cavity of **1**. Noteworthy is that plots of the absorbance changes at 424 nm versus tetrabutylammonium fluoride (TBAF) concentration show a sigmoidal curve, which indicates that the binding of fluoride anion to **1** is cooperative (positive, homotropic allosterism; Figure 2). This cooperative guest binding was analyzed with the Hill equation [Eq. (1)].^[19]

$$\lg(y/(1-y)) = n \lg[\text{guest}] + \lg K \quad (1)$$

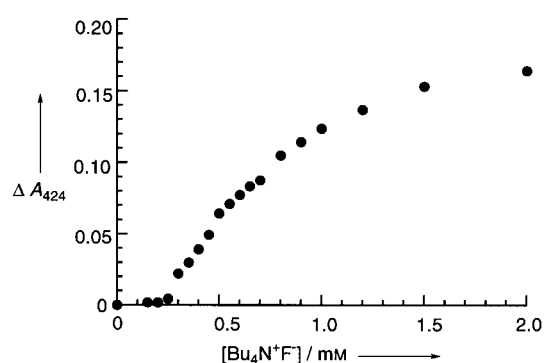


Figure 2. Plots of absorbance at 424 nm for **1** versus [TBAF].

K and n are the association constant and Hill coefficient, respectively, and $y = K/([\text{guest}]^{-n} + K)$. From the slope and the intercept of the linear plot, we obtained $\lg K = 6.6$ and $n = 2.0 \pm 0.1$ (correlation coefficient 0.997). We utilized Scatchard plots (see Supporting Information) to further characterize the binding. In these plots Hill coefficients (n) are correlated with the maximum values (y_{max}) with $n = 1/(1 - y_{\text{max}})$, and positive and negative allosterisms are expressed by the upward and downward curvatures, respectively.^[19] Consistently the y_{max} values for fluoride revealed a maximum at 0.5 with upward curvature. Given the observed 1:2 binding stoichiometry we computed energy-minimized geometries of **1** and **1**(F^-)₂.^[20] As shown in Figure 3 these results suggest that the cavities in **1** are contracted, a result of favorable π - π interactions between the strap band and the porphyrin (π -stacking distance of 4.0 Å). The calculated structure with two bound fluorides causes an expansion of the cavity that

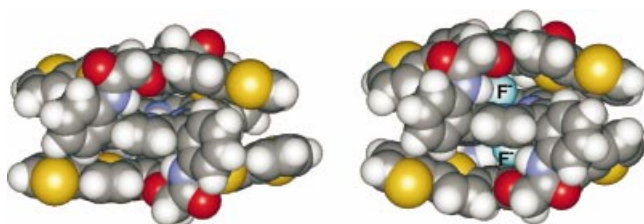


Figure 3. Energy-minimized structures of **1** (left) and **1**(F^-)₂ (right). Yellow: sulfur, red: oxygen, blue: nitrogen, and light blue: fluoride.

separates the planes of the porphyrin and the straps by 4.9 Å. This increase in distance is consistent with the transformation of the split Soret band into a single band and supports our assertion that the strap–porphyrin interactions are responsible for the splitting. The computations place the fluoride 3.6 Å from the amide N–H protons and 2.3 Å from the pyrrole N–H protons. Typical H···F hydrogen bonds range from about 2.3–2.5 Å and hence we propose that ion–dipole interactions between amide N–H and fluoride assist the hydrogen bonding between pyrrole N–H and fluoride. Our experimental findings clearly indicate that binding of a first fluoride anion facilitates a second binding. To account for this effect, we suggest the possibility of bridging F···H···F interactions through the center of the macrocycle and that the first binding distorts the macrocycle from planarity and thereby favors the binding of the second fluoride ion (Scheme 2).

To demonstrate the high selectivity of **1** to the fluoride ion we examined the influence of a second coexisting halide ion on the UV/Vis spectrum of the **1**(F[−])₂ complex. The UV/Vis spectrum of **1**(F[−])₂ ([**1**] = 5.00 μM, [fluoride] = 2.00 mM) was essentially unaffected by the added halides up to concentrations of 200 mM. From these studies the affinity of **1** to fluoride is estimated to be at least 10⁴ times higher than for other halogens. It is likely that both the allosterism and the cavity size make contributions to the fluoride selectivity toward other anions.

Compound **1** (0.05 mM in 0.1M TBAPF₆ in CH₂Cl₂) readily electropolymerized to give conducting films upon cycling (100 mVs^{−1}) electrodes between 0 and 1.1 V versus the Ag/Ag⁺ couple. Films of poly**1** thick enough for in situ conductivity studies on interdigitated microelectrodes (Figure 4) were formed in 10 cycles and the maximum conductivity was determined to be 6 S cm^{−1}.^[21, 22] The films are orange when neutral and green when oxidized. Poly**1** is highly cross-linked because of the presence of the four unsubstituted sites, α to the sulfur on the terminal thiophenes, that are active toward polymerization. The multiple sites for oxidative coupling are also responsible for the rapid growth of films. The cyclic voltammogram is a composite of the electrochemistry of the poly(bis(dithienyl)phenylene) and the porphyrin. The lower potential peak at ≈0.65 V, attributed to the conjugated polymer, also displays broad featureless electroactivity at higher potentials, a feature that is consistent with its highly

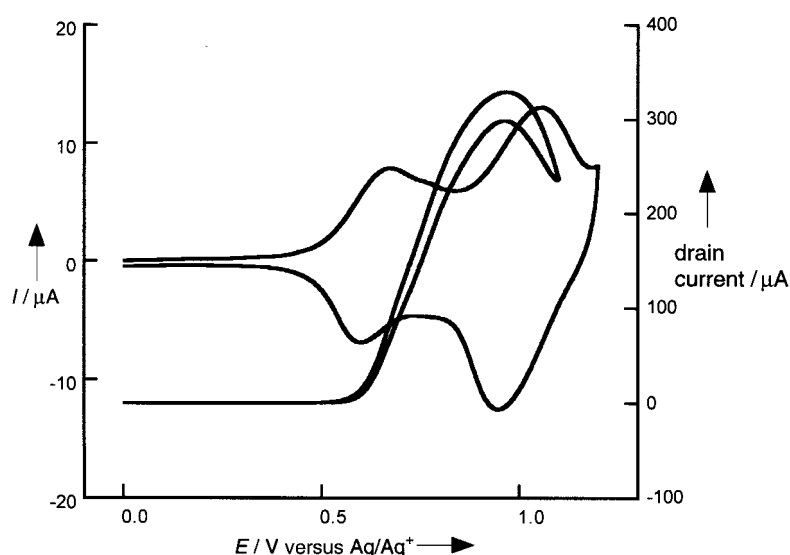


Figure 4. Cyclic voltammogram and conductivity profile using interdigitated microelectrodes (10 mVs^{−1}, offset potential of 40 mV) of a deposited film of poly**1** in CH₂Cl₂.

conductive delocalized nature. Superimposed upon this electroactivity is a redox wave at about 1 V that is assigned to the porphyrin. This potential is consistent with those reported for other porphyrins.

Treatment of poly**1** with 0.3 mM F[−] ion shifted the redox waves associated with the porphyrin (Figure 5) to lower potentials. Interestingly the electrochemical behavior of the conjugated polymer was largely unaffected. The negative shift

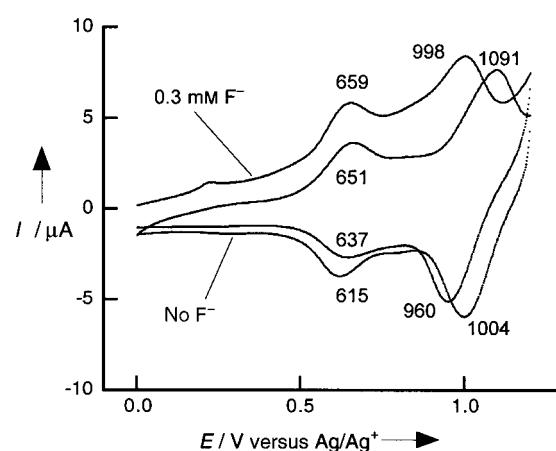
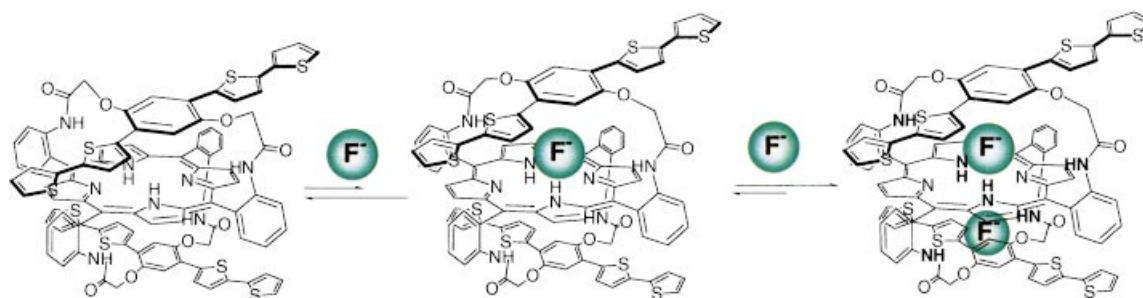


Figure 5. Cyclic voltammogram of poly**1** in 0.1M TBAPF₆ in CH₂Cl₂ with and without 0.3 mM F[−] ion. The peak potentials (mV) are labeled in the plot.



Scheme 2. Sequential binding of the fluoride ions.

of the redox potential of the porphyrin is consistent with the presence of an anion, which will stabilize proximate positive charge. Additionally, the insensitivity of the conjugated polymer is in accord with our model for fluoride-ion binding, Figure 3. In spite of the lack of interaction of the fluoride with the conjugated polymer the conductivity of the polymer is dramatically and irreversibly reduced (<50 fold reduction) upon fluoride-ion binding. The irreversible behavior can be attributed to the large binding constant, which will result in slow kinetics for the removal of the fluoride from the film. However, it is not clear why this system displays such a greatly reduced conductivity while the electrochemical response of the conjugated polymer appears unchanged. It is possible that the large effect is a result of the bulk transport properties of the conducting polymer being much more sensitive to the presence of fluoride than the electrochemical potential. We have previously found evidence for such effects in conducting polymers containing alkali metal ion receptors.^[23] However, the irreversible nature of this effect precludes the elimination of other possibilities such as the formation of cracks in the films or large interfacial resistances in response to fluoride binding.

In conclusion, we have demonstrated that **1** is a novel scaffold for the selective fluoride sensing, and shows positive homotropic allosterism with Hill coefficients of 2.0. Moreover, **1** has been easily transformed into highly conducting polymer poly**1** (6 Scm⁻¹) coatings on electrodes that show a composite electrochemical response of the polymer backbone and the porphyrin ring. Treatment of the polymer with fluoride ion produced a shift of the porphyrin electrochemistry and a large reduction in the materials conductivity. Such rational amalgamations of molecular-wire sensor schemes^[24] and allosteric systems^[9] represent a powerful design scheme for the construction of highly sensitive and selective sensory systems.

Experimental Section

General procedures: Reactions were carried out in oven-dried glassware using standard Schlenk techniques under an inert atmosphere of dry argon. ¹H NMR and UV/Vis spectra were recorded with a Varian UN-300 or Mercury 300 spectrometer and a Hewlett-Packard 8453 diode array spectrophotometer, respectively. Electrochemical measurements were performed using a computer controlled Autolab Model PGSTAT 20 potentiostat from Eco Chemie. A platinum coil was used as a counter electrode, and 5 micron spaced interdigitated microelectrodes purchased from ABTECH were used as working electrodes. Compound **5** was synthesized from the 2,5-diiodo-1,4-dihydroxybenzene and ethyl bromoacetate in DMF according to the reported method.^[25]

Synthesis of **4**: Compound **5** (530 mg, 1 mmol), 5-tributylstannyl-2,2'-bithiophene (1.14 g, 2.5 mmol), and *trans*-[PdCl₂(PPh₃)₂] (35 mg) were dissolved in dry DMF (40 mL) and the mixture was stirred overnight at 80 °C. The reaction mixture was poured into aqueous NH₄Cl solution (100 mL) and extracted with diethyl ether (150 mL). The ether layer was washed with brine (50 mL, three times) and dried over anhydrous MgSO₄. After evaporation of the solvent, the yellow residue was purified by chromatography (silica gel, CH₂Cl₂/hexane 9/1) to produce **4** (380 mg, yield 62 %). ¹H NMR (300 MHz, CDCl₃): δ = 1.34 (t, *J* = 7.2 Hz, 6H), 4.32 (q, *J* = 7.2 Hz, 4H), 4.74 (s, 4H), 7.03 (m, 2H), 7.20 (m, 8H), 7.59 (d, *J* = 3.6 Hz, 2H); HR-MS (ESI): for C₃₀H₂₇O₆S₄ [M+H]⁺ (calcd): 611.0690 (611.0680).

Synthesis of **2**: Compound **4** (200 mg, 0.33 mmol) was dissolved in THF/ethanol (15/1, 480 mL) solution and NaOH (130 mg; 10 equiv) in H₂O (5 mL) was added to the mixture. The reaction mixture was stirred for 24 h

at room temperature and 1M HCl solution (50 mL) and brine (50 mL) were added. The organic layer was extracted with diethyl ether (150 mL) and then dried over anhydrous MgSO₄. Evaporation to small volume afforded compound **2** as yellowish orange powder in 92 % yield. This compound was used for next reaction with no further purification. HR-MS (ESI): for C₂₆H₁₉O₆S₄ [M+H]⁺ (calcd): 555.0064 (555.0074).

Synthesis of **1**: In a 500 mL flask equipped with a stir bar, dry THF (350 mL) and dry pyridine (5 mL) were introduced. In another flask, compound **2** (158 mg, 0.285 mmol) was treated with oxalyl chloride (1.5 mL, large excess) in dry THF (30 mL) containing 1 drop of DMF at 60 °C for 1 h to yield the diacidchloride derivative of **2**. After removal of THF and excess oxalyl chloride under vacuum, dry THF (40 mL) was added and this solution was loaded in two 20 mL syringes. A third syringe was loaded with a dry THF solution (20 mL) of compound **3**^[15a, 26] (96 mg, 0.142 mmol). The simultaneous addition of the two reagents was driven by a syringe pump over 12 h, and the reaction mixture was stirred for an additional 24 h at room temperature. The solvent was removed by evaporation, then the red residue was purified by chromatography (silica gel, CHCl₃/acetone 98/2 and CHCl₃/ethyl acetate 95/5) to give **1** (90 mg, yield 37 %) as a brownish red powder. M.p. > 300 °C; ¹H NMR (300 MHz, CDCl₃): δ = -3.47 (s, 2H), 3.16 (d, *J* = 16.7 Hz, 4H), 4.15 (d, *J* = 16.7 Hz, 4H), 4.24 (s, 4H), 5.26 (d, *J* = 3.9 Hz, 4H), 6.74 (d, *J* = 3.8 Hz, 4H), 7.14–7.20 (m, 8H), 7.36 (d, *J* = 3.7 Hz, 4H), 7.67 (s, 4H), 7.72–7.79 (m, 8H), 8.52 (d, *J* = 8.3 Hz, 4H), 8.62 (dd, *J* = 3.5, 10.8 Hz, 8H); HR-MS (ESI) for C₉₆H₆₃N₈O₈S₈ [M+H]⁺ (calcd): 1711.2558 (1711.2534); UV/Vis (DMSO): λ_{max} (lg ε) = 408 (5.23), 424 (5.27), 521 (4.03), 555 (3.64), 598 (3.62), 653 nm (3.43).

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- [18] The titration using the fluoride salts introduced stoichiometric amounts of water which is known to interfere with fluoride complexation.^[1] In this system, no absorbance changes in either the Soret or Q band was observed upon exposure of **1** to 5% (v/v) water. Moreover 40% of $\text{1(F}^-\text{)}_2$ complex formed even when 3% (v/v) (1.67M) of water was added to the complex solution ($[\text{1}] = 5.00 \mu\text{M}$, $[\text{fluoride}] = 2.00 \text{ mM}$).
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Radical Shuttling in a Protein: Ribose Pseudorotation Controls Alkyl-Radical Transfer in the Coenzyme B₁₂ Dependent Enzyme Glutamate Mutase**

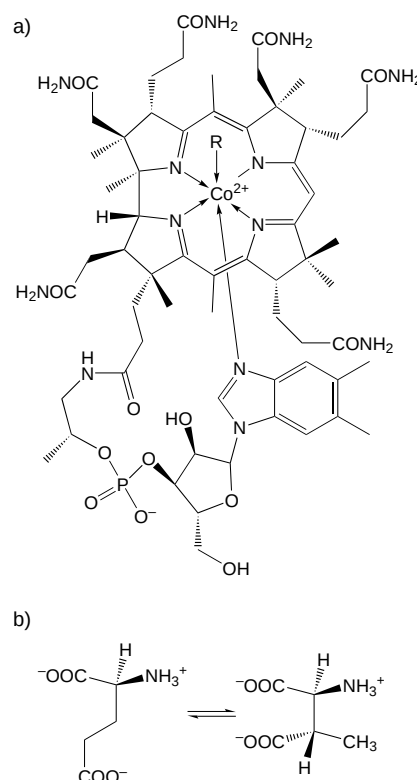
Karl Gruber,* Riikka Reitzer, and Christoph Kratky

Enzymatic reactions involving organic-radical intermediates are relatively rare, and occur when alternative (heterolytic) pathways would lead across prohibitively high activation barriers, for example, reactions at unactivated carbon centers.^[1] Because of their substantial and unspecific reactivity, organic-radical intermediates have to be scrupulously controlled by the enzyme to prevent side reactions that may result in enzyme inactivation or damage, unwanted products, or the release of toxic radicals. Once a radical is generated, the major problem for the enzyme is to guide the “hot” intermediate along the desired reaction path, and not the enhancement the rate of subsequent steps. This constitutes Rétey’s concept of “negative catalysis”.^[2]

In coenzyme B₁₂ dependent enzymes^[3] (Scheme 1 a) a catalytically relevant 5'-desoxyadenosyl (Ado) radical has long been known to be one of the products of substrate-induced homolytic cleavage of the cobalt–carbon bond of the cofactor.^[4] This class of enzymes can therefore be regarded as a prime example of enzymatic utilization of an organic radical.

The Ado radical, formed by homolytic cleavage of the Co–C bond, abstracts a hydrogen atom from the substrate in the next reaction step. While this hydrogen abstraction involves, in some cases, the intermediate formation of a protein-bound thiyl radical (e.g. in ribonucleotide reductases),^[5] direct interaction between the Ado radical and the substrate has been implicated for the majority of coenzyme B₁₂ dependent enzymes. Whereas the first steps are conceptually similar in all coenzyme B₁₂ dependent enzymes, the subsequent transformation of the substrate-derived radical to yield a product-related radical differs, although there are some mechanistic similarities.^[6]

A long-standing question concerns the way in which the enzyme couples cleavage of the cobalt–carbon bond with substrate binding. In the case of methylmalonyl-CoA mutase^[7,8] this coupling has been attributed to a crystallographically detected substrate-induced conformational change. Equally important for the catalytic mechanism is the safe



Scheme 1. a) Structural formula of some B₁₂ derivatives: 5'-desoxyadenosylcobalamin (coenzyme B₁₂, R = 5'-desoxyadenosyl[−]), methylcobalamin (R = CH₃[−]), cyanocobalamin (vitamin B₁₂, R = CN[−]), and cob(II)alamin (B_{12r}, R = e[−]). Note that the structural formula given correspond to the cofactor in solution. Upon binding to glutamate mutase, the dimethylbenzimidazole base is replaced by a protein-derived imidazole ring. b) Reaction catalyzed by glutamate mutase.

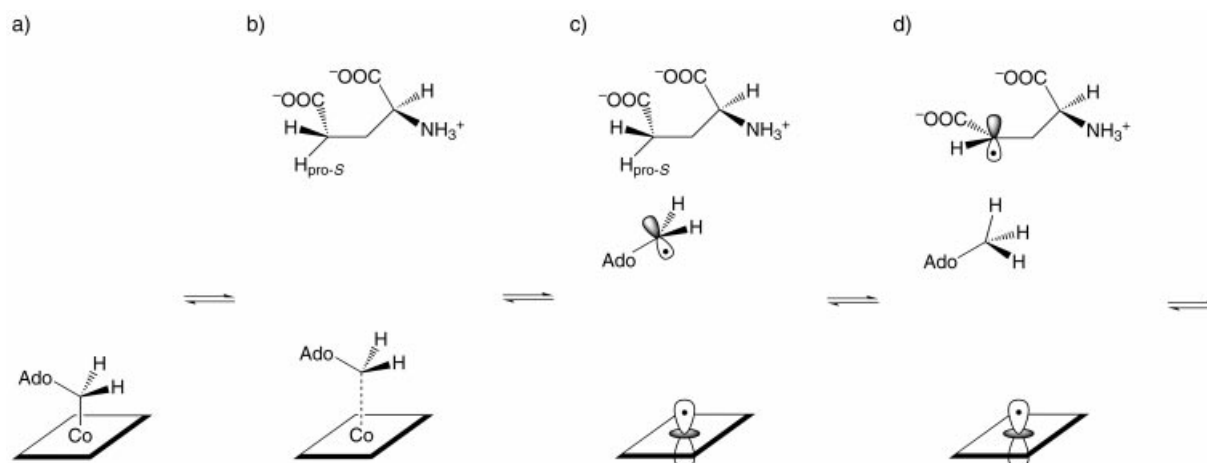
transfer of the “hot” C₅-methylene radical from the cobalt to the substrate (Scheme 2). Structural^[7,9,10] and EPR spectroscopy data^[11–13] indicate that the reaction partner for this radical is 6–8 Å away from the cobalt center and kinetic data further suggest that the Ado radical is only present as an extremely short-lived intermediate.^[14,15]

The coenzyme B₁₂ dependent glutamate mutase (Glm) catalyzes the interconversion of (S)-glutamate to (2S,3S)-3-methylaspartate (Scheme 1b). We recently determined the three-dimensional structure of the enzyme from *Clostridium cochlearium* by using inactive enzyme reconstituted with cyanocobalamin (vitamin B₁₂) and methylcobalamin^[9] (Scheme 1a). Here, we report the crystal structure analysis at 1.9 Å resolution of active Glm with 5'-desoxyadenosylcobalamin (coenzyme B₁₂, Scheme 1) as cofactor in the presence of the substrate glutamate.

Residual electron density is clearly observed above the “upper” β-face of the corrin ring (Figure 1a) and can be attributed to the adenosyl group. However, to model the ribose portion of this density, a superposition of two species has to be invoked: species A with the ribose in a C₂-endo and species B in a C₃-endo conformation. In addition, the two conformers are shifted slightly with respect to each other and have an approximately 25° difference in the torsion angle about the glycosidic bond, which leads to a distance of 1.7 Å between the 5'-carbon atoms of the two conformers. In the

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Scheme 2. The first steps along the reaction sequence catalyzed by glutamate mutase: a) the B_{12} cofactor without substrate; b) substrate binding induces Co–C homolysis through steric strain; c) following homolysis, the 5'-end of the Ado radical is transferred to the substrate; d) abstraction of a hydrogen atom from the substrate by the Ado radical leads to the formation of a 4-glutamyl radical and a protein-associated 5'-deoxyadenosine molecule.

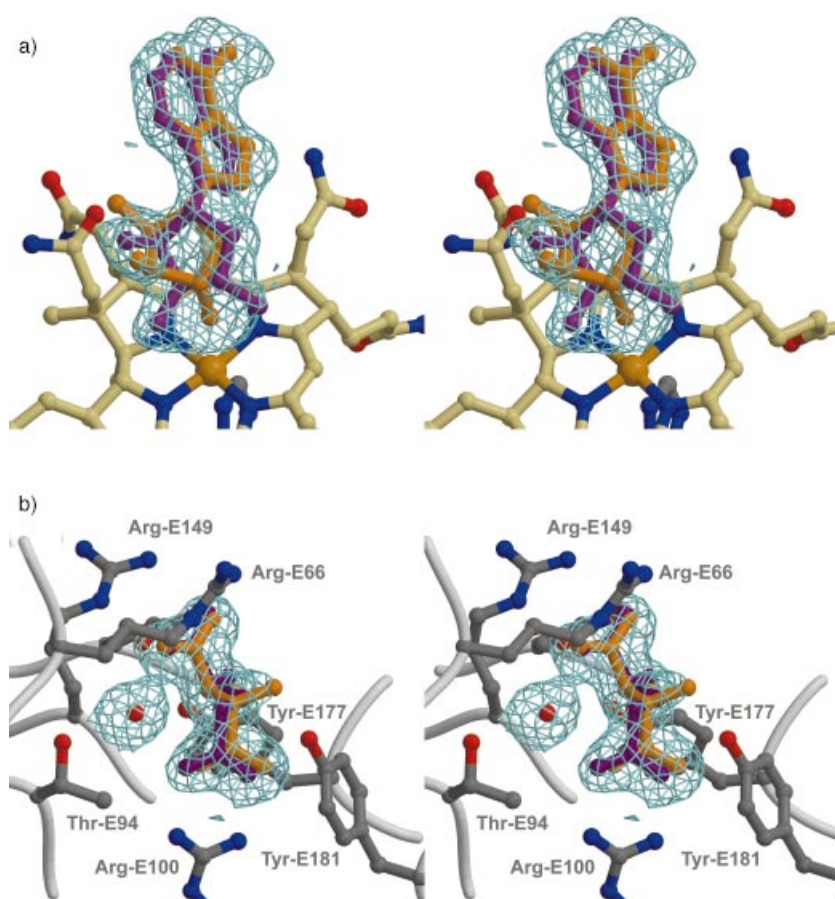


Figure 1. a) Difference electron density for the Ado ligand with the refined model of conformer A represented in orange, and conformer B in magenta. The mean B value for the ligand of $12 \text{ \AA}^2$ is comparable to the $8 \text{ \AA}^2$ observed for the surrounding protein and solvent atoms (within a maximum distance of $6 \text{ \AA}$). The largest positional differences between the two conformers are observed for O_2 ($1.7 \text{ \AA}$), O_3 ($2.9 \text{ \AA}$), C_3 ($1.3 \text{ \AA}$), and C_5 ($1.7 \text{ \AA}$). The differences for the remaining atoms of the Ado group are around $0.6 \text{ \AA}$ or less. b) Difference electron density in the substrate binding site with glutamate shown in magenta and methylaspartate in orange. The relative occupancies after refinement were 30% for glutamate and 70% for methylaspartate with a mean B value of $7 \text{ \AA}^2$. The density in (a) and (b) was calculated after the initial refinement without the Ado ligand and the substrate. Figure 1 and 2 were generated with the programs Molscript^[23] and Raster3D.^[24]

crystal investigated, conformations A and B occur in a 40:60 ratio, but different relative occupancies were found in other crystal specimens. Although the enzyme was crystallized in the presence of glutamate, the experimental density clearly shows that most of the glutamate has been converted into methylaspartate (Figure 1 b).

The distance between the 5'-carbon atom and the cobalt atom differs dramatically between the two conformers ($3.1\text{--}3.2 \text{ \AA}$ for A and about $4.5 \text{ \AA}$ for B). While C_5 is positioned above the metal center in A (Figure 2 a), it is displaced towards the substrate binding site in B (Figure 2 b), where it is within van der Waals distance from the substrate and would thus be ideally positioned for hydrogen atom exchange with the substrate.

The interaction between the Ado group and the protein is very similar for the two conformers and involves hydrogen bonds from the adenine to main-chain carbonyl atoms, to the *c*-acetamide side chain of the cofactor, and to two water molecules (Figure 2). The ribose hydroxyl groups form hydrogen bonds to Glu330, Lys326, and one water molecule. The latter hydrogen-bonding pattern differs somewhat between the two conformers (Figure 2).

The two crystallographically observed Ado species can be interpreted to correspond to intermediates along the Glm-catalyzed reaction path. If this interpretation is correct, species A is an activated cofactor molecule with a highly strained ($3.2 \text{ \AA}$ long) Co–C bond (Scheme 2b) and species B is a 5'-deoxyadenosine resulting from H-atom abstraction by the Ado radical from the substrate (Scheme 2 c).^[16]

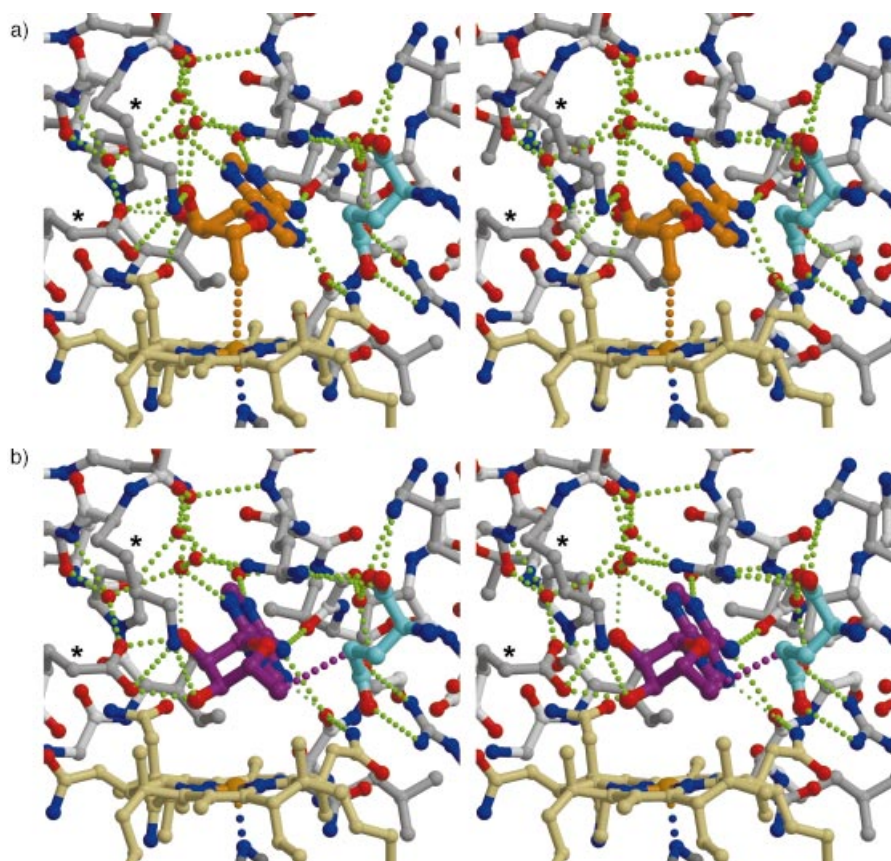


Figure 2. Stereoscopic views of the cofactor and substrate binding site and the interactions of conformers A (a) and B (b) of the adenosyl ligand. The coloring scheme for carbon atoms is as follows: protein main chain: white; side chain: light gray; Ado group: orange and magenta; corrin ring: yellow; substrate (glutamate): cyan. Nitrogen and oxygen atoms are colored blue and red, respectively. Lines of small green spheres denote hydrogen bonding interactions, lines of orange and magenta spheres indicate interactions of the ribose-C₅ with the cofactor (a) and the substrate (b), respectively, lines of small blue spheres indicate the interaction of the cobalt atom with the imidazole of His16. Residues Glu330 and Lys326 are marked with asterisks.

These two observed species suggest how glutamate mutase affords a safe and energetically facile transport of the C₅-radical atom from the cobalt center to the substrate: Ribose pseudorotation between the two minimum-energy conformations C₂-*endo* and C₃-*endo* involves a very low energy barrier,^[17] yet it rigidly constrains the trajectory of the C₅-atom with well defined endpoints. The overall position and orientation of the ribose ring is restrained by hydrogen bonds to the side chains of residues Glu330 and Lys326 (Figure 2). We believe that this discovery constitutes a perfect example for the concept of “negative catalysis”: enzymes have to exert tight control on high-energy intermediates to prevent them from entering into deactivating side reactions.^[2]

The radical-shuttling mechanism proposed here almost certainly also applies to the coenzyme B₁₂ dependent methylmalonyl-CoA mutase, for which the concept of “negative catalysis”^[2] was originally suggested. The structure of the active site (including the conserved glutamate) and the relative arrangement of cofactor and substrate^[7, 18] are strikingly similar to those of Glm. It remains to be seen which other coenzyme B₁₂ dependent enzymes have solved the radical-transfer problem in a similar fashion.^[19]

Experimental Section

Recombinant glutamate mutase from *Clostridium cochlearium* was overproduced, reconstituted with 5'-desoxyadenosylcobalamin, and purified as described previously.^[9] Crystals of up to 0.2 × 0.2 × 0.05 mm were obtained by using the sitting-drop method. Drops of 4 μL containing protein (3.5 mg mL⁻¹), coenzyme B₁₂ (0.5 mg mL⁻¹), and CdCl₂ (2 mM) were mixed with reservoir solution (4 μL containing 6% (w/v) PEG-4000 in 0.1 M Na-S-glutamate, pH 4.5) and equilibrated. Purification and crystallization were performed under red light to prevent photolytic degradation of the cofactor. This precaution was somewhat relaxed once the crystals were frozen.

Diffraction data were collected from a flash-cooled crystal (cryoprotected with 30% glycerol) at EMBL-beamline BW7B ($\lambda = 0.8439$ Å) at DESY in Hamburg, Germany, and were processed and scaled using DENZO and SCALEPACK.^[20] A starting model was obtained by using the coordinates of the cyanocobalamin reconstituted glutamate mutase (PDB-entry 1CCW)^[9] after removal of solvent, cofactor, and substrate atoms. After rigid-body refinement strong residual density was observed for the cofactor (including the Ado ligand) as well as the substrate. The completed model was refined with SHELXL-97^[21] as described previously.^[9] Details of the structure determination are listed in Table 1. Coordinates have been deposited with the Protein Data Bank with accession code 1I9C.

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Table 1. Data collection and refinement statistics.

space group	P2 ₁
unit cell dimensions	$a = 64.3$ Å $b = 113.1$ Å $c = 108.4$ Å $\beta = 96.0^\circ$
temperature	–170 °C
resolution	30–1.9 Å
observed reflections ^[a]	555 148 (30 037)
unique reflections ^[a]	121 680 (8102)
completeness ^[a] (theoretically possible reflections actually collected)	99.9% (100%)
mean $I/\sigma(I)$ ^[a]	18.4 (5.0)
R_{sym} ^[a]	0.073 (0.281)
$I > 2\sigma(I)$ ^[a]	92.1% (91.4%)
number of protein atoms	9738
number of solvent molecules (H ₂ O)	1258
number of B ₁₂ cofactor and substrate atoms	294
R_{cryst} ^[b]	0.168 (0.157)
R_{free} ^[b,c]	0.221 (0.201)
average B values	
protein	12 Å ²
solvent	24 Å ²
B ₁₂ cofactor, substrate	9 Å ²

[a] Values for the highest resolution shell (1.94–1.90 Å) are given in parentheses. [b] Numbers in parentheses correspond to observations with $I > 2\sigma(I)$. [c] Using a randomly chosen set of 5% of the reflections.^[22]

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“Carving on the Nanoscale”: Polymers for the Site-Specific Dissolution of Calcium Phosphate**

Anna Peytcheva and Markus Antonietti*

In biologically mineralized systems the constructive interaction of the inorganic crystal phase with the organic matrix material results in composite materials that are exceptional in their outward appearance and material properties.^[1, 2] Of the 60 minerals known to be “processed” by living organisms, the most frequently used are calcium carbonate, calcium phosphate, and hydrous silica. They are used for the formation of scales, prickles, shells, or endoskeletons. The mineral is usually crystallized within a protein or polysaccharide matrix which has a number of functions: it shapes and aligns the single-crystalline building blocks, and it serves as a ductile component for mechanical stress dissipation.^[3, 4] The resulting nanoscale composite structures make biominerals such as bone or teeth superior to most artificial ceramics.

One of the reoccurring motives in biomineralization is that the polymer controls the crystal structure or the biomineral shape, in that the crystal surface is stabilized by an opposite, complementary form of the polymer, which results in an overall structure of minimal energy.^[5, 6] This method can be described by ideas that originate from solid-state physics (“finding epitaxial relations”), but that do not consider polymer flexibility or adaptability. For simplicity, this class of models is called “energetical” models.

On the other hand, it is well known in biomedicine and colloid chemistry that crystals in equilibrium with a supporting aqueous phase are not static objects but are continuously reconstructed. Thus the crystal shape is the result of dynamic processes, that is, the crystal geometries which are built up fastest and dissolved slowest are those that survive. A model based upon interfacial energies and kinetics was introduced and improved over the years by Nancollas and Wu^[7] with a special emphasis on calcium phosphates. Here, the influence of a polymer or the biomatrix is purely kinetic, the structure-controlling molecules are not necessarily bound to the surface, but act by lowering the averaged surface energy, and an epitaxial fit of polymer and crystal is not required. We call this mode of operation the “kinetic” mode.

Although important for biomedicine and for controlled nanoparticle synthesis, there is still some controversy about the validity of both mechanisms. The development of scanning force microscopy (SFM) allowed the visualization of dissolution and growth processes in water in a time-resolved fashion.^[8, 9] First experiments focused on the influence of low molecular weight components such as aspartic acid^[10] or magnesium ions^[11] on the growth of calcium carbonate. Also

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protein fractions extracted from seashells^[12] or from algae^[13] have been added to growing calcite crystals so that these molecular controlling units can be observed “at work” in solution.

Herein, we focus on the biomedically relevant calcium phosphate (bone and teeth) and examine the controlled redissolution of model crystals. These crystals were grown by the double-jet technique^[14] which allows a high monodispersity and structural uniformity of the particles.^[15, 16] The observation of dissolution instead of crystallization is experimentally much more simple and also reproducible than crystal growth. The results of such experiments can be understood as a “structural inversion of kinetics”, that is, the sites and faces being dissolved fastest will never be found exposed in a crystallization experiment, whereas the remaining faces are most favored and are also those which dominate a crystal formed under similar conditions. The platelike crystals of brushite were chosen as a good model system, since they are very stable against other structural rearrangements, sufficiently thick to be not immediately dissolved, and exhibit a smooth and even (010) plane with an area of 1–2 mm² suitable as a substrate for SFM. However, we expect that the general trends found for brushite also hold for the other calcium phosphate polymorphs.

Droplets of a solution containing crystals are allowed to dry on a freshly cleaved mica surface. On top of the mica, a fluid cell for SFM is placed. Different polymer solutions are injected through a thin capillary so that they thoroughly wet the crystal surface. Images of the dissolution of the crystal surface under the influence of the polymer solution are recorded with a scan rate of 0.5 lines per second. The digital resolution of all the pictures is 256 × 256 points.

The experiments were repeated on different crystals of one sample as well as with different samples to show that the single crystal behaves as the ensemble of crystals. Thus, the presented “single site” experiments represent a sufficiently averaged limit in time and scale for the kinetic–thermodynamic results to be reproducible. This is different to mechanical atomic force microscopy (AFM) experiments with single molecules.

The scanning force microscope used is a commercial Nanoscope IIIa (Digital Instruments, Santa Barbara, USA) operating in contact mode in air and in tapping mode in liquid. It was equipped with a 100 × 100 μm J-scanner, with commercial silicon cantilevers (NT-MDT, Moscow) used in liquid at resonance frequencies of 5–40 kHz and with a radius of curvature ≤ 10 nm.

As polymers with specific mineral interactions (as opposed to the extracted, chemically uniform, biomaterial species investigated to date), two thermally polymerized poly(sodium)aspartate samples (which have been optimized for their application as lime-scale inhibitors; Rohm & Haas)^[17] with different molecular weights, as well as poly(L-)ly-

sin and bovine serum albumin (BSA) from Sigma Chemicals were chosen.

As a reference experiment, the unspecific dissolution of the (010) plane of brushite in deionized water was examined. The SFM image in Figure 1 presents the characteristic pattern

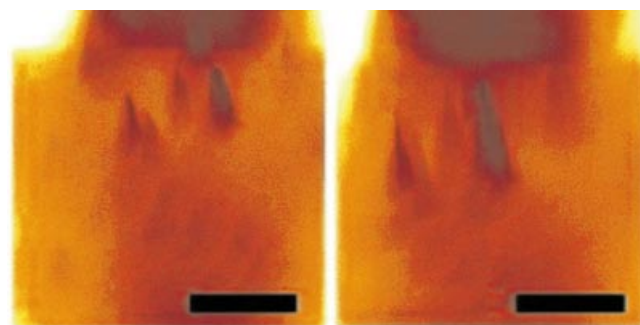


Figure 1. SFM height images of the (010) plane of brushite in deionized water, taken with a time interval of 2048 s. Scale bar = 2 μm.

which accompanies the dissolution. Triangular etch pits are framed by three characteristic steps which are parallel to the crystallographic directions (201), (001), and (101).^[18] These directions coincide with partly or fully double bonded ion rows within the crystal lattice^[19] (see also Figure 5). The analysis of the SFM height image shows that the (010) plane is dissolved in terraces of 7 Å height which correspond to one monolayer of the brushite crystal. This is in good agreement with data published by other authors.^[9] Recording successive pictures results in a movie of the time-dependence of dissolution.^[24] From the series of images, a velocity of dissolution can be calculated which along the (201) step is estimated to 0.16 nm s⁻¹.

The same experiment was repeated in the presence of a dilute aqueous solution (0.63 μmol L⁻¹) of poly(sodium)aspartate with molecular weight 18 000 g mol⁻¹ (pH of 8.1). The SFM height images in Figure 2 are selected from a series of 24.^[24] Noteworthy is that there is no polymer adsorption onto the crystal surface. This finding is in good agreement with equilibrium binding constants determined by Nancollas and Tsortos for a similar polyaspartate on the related hydroxyapatite.^[20] All interactions of the polymer with the (010) plane are therefore temporary and kinetic.

The dissolution of the (010) plane follows the triangular pattern known from the experiments in deionized water.

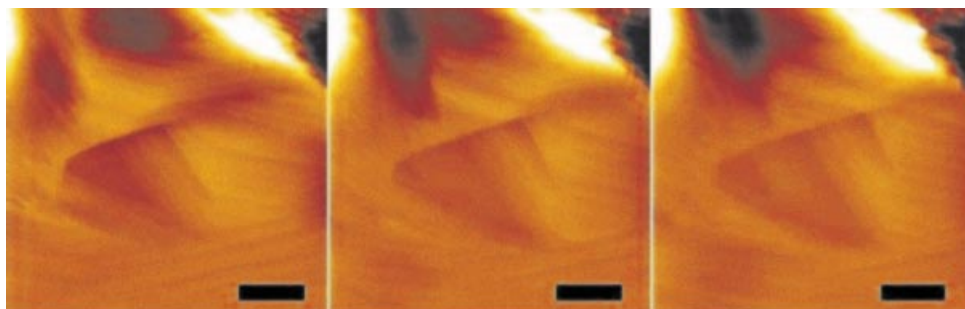


Figure 2. SFM height images of the (010) plane of brushite in 0.63 μmol L⁻¹ polyaspartate solution selected from a series of 24 pictures taken at 512 s intervals. Scale bar = 1 μm.

Pronounced complexation of Ca^{2+} ions by the carboxylate groups of the poly(sodium)aspartate can be assumed while no flocculation of the polymer is found. However, major differences are found for the dissolution of the (010) plane of brushite in poly(sodium)aspartate compared to the dissolution in deionized water:

- The velocity of dissolution along the (201) step rises to 0.45 nm s^{-1} , that is, the complexing carboxylic acid units promote dissolution (the expected result).
- Dissolution perpendicular to the (010) plane occurs, that is, the polymer works itself into the plane of observation. In water, these planes just dissolve layer-by-layer from the side, whereas the polymer allows penetration into a bare and otherwise intact crystal plane.
- The conversion of brushite into hydroxyapatite is completely suppressed (not seen by SFM, but seen in the macroscopic crystallization experiments).

This attack of the bare crystal planes is also nicely seen by light microscopy after longer contact times with the polymer solution (Figure 3); the molecular holes seen by SFM have grown to create a typical surface texture of the crystal.

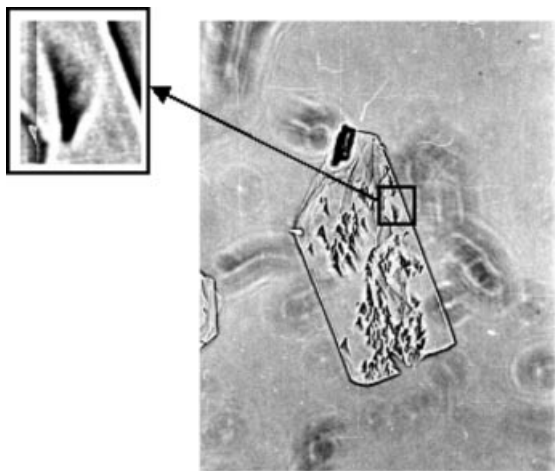


Figure 3. Light microscopy of a brushite crystal (length 0.5 mm) after contact with a polyaspartate solution of 18000 g mol^{-1} molecular weight.

The obvious question to ask is, are both the increase of the dissolution rate as well as the ability of the polymer to attack moieties in the plane really a result of the added polymer and not the result of the change of pH? Reference experiments with a low molecular weight acid or even the poly(sodium)aspartate with lower molecular weight (10000 g mol^{-1}) showed at the same molar concentration a much slower dissolution (very close to the behavior in pure water, and no considerable dissolution perpendicular to the (010) plane was detected. This is clear proof that the effect is because of the specific polymer and relies on a distinct mesoscopic size and a distinct pattern

of functional groups. These observations also support the kinetic model and can be explained in that the accumulation of polymers close to attracting surfaces or the probability of contact increase with increasing polymer size and molecular weight.

Beside of the industrially relevant poly(sodium)aspartate, it is interesting to follow polypeptides throughout the regulation of mineralization/demineralization processes. In contrast to previous work we focus on the use of very simple and common model peptide structures. Albumines are an essential component of biological mineralization reactions (as shown for bone^[21]). Taking into account that the known and characterized bovine serum albumine (BSA) consists of 582 amino acid residues, 41 of which are aspartic acid, 59 glutamic acid, and 59 lysine units,^[22] its ability to interact with minerals becomes obvious.

Again, a BSA solution was prepared in the concentration $0.63 \mu\text{mol L}^{-1}$ at pH 5.5 and brought into contact with the (010) plane of brushite. The result of the dissolution experiment is shown in three SFM amplitude images in Figure 4 which were taken at 512 s intervals. Again no protein adsorption onto the crystal surface is observed; the protein essentially stays in solution. The triangular pits, already known from the poly(sodium)aspartate solutions, confirm that step edges along the same strongly bonded ion rows are formed as seen in Figure 1. Also, to a minor extent, dissolution from sites within the plane is observed, which we attribute to the carboxylic sites and the sufficiently high molecular weight of BSA. In addition, there is a set of new characteristic steps appearing with time, indicated with arrows in Figure 4, which were not seen in the previous dissolution experiments. Knowing the crystal structure and the orientation of the brushite crystals (fixed by the direction of the pits, which are the Ca-rich [201] and [101] edges), it is straightforward to index these newly occurring stable edges: Figure 5 shows that they are formed along directions lacking doubly bonded calcium ions in the crystal lattice. Obviously, the binding between calcium sites and the carboxyl groups of the amino acids can not account for the stabilization of these steps.

The cationic groups of BSA, mainly the 59 lysine residues, have the possibility to interact selectively with the phosphate ions of the crystal lattice. To check this hypothesis, a pure poly(L+)lysine with a molecular weight of 27000 g mol^{-1} was independently used as crystallization modifier. Since our SFM

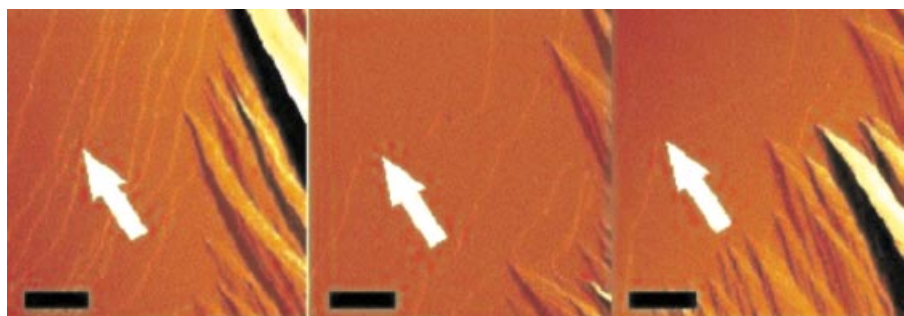


Figure 4. SFM amplitude images of the (010) plane of brushite in $0.63 \mu\text{mol L}^{-1}$ BSA solution. Scale bar = $1 \mu\text{m}$.

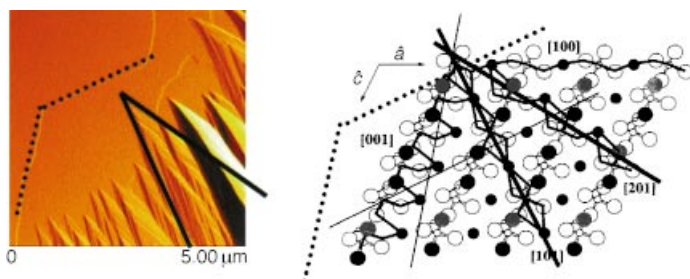


Figure 5. Assignment of the AFM image of Figure 4 to the crystallographic directions of brushite. The newly exposed edges correspond to phosphate-rich sections through the unit cell.

setup does not allow molecular resolution with cationic polyelectrolytes (they bind to the tip and most other surfaces), images of the crystals taken in a light microscope were analyzed. Figure 6 shows a brushite crystal aged for two days in a poly(L+)-lysine solution.

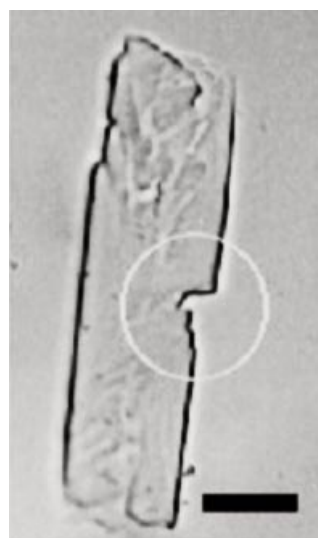


Figure 6. Light microscope image of the (010) plane of brushite in poly(L+)-lysine solution. Scale bar = 200 μm.

First, it is underlined that the developing surface structures are not induced by cracking, since those structural motives develop uniformly from whole crystals in a thermo-controlled, sealed liquid cell. The pattern found also has nothing in common with the triangular pits on the crystal surface caused by poly-(sodium)aspartate or BSA (anionic polymers). This reflects the obvious fact that a cationic polymer can interact with a number of faces or edges but does not stabilize those where Ca^{2+} ions are exposed. In addition, no dissolution from the surfaces, only from the edges is found, that is, the attack of intact planes is indeed specific for the polycarboxylic acids. The measured angles being exposed in these crystals (Figure 5) correspond to those of the additional step edges on the crystals aged in the BSA solution. This comparison supports the idea that these additional step edges were indeed generated by interaction promoted by the lysine moieties in the BSA.

At the low concentrations applied in our experiments, the adsorption of polymers onto the brushite (010) plane by interaction of their functional groups with the lattice ions is not observed in any experiment. Instead, a highly selective dissolution of the crystal surface takes place, which is not caused by pH changes or local functional patterns, as shown by the use of polymers with different molecular weights. Presumably the modifier accumulates close to the crystal because of partial binding and controls the surface by promoting dissolution of different sites with different selec-

tivity. The proximity of a polymer lowers the interfacial energy of the different crystal surfaces, which gives rise to an altered dissolution and growth behavior. Our experiments agree well with the observations of Nancollas et al. who described effective blocking of crystal growth of calcium phosphate octahydrate with polyaspartic acids at a surface occupation of 1 % of the total crystal area,^[23] which precludes a merely static structural explanation.

The ability to attack even the intact (010) plane is clearly a function of the molecular weight of the acidic polymer, and different functional polypeptides act as different "carving tools" exposing different crystal faces after their application. Interestingly, the rather simple and well known BSA interacts and modifies calcium phosphate by two operational modes, either employing the acidic or basic groups of the amino acids.

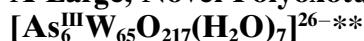
Clearly in our experiments kinetic considerations are more significant than epitaxial matching between the crystal lattice and the polymer. This points to a more flexible approach to the processes of controlled crystallization or dissolution by using the concept of interface energies and dynamic crystallization/dissolution equilibria.

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A Large, Novel Polyoxotungstate:



Ulrich Kortz,* Masha G. Savelieff, Bassem S. Bassil, and Michael H. Dickman

Polyoxoanions comprise a unique family of metal–oxygen cluster species considering the variety of structures and interesting properties in catalysis, materials science, and medicine.^[1–4] Polyoxometalates were first reported more than 100 years ago, but the structures of many species were only discovered recently by using single-crystal X-ray diffraction. The availability of this analytical tool has led to the structural characterization of a large number of novel polyoxoanions with different shapes and sizes.

It is apparent that during the last couple of years not only the number but also the size of many novel structures has been increasing dramatically. Until recently the isopolyanion $[\text{Mo}_{36}\text{O}_{112}(\text{H}_2\text{O})_{18}]^{8-}$ ^[5] as well as the heteropolyanions $[\text{As}_4\text{W}_{40}\text{O}_{140}]^{28-}$ ^[6] and $[\text{H}_7\text{P}_8\text{W}_{48}\text{O}_{148}]^{33-}$ ^[7] were examples of the largest polyoxometalates reported. However, very recently Müller et al. reported a class of gigantic polyoxomolybdate spheres, baskets, and rings containing up to 248 molybdenum atoms,^[8] and Pope et al. reported on a polyoxotungstate with 148 tungsten atoms, $[\text{As}_{12}\text{Ce}_{16}(\text{H}_2\text{O})_{36}\text{W}_{148}\text{O}_{524}]^{76-}$.^[9]

At this point it is important to realize that the mechanism of formation of polyoxometalates is still not well understood and commonly described as self-assembly. Therefore, it is usually not possible to design a multistep sequence for the synthesis of a novel polyoxometalate. As a result, the design of novel polyoxometalates remains a challenge.

Herein we report the synthesis and structural characterization of the largest tungstoarsenate(III) known to date, $[\text{As}_6^{\text{III}}\text{W}_{65}\text{O}_{217}(\text{H}_2\text{O})_7]^{26-}$ (**1**). This novel polyoxoanion is isolated in an acidic medium (pH 2) with good yield (71 %). We have also synthesized the antimony(III) analogue, $[\text{Sb}_6^{\text{III}}\text{W}_{65}\text{O}_{217}(\text{H}_2\text{O})_7]^{26-}$ (**2**), in very good yield (90 %). This compound was characterized by FTIR spectroscopy and elemental analysis.

The compact structure of **1** with idealized C_{2h} symmetry (Figures 1 and 2) consists of four inner (β - $\text{AsW}_9\text{O}_{33}$) and two outer (α - $\text{AsW}_9\text{O}_{33}$) fragments, which are linked together by a total of eleven corner-sharing WO_6 octahedra, so that **1** may be formulated as $[(\beta\text{-AsW}_9\text{O}_{33})_4(\alpha\text{-AsW}_9\text{O}_{33})_2(\text{WO}_2)_4(\text{WO}(\text{H}_2\text{O}))_6\text{WO}_5(\text{H}_2\text{O})]^{26-}$. The arrangement of the six

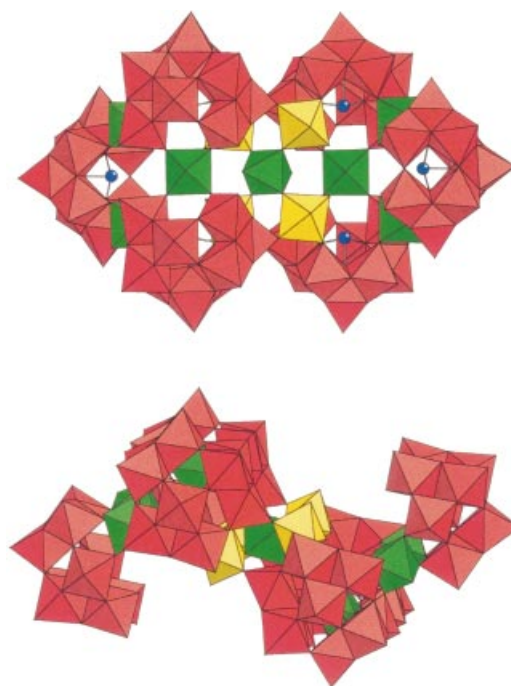


Figure 1. Polyhedral representation of $[\text{As}_6^{\text{III}}\text{W}_{65}\text{O}_{217}(\text{H}_2\text{O})_7]^{26-}$ (**1**); top view (upper) and side view (lower). Arsenic atoms are blue and all WO_6 octahedra composing $(\text{AsW}_9\text{O}_{33})$ fragments are red. The remaining WO_6 octahedra are either $\text{cis-O}_2\text{W}(\mu_2\text{-O})_4$ (yellow) or $\text{trans-O}(\text{H}_2\text{O})\text{W}(\mu_2\text{-O})_4$ (green).

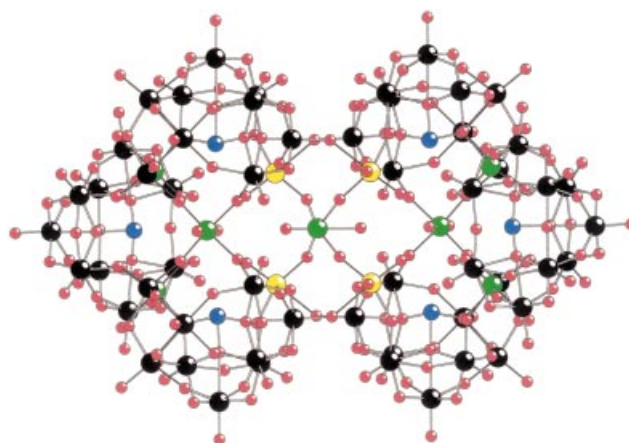


Figure 2. Ball-and-stick representation of $[\text{As}_6^{\text{III}}\text{W}_{65}\text{O}_{217}(\text{H}_2\text{O})_7]^{26-}$ (**1**). The color code is as follows: O: red, As: blue, W atoms of $(\text{AsW}_9\text{O}_{33})$ fragments: black, remaining W atoms of $\text{cis-O}_2\text{W}(\mu_2\text{-O})_4$ or $\text{trans-O}(\text{H}_2\text{O})\text{W}(\mu_2\text{-O})_4$: yellow or green, respectively.

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($\text{AsW}_9\text{O}_{33}$) subunits resembles a chair conformation of cyclohexane.

There are also alternative possibilities of rationalizing the structure of **1**. The dimeric nature of **1** could be emphasized by the formula $[\text{WO}(\text{H}_2\text{O})\{\text{As}_3\text{W}_{32}\text{O}_{108}(\text{H}_2\text{O})_3\}_2]^{26-}$. On the other hand, it should be remembered that **1** was synthesized from $[\text{As}_2\text{W}_{19}\text{O}_{67}(\text{H}_2\text{O})]^{14-}$ and such fragments can easily be identified in the structure of **1** (Figures 2 and 3). However, it must be realized that the dilacunary polyanion $[\text{As}_2\text{W}_{19}\text{O}_{67}(\text{H}_2\text{O})]^{14-}$ consists of two (α - $\text{AsW}_9\text{O}_{33}$) subunits linked by a $\text{WO}(\text{H}_2\text{O})$ center. This is best represented by the formula $[\text{WO}(\text{H}_2\text{O})(\alpha\text{-AsW}_9\text{O}_{33})_2]^{14-}$ (Figure 4). In the

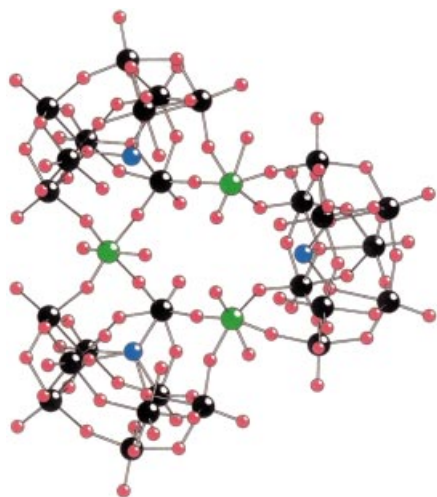


Figure 3. Ball-and-stick representation showing a trimeric cyclic subunit of **1**. The (α -AsW₉O₃₃) unit (right) and the two (β -AsW₉O₃₃) units (left) are linked to each other by three *trans*-O(H₂O)W(μ_2 -O)₄ groups. The color code is the same as in Figure 2.

structure of **1**, two types of [As₂W₁₉O₆₇(H₂O)]¹⁴⁻ fragments can be identified, [WO(H₂O)(α -AsW₉O₃₃)(β -AsW₉O₃₃)]¹⁴⁻ and [WO(H₂O)(β -AsW₉O₃₃)₂]¹⁴⁻. This means that the mech-

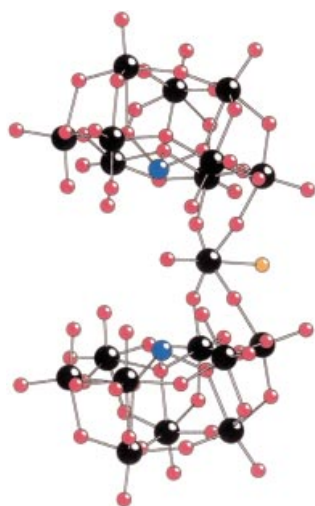
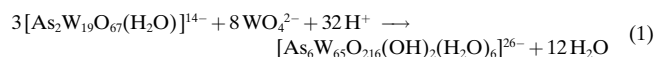


Figure 4. Ball-and-stick representation of [As₂W₁₉O₆₇(H₂O)]¹⁴⁻ as a result of a single crystal X-ray analysis for K_{6.25}NaNi[As₂W₁₉O₆₇(H₂O)] · 31.5 H₂O. W: black, As: blue, O: red. The single water molecule (O: orange) is bound to the tungsten atom bridging the two (α -AsW₉O₃₃) fragments.

anism of formation of **1** can be described as a condensation of [WO(H₂O)(α -AsW₉O₃₃)₂]¹⁴⁻ polyanions accompanied by 60° rotation of groups of edge-shared WO₆ octahedra. It might also be useful to represent the synthesis of **1** by Equation (1).



This shows clearly that some extra tungsten atoms are needed, which must become available during the course of the reaction, most likely as a result of [As₂W₁₉O₆₇(H₂O)]¹⁴⁻ rearrangement. This polyanion is synthesized at pH 6–6.5, and it is known that upon acidification the two lacunary sites are consecutively filled by tungsten atoms. The monolacunary

polyanion [As₂W₂₀O₆₈(H₂O)]¹⁰⁻ is stable at pH 2–4 and in very acidic medium (around pH 0) the polyanion [As₂W₂₁O₆₉(H₂O)]⁶⁻ is dominant. Apparently neither of the two polyanions is stable between pH 0–2. Interestingly we discovered that **1** can only be synthesized in the pH range 1.5–2.0.

The structure of **1** may be considered as the larger relative of the crown polyanion [As₄W₄₀O₁₄₀]²⁸⁻ reported by Hervé et al. some time ago.^[6] This species is composed of four (α -AsW₉O₃₃) fragments linked by four WO₆ octahedra. However, the synthesis of [As₄W₄₀O₁₄₀]²⁸⁻ involved stoichiometric interaction of tungstate and metaarsenite at pH 4. Another difference is that **1**, as opposed to [As₄W₄₀O₁₄₀]²⁸⁻, is a rather closed structure. Furthermore for [As₄W₄₀O₁₄₀]²⁸⁻ there is only one type of tungsten atom linking the (α -AsW₉O₃₃) units, *cis*-O₂W(μ_2 -O)₄, whereas in **1** there are two types, four *cis*-O₂W(μ_2 -O)₄ and seven *trans*-O(H₂O)W(μ_2 -O)₄ (see Figure 1).

Pope et al. recently reported the very large polyoxotungstate [As₁₂Ce₁₆(H₂O)₃₆W₁₄₈O₅₂₄]⁷⁶⁻ with a compact structure based on twelve [AsW₉O₃₃]⁹⁻ and four [W₅O₁₈]⁶⁻ units.^[9] These are held together by an extra 30 WO₆ octahedra and 16 Ce³⁺ (or La³⁺) ions. It is amazing that just like [As₄W₄₀O₁₄₀]²⁸⁻ this compound is synthesized from the composing elements rather than from a preformed polyoxoanion.

The protonation sites of **1** were identified by bond valence sum calculations.^[10] As a result, seven of the 65 tungsten atoms in **1** are bound to a terminal water molecule, which is *trans* to a terminal oxo group (green octahedra in Figure 1). The central W atom (W33) belongs to this group, because crystallographic refinement indicated that it is disordered over two symmetry-related positions slightly off the crystallographic inversion center. Therefore the coordination of W33 is better described as *trans*-O(H₂O)W(μ_2 -O)₄ instead of *trans*-(HO)₂W(μ_2 -O)₄. The result of an elemental analysis indicated that the remaining 26 negative charges of **1** are balanced by potassium cations. However, single crystal X-ray diffraction allowed identification of only half of the potassium ions, probably as a result of disorder. Four of these cations (K1, K1', K4, K4') are located inside the two pockets of **1** apparently stabilizing the chair structure of the polyanion. The other nine potassium ions occupy external positions and they are bound to oxo groups of neighboring polyoxoanions as well as to terminal water molecules.

Since **1** is diamagnetic, we decided to study its solution properties by ¹⁸³W NMR. Solid LiClO₄ was added to an aqueous solution of the potassium salt of **1** in order to increase its solubility. If the solid-state structure of **1** is retained in solution, 18 peaks with intensity ratios 4:4:4:4:4:4:4:4:4:4:4:4:2:2:1 are expected. The ¹⁸³W NMR spectrum (12.5 MHz, Bruker AM-300WB spectrometer) resulted in the expected number of peaks between $\delta = -80$ and -230 . However, the spectrum is quite congested and peak overlap does not allow a more detailed interpretation. Interestingly Pope et al. encountered the same problem for [As₁₂La₁₆(H₂O)₃₆W₁₄₈O₅₂₄]⁷⁶⁻ with at least 15 of the 21 expected ¹⁸³W NMR signals in a very similar chemical shift range between $\delta = -60$ and -260 .^[9]

A detailed investigation of the chemical and physical properties of **1** and **2** is currently underway.

Experimental Section

The potassium salt of **1** was synthesized by using $K_{14}[As_2W_{19}O_{67}(H_2O)]$ as a precursor. The existence of the dilacunary polyanion $[As_2W_{19}O_{67}]^{14-}$ was proposed by Tourné et al. many years ago, but the synthetic conditions were only qualitatively described and no structural characterization by single-crystal X-ray diffraction has been reported.^[11] We synthesized $K_{14}[As_2W_{19}O_{67}(H_2O)]$ by addition of As_2O_3 (0.89 g, 4.5 mmol), $Na_2WO_4 \cdot 2H_2O$ (18.8 g, 57 mmol), and KCl (0.67 g, 9.0 mmol) to 50 mL H_2O at 80 °C with stirring. After dissolution the pH was adjusted to 6.3 by adding 12 M HCl dropwise. The solution was kept at 80 °C for 10 min and then filtered. Finally 15 g KCl was added and the solution stirred for 15 minutes. The white precipitate formed was isolated by filtration and dried at 80 °C resulting in 15.4 g product (yield 95 %). Purity of this material was verified by FTIR spectroscopy. IR (KBr disk): $\tilde{\nu}$ = 941, 892, 803, 742, 629, 504, 462, 442 cm^{-1} . We were also able to crystallize $[As_2W_{19}O_{67}(H_2O)]^{14-}$ from a sodium acetate buffer (0.5 M, pH 5.5) as a mixed potassium-sodium-nickel salt, $K_{6.25}NaNi[As_2W_{19}O_{67}(H_2O)] \cdot 31.5H_2O$. Single-crystal X-ray diffraction showed the expected dilacunary polyanion (see Figure 4).^[12] It becomes apparent that in the solid state (and most likely also in solution) the structure is stabilized by potassium ions. X-ray analysis suggested that a potassium ion was disordered over the two lacunary sites (occupancy 0.5 for each). Probably for steric reasons, each polyanion can accommodate only one potassium ion. The nickel ion was disordered over three positions (occupancy 0.33 for each) external from the lacunary sites.

The synthesis of the title anion **1** was accomplished by dissolving 3.0 g (0.57 mmol) of $K_{14}[As_2W_{19}O_{67}(H_2O)]$ (synthesized as described above) in 40 mL H_2O . The pH was adjusted to 1.9 by addition of 4 M HCl, then the solution was heated and boiled for 15 min. The hot solution was filtered and allowed to stand in a closed container at room temperature. At this point the pH was 2.1. The following day colorless crystals started to form, and after 2 weeks 1.8 g (yield 71 %) of crystalline product was isolated. The synthetic procedure for **1** is very similar to that of Hervé et al. for $K_{10}[As_2W_{20}O_{68}(H_2O)]$ and the only, but important, difference is the pH after acidification. If the pH is less than 1.5 before heating, then $[As_2W_{20}O_{68}(H_2O)]^{10-}$ is formed; if the pH is between 1.5–2.0 then **1** is formed. $K_{26}[As_6W_{65}O_{217}(H_2O)_7] \cdot 15H_2O$: elemental analysis (%): calcd (found): K 5.9 (6.7), As 2.6 (2.8), W 69.1 (69.0); IR (KBr disk): $\tilde{\nu}$ = 969, 914, 887, 824, 769, 714, 670, 630, 485, 442 cm^{-1} . Crystal structure analysis for $K_{26}[As_6W_{65}O_{217}(H_2O)_7] \cdot 76H_2O$: crystal dimensions 0.28 × 0.22 × 0.22 mm, mounted on a glass fiber; T = 171(2) K; Siemens SMART CCD single-crystal diffractometer equipped with a Mo anode and a graphite monochromator (λ = 0.71073 Å). An approximate sphere of data was collected out to a $2\theta_{max}$ value of 56.9°; ω scans; Lorentzian and polarization correction; semi-empirical (SADABS) absorption correction (μ = 27.183 mm^{-1} ; min/max transmission 0.0138/0.0480); total reflections collected: 58950; independent reflections: 32038; $R(int)$ = 0.091; $R(sig)$ = 0.129. Space group $P\bar{1}$; a = 18.272(3), b = 19.141(4), c = 24.156(5) Å, α = 100.034(3), β = 106.969(3), γ = 111.885(3)°, V = 7107(2) Å³, Z = 1; ρ_{calcd} = 4.180 Mgm^{-3} ; structure solution by direct methods, refinement against $|F^2|$ over all reflections, R_1 = 0.076 for 20200 independent reflections with $I > 2\sigma(I)$ and 988 parameters, Rw_2 = 0.184 for all reflections; highest residual electron density 6.74 $e \text{ \AA}^{-3}$. Software used: Siemens SMART, SAINT, SHELXTL.^[13] No hydrogen atoms were included.^[12b]

The synthesis of **2** was accomplished by dissolving $Na_9[AsSbW_9O_{33}] \cdot 19.5H_2O$ ^[14] (6.5 g, 2.3 mmol), $Na_2WO_4 \cdot 2H_2O$ (0.76 g, 2.3 mmol), and KCl (1.5 g, 20.7 mmol) in 40 mL H_2O . The pH was adjusted to 1.0 by addition of 4 M HCl and the solution was boiled for 15 min. The hot solution was then filtered and 3.0 g CsCl were added. After stirring for 15 min the light-yellow precipitate was isolated by filtration and air dried resulting in 6.5 g (yield 90 %) of product. $Cs_{26}[Sb_6W_{65}O_{217}(H_2O)_7] \cdot 50H_2O$: elemental analysis (%): calcd (found): Cs 16.7 (17.0), Sb 3.5 (3.9), W 57.9 (56.0); IR (KBr disk): $\tilde{\nu}$ = 965, 894, 814, 746, 716, 677, 628, 533, 437 cm^{-1} .

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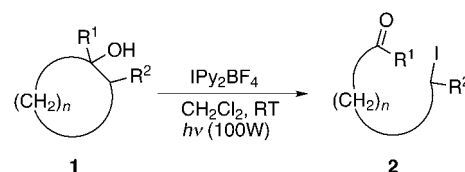
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Oxidative Opening of Cycloalkanols: An Efficient Entry to ω -Iodocarbonyl Compounds**

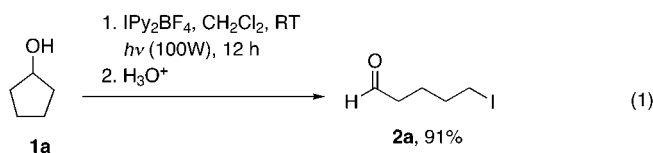
José Barluenga,* Francisco González-Bobes, Sreenivasa R. Ananthoju, Miguel A. García-Martín, and José M. González

The development of new approaches to elaborate the carbon backbone of organic compounds is among the central issues in organic synthesis. In this regard, a general method for the direct conversion of readily available cyclic alcohols to bifunctional derivatives, such as ω -iodocarbonyl compounds, is an attractive synthetic goal.^[1] Herein, we report on a powerful method for synthesizing iodinated derivatives of both, aldehydes and ketones.^[2] Thus, bis(pyridine)iodonium(i) tetrafluoroborate (IPy₂BF₄)^[3] promotes a regioselective opening reaction of **1** upon irradiation leading to compounds **2** (Scheme 1).^[4]



Scheme 1. IPy₂BF₄-promoted regioselective opening of cycloalkanols. R¹, R²: H, CH₃; n: 2, 3, 4, 10; nine examples (76–94 %).

In preliminary studies, cyclopentanol (**1a**) was recovered unreacted upon treatment with IPy₂BF₄. Moreover, NMR experiments revealed that in the absence of visible light no change had taken place even after several days at room temperature. Interestingly, when the mixture was photoactivated (irradiation, 100 W lamp) a clean transformation took place affording 5-iodopentanal (**2a**)^[5] in excellent yield after hydrolysis [Eq. (1)].



This is an elusive transformation for a secondary alcohol, only accomplished previously for cyclopentanol derivatives.^[6] Our method offers a more efficient entry to achieve this scission reaction even from **1a**.^[7] This stimulated us to further investigate its scope and convenience, in search for a clean

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and general strategy to produce aldehydes from this rearrangement.^[8] It also provided an opportunity to explore the behavior of the iodinating reagent IPy₂BF₄ under this new type of activation; thus, expanding the application profile already established for IPy₂BF₄ in electrophilic chemistry.^[3]

The photolytic reactions were conducted using 2 mmol of the starting cycloalkanol, typically dissolved in CH₂Cl₂ (20 mL), unless otherwise specified (see Table 1). Under photoactivation conditions, ring-opening of cyclobutanol (**1b**) by reaction with IPy₂BF₄ was successful, furnishing **2b**^[9] in 92% yield, as sole reaction product (Table 1, entry 2). Cyclohexanol (**1c**) was found to undergo a sluggish reaction in which a significant amount of cyclohexanone (**3**) was formed as by-product. The opening of **1c** required excess of the iodonium source, but even in this case, a considerable amount of unreacted starting material was recovered.

Alkoxy radicals are widely accepted as intermediates in β -scission processes.^[10] Thus, upon activation of the substrate by the iodinating reagent, a base might assist the process by removing hydrogen. This deprotonation might be crucial to drive the process when the ring-opening of the corresponding cycloalkanol is not a particularly favored reaction.

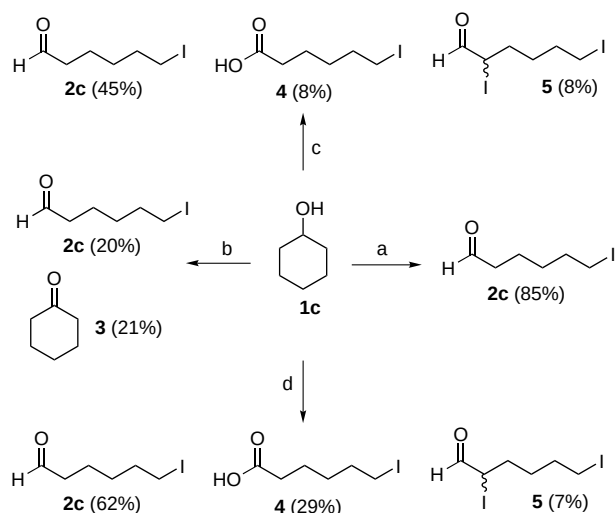
In agreement with this, the reaction of **1c** went to completion by using an excess of cesium carbonate (Table 1, entry 3, Scheme 2a).

We were interested in comparing this approach to **2c** with that based on the reaction of PhI(OAc)₂/I₂ with cyclohexanol, which has not been reported to date. When the reaction was performed in cyclohexane as solvent,^[11] the β -scission product **2c** was obtained in 20% yield from a mixture that contains cyclohexanone (**3**) (21%), iodocyclohexane as the major reaction product, as well as the expected iodobenzene (Scheme 2b). The experiment results in a noteworthy C–H activation of the alkane used as solvent,^[12] but it reveals limitations for the opening of cyclohexanol, subject of comparison. We also tested the reaction using CH₂Cl₂ as solvent,^[2b] in this case **2c** was isolated in better yield (45%; Scheme 2c); however, the formation of two new by-products (**4** and **5**) was also observed, arising from the evolution of **2c** in the reaction media. A slight improvement of the process was accomplished by increasing the amount of oxidant system to achieve full conversion of **1c**. Under these conditions, **2c** was obtained in 62% yield but significant overoxidation to **4** and **5** took place (Scheme 2d).

Table 1. Synthesis of ω -iodocarbonyl compounds **2** from cycloalkanols **1**.

Entry	Substrate 1	IPy ₂ BF ₄ [equiv]	Cs ₂ CO ₃ [equiv]	Product 2	Reaction time [h]	Yield ^[a] [%]
1		1.25	–		12	91
2		1.25	–		12	92
3		2.5	10		20	85 ^[b]
4		2.5	10		20	76 ^[b]
5		1.25	5		8	93
6		1.25	5		8	88
7		1.25	–		12	92
8		1.25	5		14	89
9		1.25	5		7	94

[a] Yield of **2** based on starting cycloalkanol **1**. [b] A 0.02 M solution of the alcohol was used.



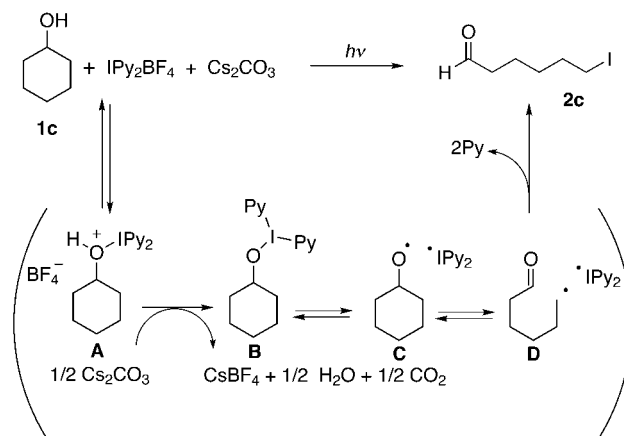
Scheme 2. Approaches for the conversion of cyclohexanol (**1c**) to 6-iodohexanal (**2c**). a) 1. IPy_2BF_4 (2.5 equiv), Cs_2CO_3 (10 equiv), CH_2Cl_2 , RT, $h\nu$ (100 W), 20 h; 2. H_3O^+ ; b) $\text{PhI}(\text{OAc})_2/\text{I}_2$ (1.1/1 equiv), cyclohexane, RT, $h\nu$ (2×100 W), 3 h; c) $\text{PhI}(\text{OAc})_2/\text{I}_2$ (1.1/1 equiv), CH_2Cl_2 , RT, $h\nu$ (2×100 W), 4 h; d) $\text{PhI}(\text{OAc})_2/\text{I}_2$ (2.2/2 equiv), CH_2Cl_2 , RT, $h\nu$ (2×100 W), 4 h.

The efficiency of IPy_2BF_4 to promote the conversion of cycloalkanol to iodocarbonyl compounds was also tested on larger ring-size cycloalkanol, such as **1d**. This process opens a new entry to long-chain ω -bifunctional compounds, such as **2d** (Table 1, entry 4). Moreover, entries 5 and 6 in Table 1 show that tertiary cycloalkanol **1e** and **1f** can be smoothly ring-opened to furnish iodoketones,^[2] proving the generality of the methodology.

Another interesting feature was observed in reactions of racemic **1g** or **1h** which, upon treatment with IPy_2BF_4 , afforded only one regioisomer of pure aldehydes **2g** and **2h**, respectively, in excellent yield^[13] (Table 1, entries 7 and 8). The preparation of **2h** required cesium carbonate to accomplish total conversion of the alcohol. The opening of the terpene derivative **1i**^[14] gives a remarkable example of the efficiency and regioselectivity offered by the $\text{IPy}_2\text{BF}_4/\text{Cs}_2\text{CO}_3$ system to generate aldehydes featuring remote iodine functionality (Table 1, entry 9). Again, the structure of the substrate exerts full control over which C–C bond is cleaved. This leads to the interesting cyclopropanecarbaldehyde framework of **2i**, as 1:1 mixture of diastereoisomers with respect to the newly formed stereocenter.^[15]

In terms of reaction mechanism, this process could involve the generation of different reactive species in a sequential manner, as depicted in Scheme 3.^[16] Thus, upon mixing the reagents, **1c** would give the oxonium ion **A** and Cs_2CO_3 might promote its evolution to the λ^3 -iodane **B**. The key alkoxy radical **C**, obtained by photolytic homolysis of **B**,^[10, 17] could undergo a well documented β -scission reaction to afford **D**.^[18] Finally, radical capture by the λ^3 -iodanyl radical would lead to **2c**. This mechanism would account for the formation of a mixture of diastereomers from **2i** as well as for the observed regioselectivity.^[19]

In summary, a mild and efficient process is reported to smoothly conduct the conversion of representative cyclic



Scheme 3. Proposed reaction mechanism for the conversion of cycloalkanol **1** to the iodocarbonyl compounds **2** by means of IPy_2BF_4 .

alcohols to useful ω -iodocarbonyl derivatives under environmentally friendly and practicable conditions, using simple commercial reagents and visible light. Further studies on the reactivity of IPy_2BF_4 with alcohols are in progress.

Experimental Section

All reactions were carried out under a positive pressure of nitrogen. Dichloromethane was dried by refluxing over CaH_2 and distilled under a nitrogen atmosphere.

Synthesis of **2f**: Cs_2CO_3 (3.72 g, 10 mmol, 5 equiv) was added to a well stirred solution of IPy_2BF_4 (930 mg, 2.5 mmol, 1.25 equiv) in dry CH_2Cl_2 (20 mL) at room temperature. Then, **1f** (228 mg, 2.0 mmol, 1.0 equiv) was added, and the mixture was irradiated (100 W) for 8 h. The mixture was then cooled in an ice–water bath and then quenched with 1M H_2SO_4 (50 mL). The mixture was extracted by using CH_2Cl_2 (4×20 mL), and the combined organic layers were washed with sodium thiosulfate (5% solution in water, 2×30 mL) and water (2×30 mL), and dried over sodium sulfate. The solvent was removed at reduced pressure. The resultant oil was further purified by column chromatography (hexane/ethyl acetate 10/1) yielding **2f** as a pale-yellow oil (422 mg, 88%). Representative data for **2f**: IR (neat): $\tilde{\nu}$: 1714 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ = 3.12 (t, J = 6.9 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 2.07 (s, 3H), 1.76 (q, J = 6.9 Hz, 2H), 1.5–1.1 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3): δ = 208.3, 43.1, 32.9, 29.7 ($\times 2$), 22.3, 6.5; elemental analysis (%) calcd for $\text{C}_7\text{H}_{13}\text{IO}$: C 35.02, H 5.45; found: C 35.17, H 5.31.

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- [1] This is in sharp contrast to the existence of many synthetic alternatives to conduct the reverse process, namely the reductive ring-closing reaction of ω -iodocarbonyl compounds by metal-based Barbier-type approaches and radical cyclization reactions.
- [2] The existing methodology is suitable only for the synthesis of ketones from tertiary cycloalkanol. For this purpose, the $\text{PhI}(\text{OAc})_2/\text{I}_2$ system, known as Suárez's reagent, has been used, mainly for compounds featuring well defined stereoelectronic requirements; for representative examples, see: a) P. Wipf, D. A. Mareska, *Tetrahedron Lett.* **2000**, 41, 4723–4727; b) R. Hernández, E. I. León, P. Moreno, E. Suárez, *J. Org. Chem.* **1997**, 62, 8974–8975; c) A. Boto, C. Betancor, E. Suárez, *Tetrahedron Lett.* **1994**, 35, 5509–5512; d) C. W. Elwood, G. Patten-den, *Tetrahedron Lett.* **1991**, 32, 1591–1594; for the use of HgO/I_2 see: e) S. Bond, P. Perlmutter, *Chem. Commun.* **2000**, 567–568; f) H. Sugimoto, S. Yamada, *Tetrahedron Lett.* **1987**, 28, 3963–3966.
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- [4] All compounds **2** were characterized by IR, ^1H , and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis and/or high-resolution mass spectrometry data.
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Chromium–Copper Exchange of Fischer Carbene Complexes: X-Ray Crystal Structure of a $[\text{Cu}\{\text{C}(\text{R})=\text{CR}^1(\text{OR}^2)\}(\text{MeCN})(\text{Et}_2\text{O})][\text{PF}_6] \text{Complex}^{**}$

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Over the last two decades Fischer-type carbene complexes have become valuable tools in stoichiometric transition metal mediated organic synthesis.^[1] The carbene nature of these complexes is demonstrated by their ability to transfer the carbene ligand not only to alkenes (cyclopropanation),^[2] but also to oxygen, nitrogen, and carbon through the corresponding ylides (amine oxides and dimethyl sulfoxide; sulfilimines; P–C, S–C, and N_2 –C ylides).^[3] Although transmetalation is probably the most important single process in organometallic chemistry, particularly for transition metal catalyzed reactions,^[4] the simple transfer of a carbene ligand from a metal–carbene complex to another metallic center is rare.^[5] Carbene complexes of Rh, Pd, Pt, Cu, and Ag have been formed by transfer of diaminocarbene ligands (imidazolinyldene or imidazolidinyldene ligands, which are found to be readily transferred between metal centers) from Group 6 metal carbene complexes.^[5–7] However, the transfer of alkoxycarbene ligands has been reported only for $[\text{Mo}\{\text{CPh}(\text{OMe})\}(\text{CO})(\text{Cp})(\text{NO})]$ to $[\text{Fe}(\text{CO})_5]$ ^[8] and $[\text{W}\{\text{CPh}(\text{OMe})\}(\text{CO})_5]$ to $\text{H}[\text{AuCl}_4]$.^[9] Very recently Sierra et al. have proposed palladium biscarbene complexes as the active intermediates in the palladium-catalyzed dimerization of alkoxycarbene–chromium complexes.^[10, 11]

In the context of our studies on copper-catalyzed coupling reactions of Fischer carbene complexes we report here on 1) the cross-coupling of Fischer chromium–carbene complexes with ethyl diazoacetate (EDA), and 2) the isolation and full characterization of the first alkoxycarbene complex of copper(I). A recent, excellent report by Hofmann and Straub^[12] on the characterization of an alkoxycarbonylcarbene–copper complex has prompted us to release our results.

When chromium–carbene complexes **1** were treated with EDA (2.5 equiv) and CuBr (15% mol) in THF at room temperature the alkenes **2** were isolated with high yields (80–95%) along with ethyl maleate and ethyl fumarate

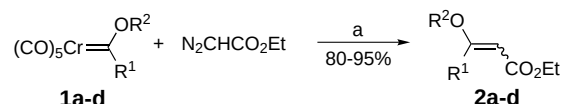
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[+] X-ray crystal structure analysis.

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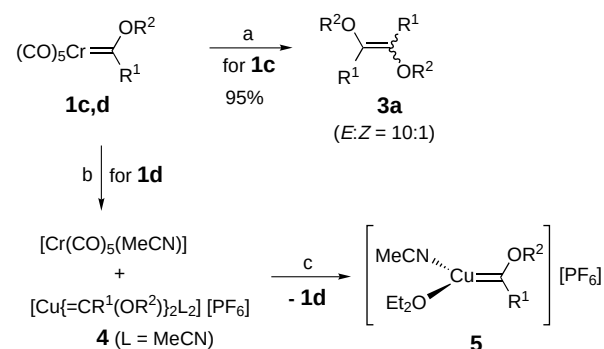
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(Scheme 1). This heterocoupling reaction worked well for phenyl- (**1a**), butyl- (**1b**) and alkenyl(methoxy)carbene (**1c**) complexes, as well as for the nonracemic alkenyl(menthoxy)-carbene **1d**, giving rise to alkenes and dienes with a high degree of functionality.^[13] The methoxy complexes **1a–c** afforded a 1:1 *E/Z* mixture, whereas only the *E* stereoisomer was formed in the case of the menthoxy derivative **1d**.



Scheme 1. CuBr-catalyzed cross-coupling reaction of alkoxychromium carbene complexes **1** with ethyl diazoacetate (**a**: $\text{R}^1 = \text{Ph}$, $\text{R}^2 = \text{Me}$; **b**: $\text{R}^1 = \text{Bu}$, $\text{R}^2 = \text{Me}$; **c**: $\text{R}^1 = (E)\text{-CH=CH-2-furyl}$, $\text{R}^2 = \text{Me}$; **d**: $\text{R}^1 = (E)\text{-CH=CH-2-furyl}$, $\text{R}^2 = (1R,2S,5R)\text{-menthyl}$): **a**) 15 mol % CuBr, THF, 25 °C, 80–95%; *E/Z* = 1:1 ($\text{R}^2 = \text{Me}$), > 30:1 ($\text{R}^2 = (1R,2S,5R)\text{-menthyl}$).

On the other hand, when the (methoxy)carbene **1c** was stirred with 15 mol % $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$ in the absence, or even in the presence, of EDA and under homogeneous conditions (CH_2Cl_2 , 25 °C) a clean dimerization to triene **3a** took place (95 % yield; *E/Z* = 10:1; Scheme 2). On the basis of



Scheme 2. Formation of the copper–carbene complex **5** from **1d** and $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$ (**3a**: $\text{R}^1 = (E)\text{-CH=CH-2-furyl}$, $\text{R}^2 = \text{Me}$; **3b**, **4**, **5**: $\text{R}^1 = (E)\text{-CH=CH-2-furyl}$, $\text{R}^2 = (1R,2S,5R)\text{-menthyl}$): **a**) 15 mol % $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$, CH_2Cl_2 , 25 °C, 95%; **b**) 50 mol % $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$, CH_2Cl_2 , 25 °C; **c**) crystallization from $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ (1/5, v/v), –20 °C, 50% (35% recovery of **1d**).

previous work^[5, 10] we assume that a copper biscarbene species of the type $[\text{Cu}=\text{CR}^1(\text{OMe})_2\text{L}_n][\text{PF}_6]$ might be the real metal intermediate. At this point, it was gratifying to find that the homochiral (menthoxy)carbene complex **1d** consumed 0.5 equivalents of $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$ (CH_2Cl_2 , 25 °C) yielding a clean crude mixture consisting of $[\text{Cr}(\text{CO})_5(\text{MeCN})]$ (identified by comparison with an authentic sample) and a copper–carbene complex, presumably of structure **4** (Scheme 2). The structure **4** is proposed on the basis of the single set of signals in the ^{13}C NMR spectrum ($\delta = 280$ (Cu=C), 4.1 (Cu–NC–CH₃)).^[14] Surprisingly, when the crude mixture was dissolved in CH_2Cl_2 and Et_2O (1/5, v/v) was allowed to diffuse into the brown solution the color slowly changed to reddish and orange crystals of the new complex **5** deposited at –20 °C (50% yield with respect to the starting copper complex). The starting carbene complex **1d** could be recovered from this solution (ca. 35% after chromatographic

purification, 70% based on the conversion). This process implies two unprecedented events on the coordination sphere of copper, 1) exchange of the carbene ligand for a diethyl ether ligand, and 2) transfer of the carbene ligand back to the chromium atom.

The structure of **5** was confirmed by a single-crystal X-ray structure analysis (Figure 1),^[15] which shows that the copper atom is three-coordinate, bonded to the carbene carbon atom

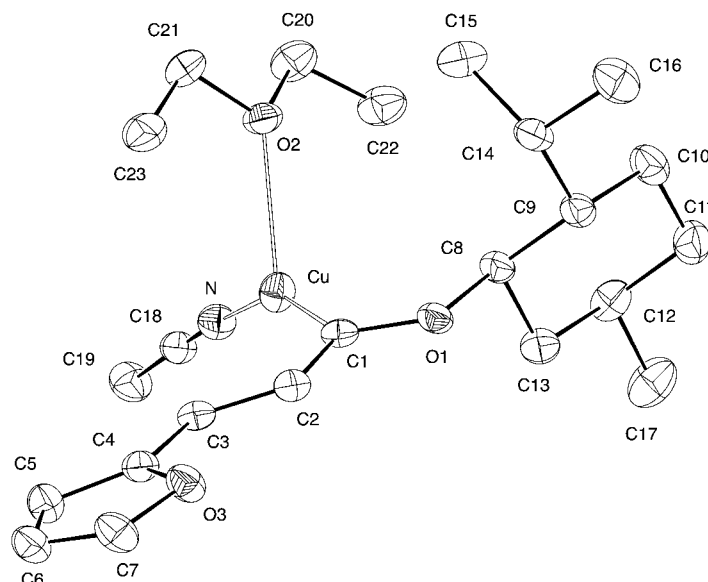


Figure 1. The structure of **5** (ORTEP view). For clarity, the hexafluorophosphate ion and hydrogen atoms are omitted. Thermal ellipsoids are drawn at 30% probability. Selected distances [Å] and angles [°]: Cu–C1 1.882(3), Cu–N 1.891(3), Cu–O2 2.345(2), C1–Cu–N 150.88(14), C1–Cu–O2 114.22(12), N–Cu–O2 94.90(11), N–Cu–C1–C2 –69.4(4), O2–Cu–C1–C2 112.5(3), Cu–C1–C2–C3 4.5(5).

(C1), the diethyl ether oxygen atom (O2), and the acetonitrile nitrogen atom (N) in an approximately trigonal fashion. The planes defined by O2, N, C1, Cu (mean deviation: 0.0050 Å) and Cu, O1, C2, C1 (mean deviation: 0.0003 Å) deviates from an orthogonal arrangement; the N–Cu–C1–C2 and O2–Cu–C1–C2 torsion angles are –69.4° and 112.5°, respectively. The alkenyl and copper carbene moieties are coplanar (Cu–C1–C2–C3 torsion angle: 4.5°). The Cu–C(carbene) distance (1.882 Å) is in agreement with that reported for X–Cu=CR₂ complexes.^[16] The Cu–C2 (2.96 Å) and Cu–C3 (3.24 Å) distances rule out a possible copper–alkene coordination.^[17]

Complex **5** is stable in the solid state or in solution (CH_2Cl_2 , toluene) for several days at –20 °C under nitrogen. The ^1H , ^{13}C , ^{31}P , and ^{19}F NMR spectra were recorded in CD_2Cl_2 at ambient temperature without decomposition. The Cu=C carbon atom resonates at $\delta = 276.5$.^[18] Despite the creation of a chirality axis, a single set of signals was observed in the variable-temperature ^1H NMR spectra between –80 °C and 25 °C. The UV spectrum of **5** ($\lambda_{\text{max}} = 383$ nm, $\epsilon = 42\,300$, in CH_2Cl_2) suggests a $\pi\text{--}\pi^*$ transition of the copper–carbon double bond with predominant metal-to-ligand charge-transfer character.

The carbene ligand of both complexes **4** and **5** dimerized stereoselectively in the presence of tributylphosphane

(3 equiv, CH_2Cl_2 , 25°C) affording the (1*E*,3*E*,5*E*)-1,3,5-triene **3b** (75% for **4**; 82% for **5**). Moreover, we found that the copper complex **5** catalyzed the dimerization of EDA (CH_2Cl_2 , 25°C) to a mixture of ethyl maleate and ethyl fumarate, with retention of the structural integrity of the catalyst.

In summary, chromium–carbene complexes efficiently transfer the carbene ligand to a copper(I) center allowing the preparation of functionalized alkenes, dienes, and trienes through homo- and heterocoupling reactions. A new copper(I)–carbene complex has been isolated and structurally characterized by X-ray diffraction that features, 1) an uncommon tricoordinate pattern, and 2) the presence of two other ligands with fair (MeCN) and very low (Et_2O) coordination capability. It should be emphasized that no X-ray structural determination has been hitherto achieved for copper(I) carbene complexes of the type $[\text{ML}_3]$,^[17] rather only the spectroscopic characterization has been reported for a few d^{10} copper carbene complexes containing either highly donor carbene ligands (*tert*-butyl-substituted imidazoline and imidazolidine carbenes)^[5–7] or extremely basic, sterically demanding iminophosphoramidate ligands.^[12] Further work directed to study the potential of these chiral copper(I)–carbene complexes in asymmetric catalysis is in progress.

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- [14] The vinylic hydrogen atoms of the carbene ligand of **4** are low-field shifted (around 1 ppm) compared with those of complex **1d**. The

HMBC experiments clearly indicate correlation between the olefinic C_β hydrogen atom and the carbene carbon atom.

- [15] Crystal structure data for **5**. $\text{C}_{23}\text{H}_{37}\text{CuF}_6\text{NO}_3\text{P}$: $M_r = 584.05$, orange plates, dimensions = $0.45 \times 0.20 \times 0.10$ mm, orthorhombic, space group $P2_12_12_1$ (determined from the systematic absences), $a = 7.3757(1)$, $b = 14.2948(2)$, $c = 26.6661(3)$ Å, $V = 2811.52(6)$ Å³, $\lambda = 1.5418$ Å, $Z = 4$, $\rho_{\text{calc}} = 1.380$ Mg m⁻³, $F(000) = 1216$, $\mu(\text{Cu}_{\text{K}\alpha}) = 2.207$ mm⁻¹. The crystals are extremely air-sensitive. The sample was mounted in an appropriately sized cryoloop, and it was completely covered with mineral oil to prevent it from being in contact with oxygen. The crystal was held at 120(2) K with an Oxford Cryosystems Cryostream Cooler. Data collection was performed on a Nonius KappaCCD single-crystal diffractometer (fine-focus sealed tube, horizontally mounted graphite crystal, 95 mm CCD camera). The crystal–detector distance was fixed at 29 mm, and a total of 1018 images were collected by using the oscillation method (φ and ω scans), with 1° oscillation and 30 s exposure time per image. The data collection strategy was calculated with the program COLLECT. Data reduction and cell refinement were performed with the programs HKL DENZO and SCALEPACK. Unit cell dimensions were determined from 30381 reflections between $\theta = 1.473^\circ$ and 70.076° . A total of 20320 reflections were measured ($\theta_{\text{min}} = 3.51^\circ$, $\theta_{\text{max}} = 69.79^\circ$; $-8 \leq h \leq 8$, $0 \leq k \leq 17$, $0 \leq l \leq 32$). Multiple observations were averaged ($R_{\text{int}} = 0.059$), resulting in 5197 unique reflections of which 4910 were observed with $I > 2\sigma(I)$. Final mosaicity was $0.616(1)^\circ$. All data completeness was 98.7%. Intensity–error ratio for all reflections was 413.0:176. The crystal structure was solved by Patterson methods and phase expansion using the program DIRDIF-99.2. An empirical absorption correction was applied ($T_{\text{min}} = 0.450$; $T_{\text{max}} = 1.000$) using XABS2. Anisotropic least-squares refinement was carried out with SHELXL-97. All non-hydrogen atoms were anisotropically refined. Hydrogen atoms were geometrically placed riding on their parent atoms with isotropic displacement parameters set to 1.2 times the U_{eq} value of the atoms to which they are attached (1.5 for methyl groups). The final cycle of full-matrix least-squares refinement based on 5197 reflections and 317 parameters converged to a final value of $R1(\text{observed}) = 0.0405$, $R1(\text{all data}) = 0.0445$, $wR2(\text{all data}) = 0.1024$, $S = 1.083$, $(\Delta/\sigma)_{\text{max}} = 0.003$, weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.0000P)^2 + 2.6320P]$, where $P = (F_o^2 + 2F_c^2)/3$. Final difference Fourier maps showed no peaks higher than 0.366 e Å^{-3} nor deeper than -0.288 e Å^{-3} . Flack parameter = $0.00(3)$. Geometrical calculations were made with PARST97. The crystallographic plots were made with PLATON. 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-163238. 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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- [18] Selected NMR signals of **5** (numbering according to Figure 1). ¹H NMR: $\delta = 8.15$ (1H, d, $J = 15$ Hz; C3-H), 7.1 (1H, d, $J = 15$ Hz; C2-H), 3.4 (4H, q, $J = 7$ Hz; C20-H, C21-H), 2.35 (3H, s; C19-H), 1.1 (6H, t, $J = 7$ Hz; C22-H, C23-H); ¹³C NMR: $\delta = 276.5$ (C1), 152.4, 152.2, 150.5, 133.3, 126.1, 115.6 (C1–C7), 119.6 (C18), 66.5 (C20, C21), 3.1 (C19).

Fmoc-Compatible Solid-Phase Peptide Synthesis of Long C-Terminal Peptide Thioesters**

Axel Sewing and Donald Hilvert*

C-Terminal peptide thioesters are valuable intermediates for the chemical synthesis of proteins by peptide fragment condensation,^[1] and for the production of cyclic peptides^[2] and peptide dendrimers.^[3] They are typically prepared either by solid-phase peptide synthesis (SPPS)^[4] or in the case of longer segments, biosynthetically, by intein technology.^[5]

Until recently, SPPS of peptide C-terminal thioesters was largely restricted to *tert*-butoxycarbonyl (Boc) methodology^[4] because of the sensitivity of peptide thioesters to the basic conditions required to remove the widely used 9-fluorenylmethoxycarbonyl (Fmoc) protecting group.^[6] However, several Fmoc-compatible routes to peptide thioesters are now available, including the use of new cleavage cocktails,^[7] backbone amide linkers,^[8] and activatable sulfonamide linkers.^[9, 10] Each approach has advantages and disadvantages, but the need for special linkers, resins and/or complicated procedures remains a general limitation.

We recently reported an alternative Fmoc-compatible strategy for production of peptide thioesters involving direct cleavage of peptides from standard resins using an excess of AlMe_2Cl and EtSH in dichloromethane.^[11] This one-pot method yields the corresponding thioesters directly in good yield and purity. However, some problems arising from epimerization of C-terminal residues other than glycine and undesired side reactions, especially formation of side chain thioesters and aspartimide rearrangement, were observed. Here we report substantial improvements in the original procedure that allow its application to long peptides of complex composition.

The synthesis of the pentapeptide thioethyl ester LYRAG-SEt was used as a model system for optimizing the reaction conditions. The influence of the resin (4-hydroxymethylphenylacetamidomethyl-polystyrene (PAM), Wang, and 4-hydroxymethylbenzoic acid (HMBA))^[6, 12] and the choice of Lewis acid on yield, side reactions, and epimerization was examined. In our original publication we reported that cleavage of LYRAG from PAM resin with AlMe_2Cl /EtSH to give LYRAG-SEt proceeded in 60% yield. With improved handling procedures and more accurate quantification of resin loading^[6] this yield could be increased to 87% (Table 1). The HMBA resin afforded comparable yields and purities (84% isolated thioester) but the more reactive Wang resin

Table 1. Yields of selected C-terminal peptide thioesters.

Peptide	Resin/ Ester	Reagent ^[a]	Thioester [%]	Acid [%]	Bis-thioester ^[b] [%]	Aspartimide ^[c] [%]
LYRAG	PAM	A	87	5	–	–
LYRAG	PAM	B	85	6	–	–
LYRAG	HMBA	A	84	5	–	–
LYRAG	OMe	C	67	17	–	–
IFKDG	PAM	A	22	n.d. ^[d]	13	25
IFKDG	PAM	D	43	n.d. ^[d]	15	6
IFKDG	PAM	B	62	n.d. ^[d]	6	3

[a] Reagent A: 0.2 M AlMe_2Cl , 0.6 M EtSH in CH_2Cl_2 ; reagent B: 0.2 M AlMe_3 , 0.6 M EtSH in CH_2Cl_2 ; reagent C: 0.02 M (2 equiv) AlMe_2Cl , 0.06 M (6 equiv) EtSH in CH_2Cl_2 ; reagent D: 0.2 M AlMe_2Cl , 0.6 M EtSH, 0.2 M THF in CH_2Cl_2 .

[b] Bis-thioester IFKD(SEt)G-SEt. [c] Aspartimide-thioester IFKD(imid)G-SEt. [d] n.d.: not determined.

always gave lower yields of the desired thioester and a higher proportion of the corresponding C-terminal acid. Because preloaded PAM resins are commercially available, they have an advantage over HMBA resins. However, the HMBA resin also allows the protected peptide to be cleaved with triethylamine in methanol to give a methyl ester.^[6] The latter can be converted into a thioester in solution with lower excesses of alkylaluminum reagent than used for direct cleavage from the resin, but this two-step procedure provided no advantage with respect to purity or yield of the final product when peptides, rather than single amino acids,^[11] were used.

By using AlMe_3 in place of the more acidic AlMe_2Cl , the formation of aspartimides and aspartic acid side chain thioesters can be greatly suppressed (Table 1). For example, attempts to cleave IFKDG-PAM with AlMe_2Cl /EtSH produced the thioester IFKDG-SEt in low yield (22%), as well as substantial amounts of the IFKD(SEt)G-SEt bis-thioester (13%) and the aspartimide generated by cyclization of the aspartyl residue (25%). In contrast, treatment of IFKDG-PAM with AlMe_3 /EtSH gave a 62% yield of the desired thioester, 6% bis-thioester, and only 3% aspartimide thioester. Addition of stoichiometric amounts tetrahydrofuran (THF) to the AlMe_2Cl reagent also reduced aspartimide formation, but did not affect bis-thioester formation. Addition of THF to AlMe_3 provided no advantage.

Although AlMe_3 is compatible with peptides of complex composition, earlier studies^[11] suggested that the extent of epimerization of susceptible C-terminal thioesters would also increase in the presence of this Lewis acid. Indeed, the ratio of L,L,L,D diastereomers of the tripeptide Ac-Gly-Ala-Phe-SEt went from 9:1 to 3.5:1 when AlMe_2Cl was replaced by AlMe_3 in the cleavage reaction. This difficulty is circumvented when a cleavage site with a C-terminal glycine can be chosen, as is often the case.^[1, 9, 13, 14]

The utility of the optimized AlMe_3 /EtSH conditions was demonstrated by synthesis of the thioester corresponding to the 37 amino acid long N-terminal fragment of bovine pancreatic trypsin inhibitor (BPTI) (Figure 1). This fragment was previously prepared by Boc methodology and used to synthesize the 58 amino acid long inhibitor by native chemical ligation.^[14]

We prepared the BPTI^{1–37} fragment by SPPS on a PAM resin with standard Fmoc chemistry. The optimized synthesis required triple couplings at difficult stretches^[15] as well as

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

RPDFCLPEPPYTGPKARIIRYFYNAKAGLCQTFVYGG↓CRAKRNNFKSAEDCMRTCGGA

Figure 1. Sequence of BPTI with ligation site indicated (↓).

increased deprotection and coupling times. After completion of the synthesis, the peptide was cleaved from the resin by treatment with AlMe_3 and thiol for 5 h. All side chain protecting groups were subsequently removed with a cocktail containing 89 % trifluoroacetic acid (TFA), 4 % water, 2 % phenol, 2 % thioanisole, 2 % ethanethiol, and 0.8 % triisopropylsilane. Analysis of the crude reaction mixture by HPLC/MS identified BPTI^{1–37}-SEt as the major product (Figure 2).

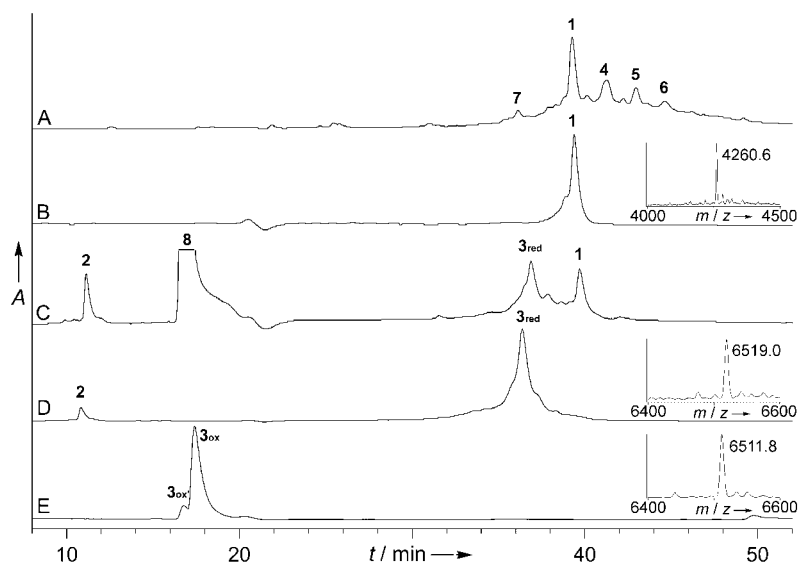


Figure 2. Synthesis of BPTI^{1–37}-SEt (**1**) and its native chemical ligation with BPTI^{38–58} (**2**) monitored by analytical HPLC. A) Crude product **1**; B) isolated and purified **1**; C) ligation ($t = 8$ h) of **1** with **2** to form BPTI^{1–58} (**3_{red}**); D) ligation mixture ($t = 16$ h) after gel filtration to remove small molecules; E) native, oxidized BPTI^{1–58} (**3_{ox}**) after gel filtration and HPLC purification. Masses of key species calculated from the multiple charged ion mass peaks are shown as insets. Side products and reagents: **3_{ox}**, oxidized BPTI^{1–58} containing methionine sulfoxide; **4**, BPTI^{1–37}-bisthioester; **5**, *t*Bu adduct of BPTI^{1–37}-SEt; **6**, acetyl-capped deletion fragment Ac-BPTI^{15–37}-SEt; **7**, acetyl-capped deletion fragment Ac-BPTI^{6–37}-SEt; **8**, thiophenol.

The Asp³(SEt) bis-thioester, a *tert*-butyl adduct of the desired thioester, and two capped deletion fragments, Ac-BPTI^{6–37}-SEt and Ac-BPTI^{15–37}-SEt, were observed as minor side products. The desired thioester is poorly soluble, making purification difficult. After two preparative HPLC runs on a C-8 column, however, it was obtained in 8 % overall yield, which can be compared with the 15 % yield for the same peptide obtained by the Boc method. The mass of this fragment was determined to be 4260.6 Da by electrospray mass spectrometry (ESI-MS), in good agreement with the calculated mass of 4261.0 Da.

To show that the synthetic BPTI^{1–37}-SEt fragment is identical to the previously prepared material, it was condensed with BPTI^{38–58} according to literature protocols to give reduced, denatured BPTI in 63 % yield (Figure 2). After oxidation and folding,^[14, 15] synthetic BPTI was purified by gel filtration and HPLC. The experimentally determined masses of the oxidized (6511.8 Da) and reduced (6519.0 Da) protein were in good agreement with the calculated values (6512.5 Da

and 6518.5 Da, respectively). Synthetic and commercial BPTI co-eluted on a C-8 HPLC column and exhibited identical circular dichroism spectra (data not shown). Moreover, the synthetic protein inhibited trypsin stoichiometrically^[16] and chymotrypsin^[14, 17] with a K_i of 1.3×10^{-8} M (lit.^[14] 1.2×10^{-8} M).

Lewis acid activated cleavage of peptides from commercial resins provides a facile route to C-terminal peptide thioesters. The method is compatible with standard Fmoc procedures, employs commercially available reagents and resins, and does not require special handling procedures. Use of AlMe_3 as the Lewis acid minimizes side reactions like aspartimide and bis-thioester formation, although some epimerization at the site of cleavage occurs. Given its simplicity, this method should be useful for preparing thioesters of a wide variety of peptide segments having a C-terminal glycine, which themselves are widely used for protein synthesis.^[1, 9, 13, 14]

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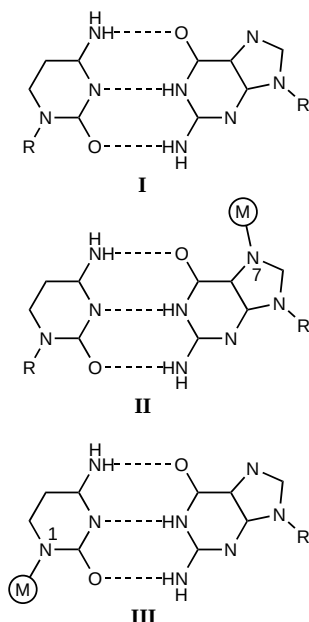
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Pt^{II} Binding to N1 of Cytosine: Strengthening the Watson–Crick Pair with Guanine and yet Confining Its pH Existence Range**

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Metal coordination to a nucleobase donor atom remote from its hydrogen bonding sites neither prevents base-pair formation for steric reasons nor diminishes their strength. On the contrary, it enhances it. This effect, which was originally proposed by theoretical chemists and attributed to polarization effects of the metal ion as well as electrostatic attraction,^[1] has recently been found to be also true for solutions of guanine nucleobases carrying a Pt^{II} entity at N7.^[2] X-ray crystallographic results are consistent with this view.^[2a, 3]

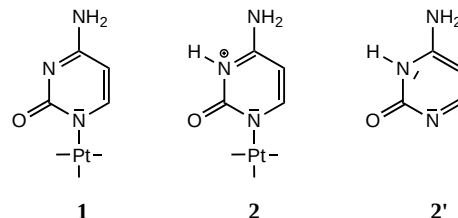
As will be shown here, the previously demonstrated case of Watson–Crick pairing between N7-platinated guanine and cytosine can be inverted by coordination of the metal to the cytosine N1 position and hydrogen bonding it with a non-metalated guanine (Scheme 1). The order of stability in



Scheme 1. The Watson–Crick base pairs C/G, C/G-Pt, and Pt-C/G.

DMSO solution is $\text{II} \approx \text{III} > \text{I}$; thus, the artificial (platinated) base pairs are more stable than the natural ones.

We have recently prepared and characterized (X-ray crystallography, NMR spectroscopy) two complexes of composition *trans*-[(CH₃NH₂)₂Pt(C-NI)₂] (**1**) and *trans*-[(CH₃NH₂)₂Pt(HC-NI)₂]²⁺ (**2**) (C = cytosine anion, HC = neutral cytosine) (Scheme 2).^[4] The cytosine ligand in **2**



Scheme 2.

represents a platinated form of a rare cytosine tautomer 2'. Cococrystallization of the neutral complex **1** with 9-ethylguanine (9-EtGH) yielded crystals of *trans*-[(CH₃NH₂)₂Pt(C-NI)₂]·2[9-EtGH]·8H₂O (**3**) (Figure 1).^[5] The two cytosi-

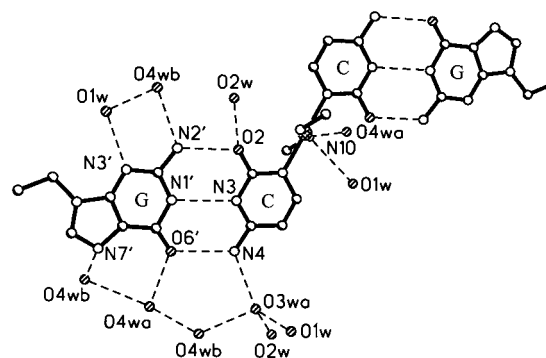


Figure 1. Structure of *trans*-[(CH₃NH₂)₂Pt(C-NI)₂]·2[9-EtGH]·8H₂O (**3**) in the crystal.

nate ligands adopt a head–tail orientation and form large (70.6(2)°) dihedral angles with the Pt coordination plane, which is a normal arrangement. There are no unusual features of the cytosine ligands compared to those in **1**.^[4] The two cytosine bases in **3** are involved in Watson–Crick pairing with the 9-EtGH nucleobases. The complementary bases are almost coplanar (2.9(2)°). Hydrogen bonds within the base pairs are 2.882(5) (N4···O6'), 2.930(4) (N3···N1'), and 2.833(4) Å (O2···N2'), thus paralleling the trend in the nonmetalated Watson–Crick pair^[6] but deviating somewhat from distances in base pairs containing N7-platinated guanine bases.^[3c, 4] The solid-state structure of **3** (Figure 1) is dominated by extensive base stacking interactions along the *x* axis: Thus the Watson–Crick pairs form stacked dimers (3.4 Å), which themselves are stacked (3.5 Å) in such a way that a layer structure with repeating units of four bases (Pt-C, 9-EtGH, 9-EtGH, Pt-C) is formed.

Concentration-dependent ¹H NMR spectra of **3** recorded in [D₆]DMSO are consistent with intermolecular hydrogen bond formation between platinated cytosine and 9-EtGH; the

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signal of the N1H proton of 9-EtGH undergoes an upfield shift of about 1 ppm in a dilution range of 0.18 M to 0.0056 M, whereas, shifts of the signals of the exocyclic NH₂ groups of both the cytosine and guanine nucleobases are expectedly smaller (ca. 0.5 ppm). These shifts are consistent with Watson–Crick pairing, since in each case only one of the two amino protons is hydrogen-bonded; however, an averaged signal of the two protons in an amino group is recorded. Stacking interactions between complexes can be ruled out since the chemical shifts of the aromatic protons of the two bases are virtually unaffected by changes in concentration. Under the assumption that the two cytosine ligands in **3** behave independently as far as hydrogen bonding with 9-EtGH is concerned, the stability of the base pair has been calculated to be $K = 10.0 \pm 1.7 \text{ M}^{-1}$ (3σ). This value is higher than for the nonplatinated base pair 9-EtGH/1-MeC (1-MeC = 1-methylcytosine) in the same solvent ($K = 6.9 \pm 1.3 \text{ M}^{-1}$) and is in the same range as found for various pairs of [Pt(9-EtGH-N7)] complexes with 1-MeC.^[2]

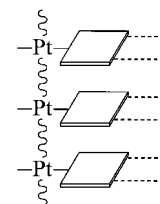
As with thymine/adenine pairs, it is not possible to quantify Hoogsteen pairing of the CH ligands in **2** with 9-EtGH in DMSO due to the presence of two hydrogen bonds only. However, the formation of hydrogen bonds between the CH ligands in **2** and free 1-MeC is evident from concentration-dependent ¹H NMR spectroscopy. The base pair formed is a metalated analogue of the well known hemiprotonated cytosinium/cytosine pair and reveals an association constant of $K = 24 \pm 8 \text{ M}^{-1}$.

Apart from base pair stability, the question of the pH range in which a pair exists, is of importance. For guanine/cytosine pairing, to a first approximation the pK_a values of guanine define the boundaries for existence in aqueous solution.^[7] Thus, Watson–Crick pairing becomes obsolete when guanine is an anion ($\text{pH} > pK_a \approx 9$) or alternatively when cytosine is protonated at N3 ($\text{pH} < pK_a \approx 4$). However, protonated cytosine can still pair with neutral guanine in the Hoogsteen fashion down to a pH value at which guanine becomes protonated at N7 ($pK_a(\text{guaninium}) \approx 3$). Base pairing between guanine and cytosine (**I**) therefore covers roughly the pH range 3–9 (Figure 2a). The same pH range is also valid for the guanine/cytosine base pair **III** with a pyrimidine ligand

platinated at N1, but as a consequence of the pK_a of about 7 for [Pt(CH-NI)],^[4] the stability range of the Watson–Crick pair becomes much narrower (pH 7–9) at the expense of the Hoogsteen range, which extends now from about pH 3–7 (Figure 2b). Using similar arguments, it can be concluded that N7-platinated guanine and cytosine have a range of pH 4–9 for Watson–Crick pairing (**II**), with Hoogsteen pairing prevented (Figure 2c) owing to the position of the platination. Finally it can be concluded that [Pt(CH-NI)] is able to form hydrogen bonds with a free cytosine base over a wider pH range (pH 4–7, Figure 2e) than two hemiprotonated free cytosine bases (Figure 2d).^[7]

In summary, the presented results show that a strengthening of the G/C Watson–Crick base pair can be accomplished by either metalation of N7 of the guanine or of N1 of the cytosine base. Similarly, the strength of the pair between Pt-CH and 1-MeC exceeds that of the regular G/C pair, even though it does not reach the record value recently observed for the hemideprotonated pair of N7-platinated guanine.^[8]

Our findings have led us to pursue the idea of creating artificial oligonucleotide analogues that consist of a backbone of metal ions and bridging ligands, with nucleobases attached either through N1 (unsubstituted pyrimidine bases), through N9 (unsubstituted purine bases), or through N7 (N9-substituted purine bases) to the metal centers. These coordination polymers might be considered relatives of purely organic neutral^[9] and cationic^[10] oligonucleotide analogues. Indeed, X-ray crystal structure studies on metal-containing oligo- or polymeric compounds of this kind have been described^[11] although the reported ones represent kinetically labile entities. The use of Pt^{II} might be advantageous in that it not only is expected to lead to kinetically more robust species, but that additionally the bases, regardless of their binding site (as long as endocyclic sites are involved), are forced in a more or less parallel fashion (Scheme 3). As outlined here, hydrogen bonding properties of nucleobases attached to Pt and the pH range available for hydrogen bonding make them attractive synthesis goals.



Scheme 3. Simplified representation of nucleobases linked through Pt^{II}.

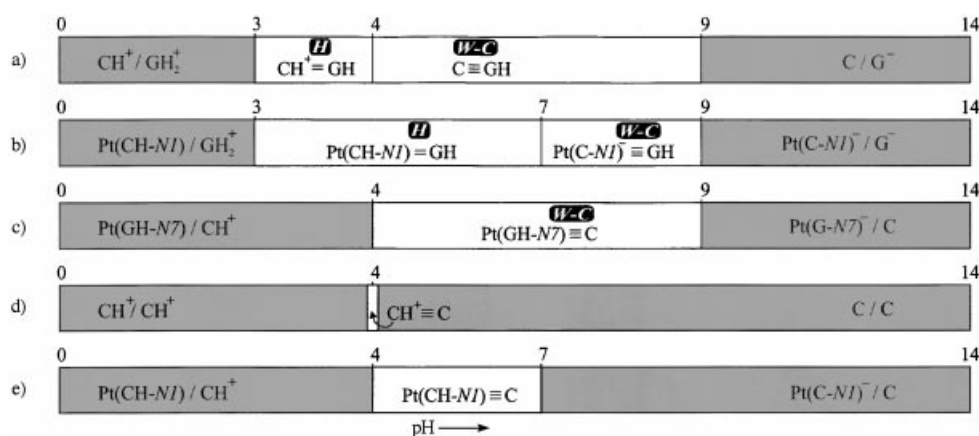


Figure 2. Approximate existence ranges of Hoogsteen (H) (=) and Watson–Crick (W–C) base pairs (≡) considering various protonation states of free and platinated cytosine (C) and 9-ethylguanine (G) nucleobases (see also text).

Experimental Section

Compounds **1** and **2** were synthesized as described in reference [4], 9-EtGH was purchased from Chemogen (Konstanz, Germany).

9-EtGH (20 mg, 116 μmol) was added to a solution of **1** (30 mg, 58 μmol) in water (10 mL). The mixture was briefly warmed to 70 °C and then allowed to cool and slowly evaporate at 4 °C. Colorless crystals of **3** were isolated in 55 % yield. Elemental analysis (%) calcd for C₂₄H₃₂N₁₈O₁₂Pt (979.8634): C 29.4, H 5.3, N 25.7; found: C 29.2, H 5.4, N 25.7.

NMR data: Concentration-dependent ¹H NMR measurements were performed on a Bruker AC200 instrument at 20 °C in [D₆]DMSO using TMS as internal standard. For calculation of K values, the two

bound cytosine nucleobases were considered independent binding sites for the partners; association constants were determined in analogy to the method described in reference [2a]. For **3** $K = 10.0 \pm 1.7 \text{ M}^{-1}$ (3σ) corresponds to the weighted mean of the individually calculated association constants of the NH_2 ($K = 10.14 \pm 2.37 \text{ M}^{-1}$, $\delta_0 = 6.472 \pm 0.023$, $\delta_\infty = 7.432 \pm 0.055$; the index 0 denotes a high dilution, the index ∞ denotes a high concentration) and N1H ($K = 9.89 \pm 2.51 \text{ M}^{-1}$, $\delta_0 = 10.576 \pm 0.051$, $\delta_\infty = 12.541 \pm 0.125$) protons of 9-EtGH. Since NH_2 of cytosine could not be detected at higher concentrations, only a rough estimate of K could be obtained; however, this value was, within the given error limits, in agreement with that for the guanine protons.

An equimolar solution of **2** (120 mM) and 1-MeC was stepwise diluted and ^1H NMR spectra were recorded. The association constant $K = 24 \pm 8 \text{ M}^{-1}$ corresponds to the weighted mean of the individually calculated association constants of the N3H(C) ($K = 37.8 \pm 14.0 \text{ M}^{-1}$, $\delta_0 = 11.899 \pm 0.035$, $\delta_\infty = 13.127 \pm 0.143$), N4H_2 (1-MeC) ($K = 16.8 \pm 4.0 \text{ M}^{-1}$, $\delta_0 = 6.925 \pm 0.012$, $\delta_\infty = 7.577 \pm 0.046$), and the N4H_2 (C) ($K = 29.6 \pm 3.7 \text{ M}^{-1}$, $\delta_0 = 7.184 \pm 0.018$, $\delta_\infty = 8.887 \pm 0.059$) protons. It should be mentioned that the N3H(C) resonance is not observable at high concentrations, and the N4H_2 resonance of the Pt-bound cytosine is split into two resonances of which only one is concentration-dependent. Since water of crystallization had not been removed in either case, we consider the reported association constants as lower limits, as the water molecules present in solution will compete for the hydrogen bonding sites.

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Electrochemical Regeneration of Low-Valent Indium(I) Species as Catalysts for C–C Bond Formations**

Gerhard Hilt* and Konstantin I. Smolko

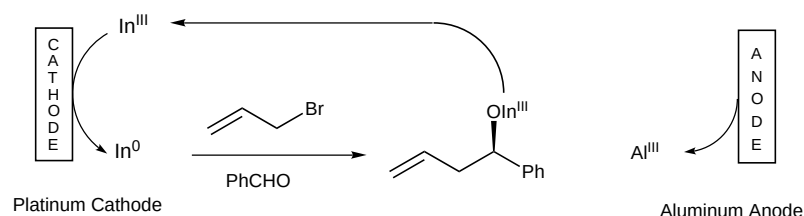
Dedicated to Professor Martin F. Semmelhack on the occasion of his 60th birthday

In recent years the chemistry of indium has become more popular in organic synthesis, partly due to the ability to perform this chemistry in water or aqueous solvent systems. The chemistry of indium metal as well as the chemistry of indium salts for C–C bond formation processes has been reviewed several times.^[1] In most cases indium metal is used in stoichiometric amounts and only in a few cases could versions be realized that were catalytic in indium, accompanied with a stoichiometric amount of a reducing agent.^[2] In addition, the chemistry of low-valent indium(I) salts has been sparsely investigated, most probably because of the high cost of these species.^[3]

Our approach to an electrochemical regeneration process for low-valent indium species was based on the idea of using the cathode as the reducing agent for the spent indium(III) reagent (Scheme 1). We chose the indium-catalyzed allylation of benzaldehyde with allyl bromide as a test reaction. We found, however, that an efficient allylation only took place when sacrificial anodes (preferably aluminum foil anodes)^[4] were used in an undivided cell, whereas the reaction proceeded only with low conversions when a divided cell (Pt cathode and Al anode) or a quasi-divided cell (Pt foil cathode and Pt wire anode)^[5] were used. We concluded that a major part of the reduction process in an undivided cell must

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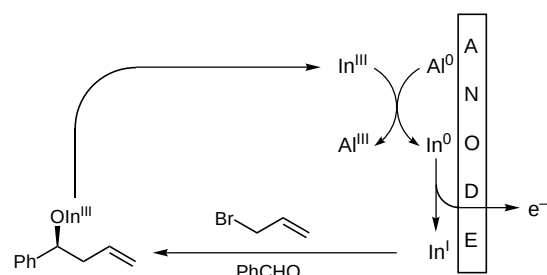
[**] This work was supported by the Deutsche Forschungsgemeinschaft. We thank Prof. W. Schnick, W. Wünschheim, and S. Rannabauer for the measurement of the Raman spectra.



Scheme 1. Planned cathodic reduction of indium(III) salts in the presence of a sacrificial anode.

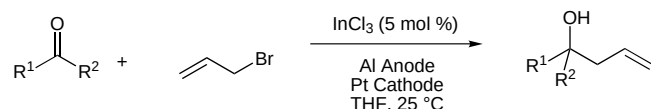
therefore take place at the anode and not at the cathode as originally envisaged.

In the absence of InCl_3 , however, we detected some conversion under electrochemical conditions, but the rate of the reaction was a factor of 10–15 slower than in the presence of indium salts (5 mol % InCl_3). The use of aluminum foil as a sacrificial anode leads to the removal of the passivation oxide layer leaving fresh aluminum behind,^[6] which is in turn able to reduce the indium(III) salts efficiently to metallic indium. However, deposition of metallic indium in the absence (or at low concentrations) of substrates could not be detected, since the polarization as anode causes a reoxidation of the indium metal to THF-insoluble indium(I) salts, which precipitate as orange-brown materials on the electrode surface. Raman spectroscopy and atom emission spectroscopy suggests that this precipitate could be InAlBr_4 .^[7] Direct reduction of the indium(III) salts to indium(I) salts, which are sparingly soluble in THF, by the aluminum foil can be excluded since the characteristic brown/red-brown precipitate is not formed under nonelectrochemical conditions. The generation of such low-valent indium species most likely proceeds by the reduction of indium(III) salts to metallic indium, as described for many non-noble metals, followed by reoxidation to indium(I) salts (Scheme 2).^[8] The resulting In^{I} undergoes oxidative addition to the allyl bromide and, in turn, mediates the formation of the new C–C bond in an indium-catalyzed Barbier-type reaction.



Scheme 2. Reductive anodic process for the generation of low-valent indium species at the aluminum anode for the electrochemical allylation of benzaldehyde.

Preparative reactions with different types of aliphatic and aromatic carbonyl compounds, and even sterically demanding ketones (Scheme 3), were carried out under these electro-



Scheme 3. Catalytic allylations of carbonyl compounds under electrochemical conditions.

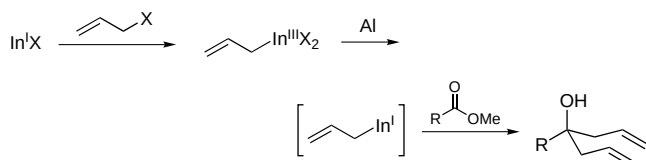
chemical conditions, and some characteristic examples are given in Table 1 (entries 1–5). The electrolyses were conducted under galvanostatic conditions (100 mA) until 1.0 F mol^{−1} of current was consumed. The conversions were in all cases complete (with the exception of the reaction in entry 4, Table 1), but the current yield for the conversions of simple aldehydes and ketones often exceeded 100% during the course of the

Table 1. Results of the electrochemical indium-catalyzed allylations of carbonyl compounds.

Entry	R ¹	R ²	Product	Yield [%]
1	Ph	H		89
2	C ₆ H ₁₁	H		76
3	Ph	CH ₃		96
4	<i>i</i> Pr	<i>i</i> Pr		50
5		–CH ₂ CH ₂ CH ₂ CH ₂ –		87
6	Ph	OCH ₃		85
7	<i>i</i> Pr	OCH ₃		82

reaction, which suggests that a chemical side reaction, which is most probably based on allylations mediated by indium or aluminum metal, cannot be discarded completely.

Of special interest are bis-allylations of aromatic and aliphatic esters (Table 1, entries 6 and 7), since such conversions cannot be achieved by using conventional stoichiometric allylations of the ester by means of indium metal or indium(I) iodide.^[9] A bis-allylation of carboxylic esters can, however, be conducted in relatively low conversion using stoichiometric amounts of allylaluminum sesquibromide (<30% after 2 h). Also, a bis-allylation of carboxylic esters with stoichiometric amounts of InAlBr_4 or a mixture of $\text{In}^{\text{I}}\text{Br}$ and AlBr_3 is not observed. In contrast, the desired product can be obtained when preformed stoichiometric amounts of allylindium sesquibromide or allylindium dihalide are treated with aluminum powder under sonication conditions (>70% conversion after 2 h, Scheme 4). Evidently, allylindium(III) species are not able to allylate esters and therefore a further reduction step, most likely to reactive, low-valent indium species, is necessary to generate a more powerful allylating agent which can complete the desired conversion. Another observation supports these findings; when freshly prepared allylmagnesium halides are treated with indium(I) iodide in THF at -40°C , the desired bis-allylated product can be

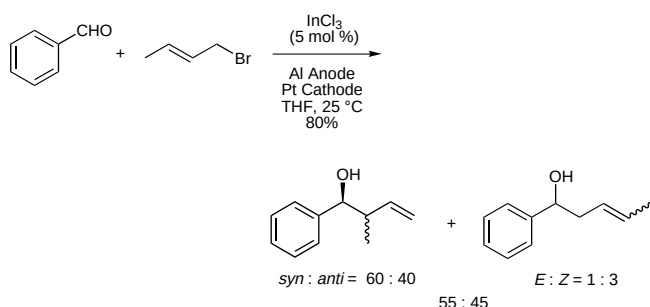


Scheme 4. Reductive activation of allylindium(III) intermediates in the electrochemical allylation of carboxylic esters.

obtained. Thus, a freshly prepared solution of allylmagnesium halide was added at -78°C to a red-purple suspension of In^{I} in THF, and upon warming to -40°C a clear solution was obtained. After the addition of the ester the solution was stirred for 10 min. Subsequent analysis indicated that the conversion was 80%.^[10] For the conversion of aldehydes and ketones, however, the initially formed allylindium(III) species seems to be sufficiently reactive to facilitate an effective allylation process, without the need for further reduction of the allylindium(III) dihalide.

Moreover, in the presence of the supporting electrolyte (0.1M Bu_4NBr) the formation of Lewis acidic AlX_3 compounds is suppressed, and AlX_4^- ions are formed immediately, and thereby the possible Oppenauer-type oxidation of the generated homoallylic alcohols to β,γ -unsaturated ketones (Table 1, entry 1) and a subsequent second allylation to bis-allylated products are not observed as a side reaction under the electrochemical conditions.^[3b]

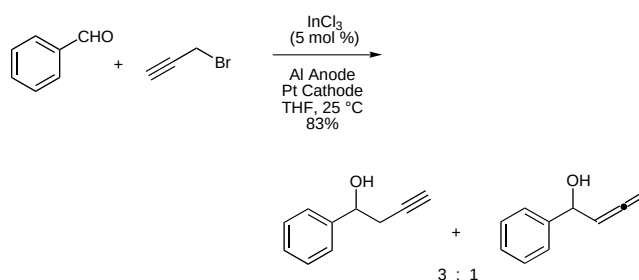
Another indication that the allylation process proceeds via indium(I) species formed on the anode is the reaction of crotyl bromide with benzaldehyde, probably one of the few reactions for which it is possible to differentiate between the nature of the allylating agents involved in the process. Presumably, when starting from indium(I) iodide, allylindium dihalides are generated, whilst the reaction of indium metal furnishes allylindium sesquihalides as the allylating agent. Analysis of the products from the crotylation of benzaldehyde mediated by indium(I) halides gives a mixture of the four possible stereoisomers (γ -substitution product: *syn/anti* isomers; α -substitution product: *E/Z* isomers).^[3] In contrast, when the crotylation of benzaldehyde is performed starting from indium metal the γ -substitution product is formed exclusively under otherwise identical reaction conditions.^[11] In our electrochemical crotylation of benzaldehyde we found the product distribution is almost identical with that obtained in the indium(I)-mediated crotylation (Scheme 5). A trans-



Scheme 5. Catalytic crotylation of benzaldehyde under electrochemical conditions.

metalation of the allyl group from indium to aluminum can also be excluded since both the stoichiometric crotylation using preformed allylaluminum sesquihalides, as well as the relatively slow electrochemical reaction in the absence of indium(III) salts, also furnishes the γ -substitution product exclusively.

Further support for the involvement of indium species and not aluminum-derived C–C bond formation is provided by the electrochemical reaction of propargyl bromide with benzaldehyde, which gives, under electrochemical conditions, a mixture of the corresponding homopropargylic alcohol and the allenylic alcohol in a ratio of 3:1 (Scheme 6), while an electrolysis in the absence of indium gave no desired product.^[12] When the analogous propargylation was performed starting from metallic indium in aqueous methanol a similar product distribution was found (6:1),^[13] but when we changed the solvent system to THF, the propargylation with metallic indium gave a complex mixture of products.



Scheme 6. Catalytic propargylation of benzaldehyde under electrochemical conditions.

These findings indicate that in the case of propargylation, the reaction does not proceed via aluminum species or via indium sesquibromides, but that reactive low-valent indium species are formed by a combination of chemical redox processes with metallic aluminum and electrochemical reactions at the anode.

In conclusion, we have achieved for the first time an electrochemical method for generating and regenerating highly reactive low-valent indium(I) species. The particularly unusual feature of these reactions is that the indium salts are reduced at the aluminum anode. Through a combination of an electrochemically activated aluminum surface for the reduction of the indium(III) salts and the anodic oxidation of the resulting metallic indium to indium(I) aluminum halides, In^{I} -catalyzed allylations could be carried out for the first time. The development of these regeneration routes for low-valent indium(I) species has facilitated catalytic allylations on a preparative scale with aldehydes, ketones, and, for the first time, even carboxylic esters as carbonyl components.

Experimental Section

1-Phenyl-3-buten-1-ol (representative procedure): In a 50-mL beaker cell benzaldehyde (212 mg, 2.0 mmol), allyl bromide (605 mg, 5.0 mmol, 2.5 equiv), indium trichloride (22 mg, 0.1 mmol, 5.0 mol %), and tetra-*n*-butylammonium bromide (0.1M) were dissolved in dry THF (20 mL) under a nitrogen atmosphere. The solution was electrolyzed at constant current (100 mA) until 1.0 F mol^{-1} was consumed on an aluminum foil anode (6 cm^2) and a platinum foil cathode (6 cm^2). After the electrolysis, the

solution was stirred for another 10 min and the solvents were removed on a rotary evaporator. The residue was taken up in water and extracted three times with diethyl ether. The organic phase was dried over sodium sulfate and concentrated. The crude product was purified by column chromatography on silica gel (eluent pentane/diethyl ether 10/1) and the product was obtained as a colorless oil (260 mg, 1.78 mmol, 89 % yield).

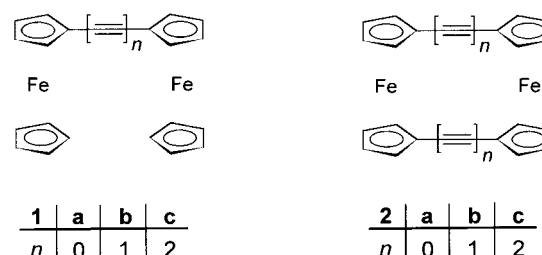
Received: March 7, 2001 [Z16729]

Synthesis and Properties of the First [4,4]Ferrocenophane-1,3,15,17-tetrayne**

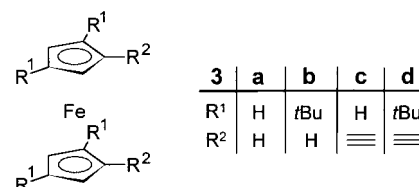
Kai H. H. Fabian, Hans-Jörg Lindner, Norbert Nimmerfroh, and Klaus Hafner*

Dedicated to Professor Siegfried Hünig on the occasion of his 80th birthday

In recent years transition metal complexes of π -conjugated oligomers and polymers have become of increasing interest as potential materials for technical applications.^[1] Studies of novel model compounds such as the bisferrocenes **1a–c** and the ferrocenophanes **2a, b** with defined geometric arrangement of the metal atoms have demonstrated that the physical properties of these complexes are determined predominantly by the extent of delocalization and by the electronic interactions between the metal centers.^[2,3] In this context



the ferrocenophane **2c** with two butadiynyl bridges, homologous with **2a, b**, is of particular interest, yet all attempts to prepare it by the oxidative coupling of 1,1'-diethynylferrocene **3c** have been unsuccessful because of its pronounced tendency towards polymerization.^[4, 5]



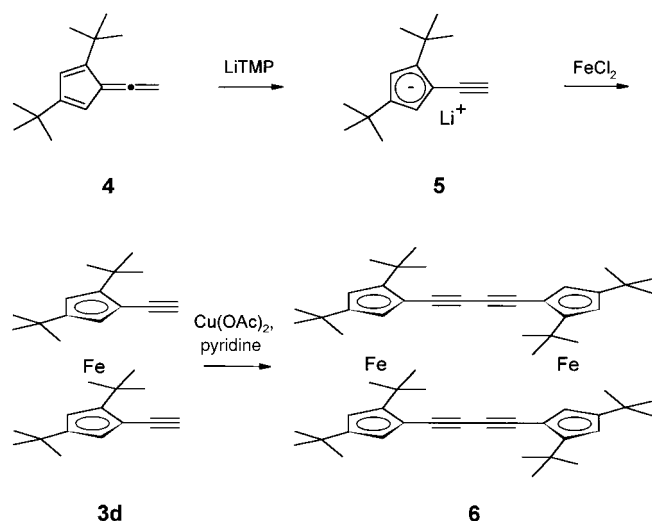
The synthesis of the stable 2,2',4,4'-tetra-*tert*-butyl-1,1'-diethynylferrocene **3d** and its Eglinton coupling^[6] has allowed access to the kinetically stabilized derivative **6** of [4,4]ferrocenophane-1,3,15,17-tetrayne **2c**^[7a] for the first time.

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The 1,1'-diethynylferrocene **3d** is readily prepared by deprotonation of 1,3-di-*tert*-butyl-5-vinylidenecyclopentadiene (**4**)^[8] with lithium 2,2,6,6-tetramethylpiperidide (LiTMP) and subsequent reaction of the lithium salt **5** with iron(II) chloride (Scheme 1).^[7b] Compound **3d** is obtained as



Scheme 1. Preparation of 1,1'-diethynylferrocene **3d** and [4,4]ferrocenophane-1,3,15,17-tetrayne **6**.

red crystals in 26% yield. Since the *tert*-butyl groups inhibit the rotation of the two cyclopentadienyl (Cp) rings the signals of the *tert*-butyl groups and the 3,3' ring protons as well as those of the terminal ethynyl protons of **3d** are broadened in the ¹H NMR spectrum at room temperature. At –40 °C these signals are split into four singlets for the *tert*-butyl groups and two singlets for the ethynyl protons, whereas the signals of each pair of the four ring protons appear as two doublets (*J* = 0.8 Hz). On the basis of this temperature dependency of the ¹H NMR data the energy barrier $\Delta G_{273-293\text{ K}}^*$ for the ring rotation in the 1,1'-diethynylferrocene **3d** is calculated to be $58.8 \pm 2.2 \text{ kJ mol}^{-1}$.^[9] With selective low-temperature ¹H NMR NOE spectroscopy at 233 K it was possible to establish an achiral *syn* configuration (*meso* form) for **3d**.^[10] The ¹³C NMR signals of the acetylene carbon atoms are located within the characteristic range of $\delta = 76.69\text{--}83.83$. The infrared (IR) spectrum exhibits the acetylene stretching frequency at $\tilde{\nu}_{(\text{C}\equiv\text{H})} = 3307$ and $\tilde{\nu}_{(\text{C}\equiv\text{C})} = 2105 \text{ cm}^{-1}$. The redox potential of **3d**, determined cyclic voltammetrically, at $E_{1/2} = 0.49 \text{ V}$ is higher than that of tetra-*tert*-butylferrocene **3b** ($E_{1/2} = 0.12 \text{ V}$) as a result of the negative inductive effect of the C–C triple bond (Table 1).^[11]

Compound **3d** can be converted into the ferrocenophane **6** by oxidative Eglinton coupling^[6] with copper(II) acetate in boiling pyridine and is isolated, by chromatography of the reaction mixture, as red needles which do not show any tendency to decompose or melt at up to 230 °C.^[12] In addition, an oligomer mixture was obtained, the separation of which has to-date not been successful.^[13] The ¹H NMR spectrum of **6** with only two singlets for the *tert*-butyl groups and two broad singlets at $\delta = 3.97$ and 4.22 for the ring protons^[14] indicates a symmetrical structure. The IR spectrum shows only weak acetylene stretching bands at $\tilde{\nu}_{(\text{C}\equiv\text{C})} = 2196 \text{ cm}^{-1}$. The intensity

Table 1. Oxidation potentials of ferrocenes **3a**, **b**, and **d**, bisferrocenes **1a–c**, and ferrocenophanes **2a**, **b**, and **6**.

	$E_{1/2}^1 [\text{V}]$	$E_{1/2}^2 [\text{V}]$	$\Delta E_{1/2} [\text{V}]$	Ref.
3a ^[11]	0.34	–	–	
3b ^[11]	0.12	–	–	
3d ^[11]	0.49	–	–	
1a	0.44	0.79	0.35	[19]
1b	0.63	0.76	0.13	[19]
1c	0.58	0.68	0.10	[19, 20]
2a	0.27	0.86	0.59	[19]
2b	0.62	0.98	0.36	[19]
6 ^[11]	0.35	0.60	0.25	

of the UV/Vis absorption of **6** at $\lambda_{\text{max}} = 232 \text{ nm}$ ($\lg \epsilon = 5.02$), is high in comparison to that of **3d**, and its characteristic ferrocene band, bathochromatically shifted relative to the “monomer” **3d** by $\Delta\lambda = 22 \text{ nm}$ (**3d**: $\lambda_{\text{max}} = 470 \text{ nm}$ ($\lg \epsilon = 2.40$); **6**: $\lambda_{\text{max}} = 492 \text{ nm}$ ($\lg \epsilon = 3.19$)), indicates delocalization through the butadiyne bridges (Figure 1).

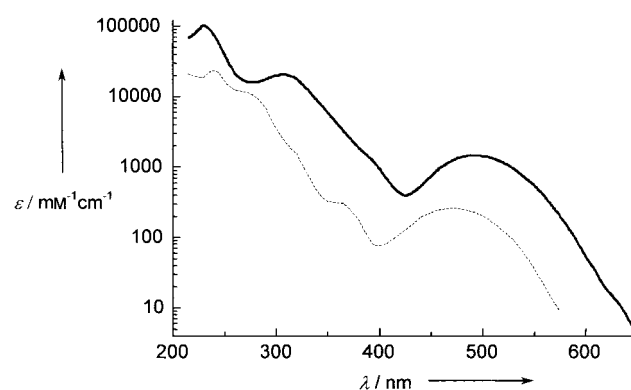


Figure 1. UV/Vis spectra of **3d** (----) and **6** (—) in *n*-hexane.

A single-crystal X-ray structural analysis of the ferrocenophane **6** was performed (Figure 2).^[15] The binuclear complex crystallizes in the space group $P2_1/n$ and has four crystallographically independent molecules per unit cell. The *tert*-butyl groups on the two ferrocene units are in each case situated in the *syn* position. Because of the opposing steric hindrance of the *tert*-butyl groups the two Cp rings lie in an almost perfect

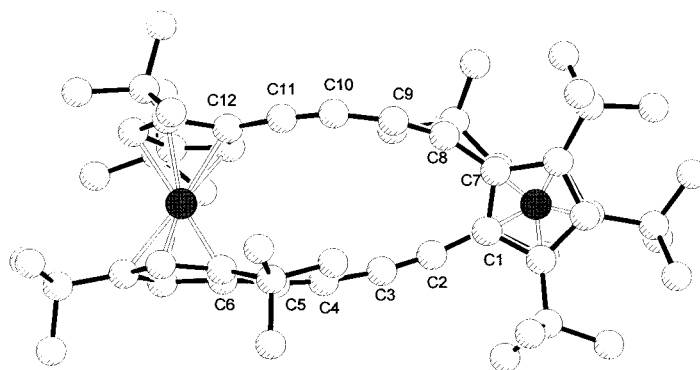


Figure 2. Molecular structure of **6**. The numbering of the atoms does not correspond to the nomenclature. Selected bond lengths [pm] and angles [°]: C1–C2 143.1(5), C2–C3 120.5(5), C3–C4 136.2(6), C4–C5 120.5(5), C5–C6 142.4(5), C7–C8 141.7(5), C8–C9 120.0(5), C9–C10 137.6(6), C10–C11 119.8(5), C11–C12 142.5(6); C1–C2–C3 162.6(4), C2–C3–C4 165.8(4), C3–C4–C5 165.0(4), C4–C5–C6 161.1(4), C7–C8–C9 169.8(4), C8–C9–C10 167.9(4), C9–C10–C11 168.6(4), C10–C11–C12 171.7(4).

antiperiplanar conformation. Consequently the two ethynyl units are twisted relative to each other by an angle of around 45° , and the bond angle along the two butadiynyl bridges deviates considerably from the ideal angle of 180° with values between 161.1 – 171.7° . Thus the two ferrocene units are twisted by about 82° relative to each other and so are almost orthogonal (“twist” form^[14]) so that the molecule exists in a helical-chiral conformation—as a racemic compound in the crystal.

The lengths of the C–C triple bonds lie within the range of $119.8(5)$ to $120.5(5)$ pm and correspond to those of the bisferrocene **1c**.^[3] The Fe–C_{cp} bonds of $203.0(4)$ to $211.6(3)$ pm are with an average length of 208 pm longer than in ferrocene (**3a**; 205 pm^[16]) and $1,1',3,3'$ -tetra-*tert*-butylferrocene (**3b**; 204 – 206 pm^[17,18]). The distance between the two iron atoms in the ferrocenophane **6** is $802.5(5)$ pm.

The cyclic voltammogram^[11] of **6** (Figure 3) with two reversible half-wave potentials at $E_{1/2} = 0.35$ V and $E_{1/2} = 0.60$ V suggests a redox interaction between the ferrocene units. The difference between the two half-waves of $\delta E = 0.25$ V is about twice as large as the value found by Cowan

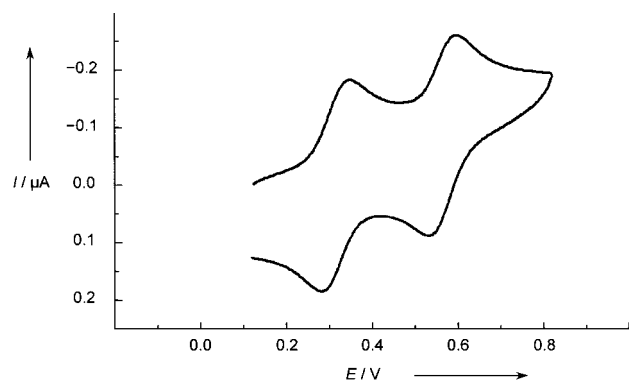


Figure 3. Cyclic voltammogram of **6**.^[11]

et al.^[19] and Hendrickson et al.^[20] for the singly bridged bisferrocenylbutadiyne **1c** ($\Delta E = 0.1$ V). This confirms results according to which in the transformation from “open” bisferrocenophane structures **1** to the “closed” ferrocenophanes **2** the differences between the two half-wave potentials approximately double (Table 1).^[19] A “through-space” interaction between the iron centers in the case of the ferrocenophane **6** can be excluded on account of their large separation.

Experimental Section

2,2',4,4'-tetra-*tert*-butyl-1,1'-diethynylferrocene **3d**: at -90°C , under purest nitrogen, *n*-butyllithium, (3.3 mL, 5.0 mmol, 1.5 M *n*BuLi solution in *n*-hexane) was added dropwise to a solution of 2,2,6,6-tetramethylpiperidine (0.71 g, 5.0 mmol) in THF (10 mL) and the mixture was allowed to warm to room temperature. The mixture was again cooled to -90°C , treated dropwise with a solution of **4** (1.01 g, 5.0 mmol) in THF (10 mL), and stirred at room temperature for 2 h. After the addition of FeCl_2 (0.63 g, 5.0 mmol) at -90°C the reaction mixture was stirred for a further 12 h at room temperature and then treated with water (10 mL) and diethyl ether (20 mL). The aqueous phase was separated, extracted with diethyl ether (20 mL), and the combined organic extracts dried with magnesium sulfate. After filtration and evaporation of the solvent in vacuum the red-brown, oily residue was purified by chromatography on silica gel eluting with *n*-hexane. After removal of the solvent in vacuum **3d** (300 mg, 26%) was

isolated from the orange band as red crystals. M.p. 148 – 150°C ; IR (KBr): $\tilde{\nu} = 3307, 2957, 2926, 2867, 2105\text{ cm}^{-1}$; UV/Vis (*n*-hexane): $\lambda_{\text{max}}(\lg \epsilon) = 216\text{ nm}$ (4.33), 239 (4.41), 266 (4.08) sh, 356 (2.49) sh, 470 (2.40); $^1\text{H NMR}$ (300 MHz, CDCl_3 , -40°C , TMS): $\delta = 1.21$ (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.26 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.34 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.45 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.93 (s, 1H, $\text{C}\equiv\text{CH}$), 3.07 (s, 1H, $\text{C}\equiv\text{CH}$), 4.00 (d, $J = 0.8\text{ Hz}$, 1H, Cp-H), 4.11 (d, $J = 0.8\text{ Hz}$, 1H, Cp-H), 4.15 (d, $J = 0.8\text{ Hz}$, 2H, Cp-H); EI-MS (70 eV): m/z (%): 458 (100) [M^+], 57 (35) [C_4H_9^+]; elemental analysis calcd (%) for $\text{C}_{30}\text{H}_{42}\text{Fe}$ (458.5): C 78.59, H 9.23; found: C 78.51, H 9.42.

6,8,11,13,19,21,24,26-octa-*tert*-butyl-[4.4]ferrocenophane-1,3,15,17-tetrayne 6: a solution of **3d** (600 mg, 1.3 mmol) in pyridine (10 mL) was added dropwise over 2 h to a suspension of copper(II) acetate (905 mg, 5.0 mmol) in boiling pyridine (70 mL) and the reaction mixture was heated under reflux for a further 5 h. After removal of the solvent in vacuum the residue was extracted with diethyl ether ($3 \times 50\text{ mL}$), the solution was filtered and washed to neutral with 2 N hydrochloric acid and water. After the organic phase was dried with magnesium sulfate the solvent was removed in vacuum and the residue purified by chromatography on silica gel eluting with *n*-pentane. After removal of the solvent in vacuum **6** (175 mg, 28%) was obtained from the light red band as red needles which do not melt at up to 230°C . IR (KBr): $\tilde{\nu} = 2959$ (C–H), 2867 (C–H), 2196 (C $\equiv$ C), 2134 cm^{-1} (C $\equiv$ C); UV/Vis (*n*-hexane): $\lambda_{\text{max}}(\lg \epsilon) = 232\text{ nm}$ (5.02), 308 (4.31), 492 (3.19); $^1\text{H NMR}$ (300 MHz, CDCl_3 , 25°C , TMS): $\delta = 1.26$ (s, 18H, $2 \times \text{C}(\text{CH}_3)_3$), 1.43 (s, 18H, $2 \times \text{C}(\text{CH}_3)_3$), 3.97 (brs, 4H, Cp-H), 4.22 (brs, 4H, Cp-H); EI-MS (70 eV): m/z (%): 912 (100) [M^+], 456 (28) [M^{2+}]; elemental analysis calcd (%) for $\text{C}_{60}\text{H}_{80}\text{Fe}_2$ (913.0): C 78.93, H 8.83; found: C 78.51, H 8.93.

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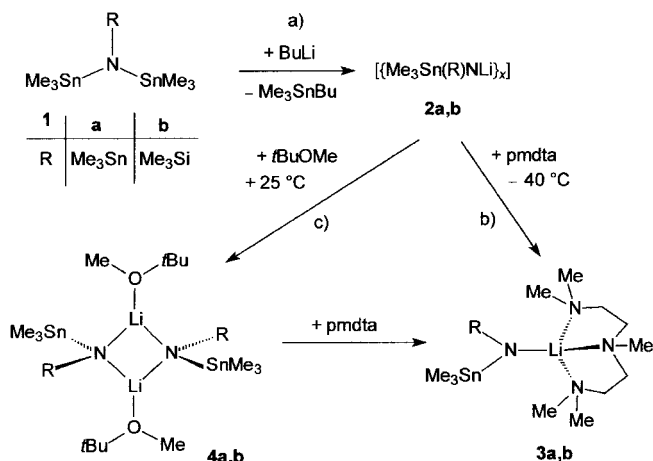
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- [11] The cyclic voltammetric measurements were carried out in CH₂Cl₂ at –20 °C with Bu₄NPF₆ (0.1 M) as supporting electrolyte and calibrated against cobaltocenium/cobaltocene ($E_{\text{ox}} = -1.04 \text{ V}_{\text{SCE}}$; SCE = saturated calomel electrode). The scan speed was 100 mV s^{–1}; working electrode: Pt button; reference electrode: Pt wire.
- [12] A differential scanning calorimetry (DSC) measurement carried out at up to 250 °C gave no indication of topochemical solid-state polymerization.
- [13] The oligomeric mixture is readily soluble in *n*-pentane and shows molecular masses of up to $M = 9200$ (molecular mass of the icosomer (20mer) of **3d**) in the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum.
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New Building Blocks in Amide Chemistry—*N*-Lithiobis(trimethylstannyl)amine and *N*-Lithiotrimethylstannyl(trimethylsilyl)amine**

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The enormous synthetic utility of *N*-lithiosilylamines, in particular of *N*-lithiobis(trimethylsilyl)amine, [LiN(SiMe₃)₂],^[1] has been well documented.^[2] In contrast, related tin derivatives have remained unknown so far, and *N*-lithiostannylamines in general have received scant attention,^[3] probably because of the greatly enhanced reactivity of the Sn–N bond^[4] when compared with Si–N bonds. However, this enhanced reactivity is desirable in metal amides for further transformations, and therefore, selective smooth syntheses of such amides bearing one or two trimethylstannyl groups at the nitrogen atom are an attractive goal. We have now succeeded in obtaining pure *N*-lithiobis(trimethylstannyl)amine, [LiN(SnMe₃)₂] (**2a**), and *N*-lithiotrimethylsilyl(trimethylstannyl)amine, [LiN(SiMe₃)SnMe₃] (**2b**), for the first time from the 1:1 reaction of tris(trimethylstannyl)amine, (Me₃Sn)₃N (**1a**),^[5] and trimethylsilylbis(trimethylstannyl)amine, (Me₃Sn)₂NSiMe₃ (**1b**),^[6] respectively, with butyllithium (Scheme 1). The low solubility of **2a,b** points to a mixture of oligomers ($x \approx \infty$ in Scheme 1).



Scheme 1. Synthesis of **2a,b**, **3a,b**, and **4a,b**. $x = 1, 2$.

The reaction of **1a** or **1b**, dissolved in hexane, with BuLi in hexane (Scheme 1 a) afforded colorless, extremely air- and moisture-sensitive powders that could be isolated and stored

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for prolonged time under argon atmosphere. After addition of *N,N',N''*-pentamethyldiethylenetriamine ((Me₂NCH₂CH₂)₂-NMe; pmdta) to a suspension of **2a** in hexane at -40 °C, crystalline [(Me₃Sn)₂NLi(pmdta)] (**3a**) was isolated (Scheme 1b). When **2a** was dissolved in *t*BuOMe, in the absence of pmdta, the dimer [{(Me₃Sn)₂NLi(*t*BuOMe)}₂] (**4a**) was obtained as a crystalline product (Scheme 1c). Compound **2b**, dissolved in *t*BuOMe (Scheme 1c), gave the analogous dimer [{Me₃Sn(Me₃Si)NLi(*t*BuOMe)}₂] (**4b**), and treatment of **4b**, dissolved in *t*BuOMe, with pmdta, afforded the mononuclear species [Me₃Sn(Me₃Si)NLi(pmdta)] (**3b**) in good yield.

The solid-state molecular structures of **3a**^[7] and **4a**^[8] are shown in Figures 1 and 2, respectively. The sterically demanding stannyl group has a special influence on the bonding properties in these metal amides. The bond length Li1–N1

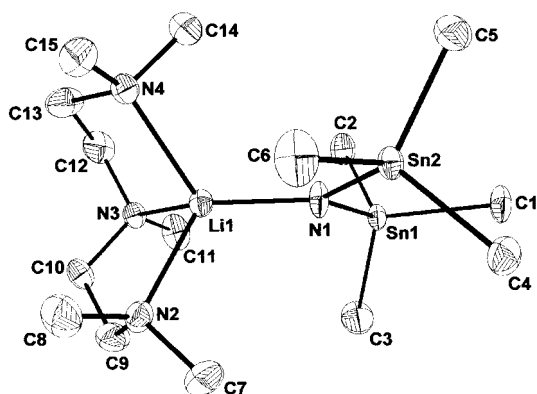


Figure 1. Molecular structure of [(Me₃Sn)₂NLi(pmdta)] (**3a**; ORTEP, thermal ellipsoids represent a 25% probability). Selected bond lengths [Å] and angles [°]: Li1–N1 1.92(1), Sn1–N1 1.994(5), Sn2–N1 1.995(5), Li1–N2 2.15(1), Li1–N3 2.18(1), Li1–N4 2.22(1), Sn1–C1 2.179(7), Sn1–C2 2.145(8), Sn1–C3 2.145(9), Sn2–C4 2.148(7), Sn2–C5 2.162(8), Sn2–C6 2.157(8), Sn1–N1–Sn2 115.5(2), Sn1–N1–Li1 123.8(4), Sn2–N1–Li1 120.6(4), N1–Li1–N2 116.1(5), N1–Li1–N3 129.4(6), N1–Li1–N4 120.2(6), C2–Sn1–C3 108.2(4), C1–Sn1–C2 103.7(3), N1–Sn1–C1 115.8(3).

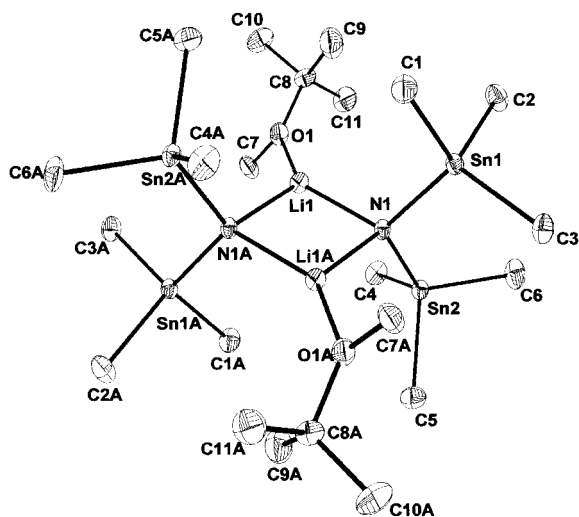


Figure 2. Molecular structure of [{(Me₃Sn)₂NLi(*t*BuOMe)}₂] (**4a**; ORTEP, thermal ellipsoids represent a 25% probability). Selected bond lengths [Å] and angles [°]: Li1–N1 2.001(5), Li1A–N1 2.022(5), Sn1–N1 2.032(2), Sn2–N1 2.032(2), Li1–O1 1.987(6), O1–C8 1.437(5), Sn1–C1 2.153(3), Sn1–C2 2.164(3), Sn1–C3 2.160(3), Sn2–C6 2.167(3), Sn1–N1–Sn2 111.0(1), Sn1–N1–Li1 115.4(2), Sn2–N1–Li1 120.2(2), N1–Li1–N1A 103.9(2), Li1–N1–Li1A 76.1(2), N1–Sn1–C1 107.4(1), N1–Sn2–C6 114.4(1).

(1.92(1) Å) in **3a** is the shortest so far for a bond to a tetracoordinate Li atom (e.g. *d*(Li–N) = 1.98(2) Å in [(Me₃Si)₂NLi(pmdta)]^[9, 10]). The Sn1–N1 (1.994(5) Å) and Sn2–N2 (1.995(5) Å) distances in **3a** are the shortest Sn–N single bonds observed as yet (see e.g. **1a**: *d*(Sn–N) = 2.037(6) Å (av)^[11]). In spite of this, the Sn–N–Sn angle (115.5(2)°) is smaller than in any known structure of bis(trimethylstannyl)amine derivatives containing a three-coordinate nitrogen atom. In contrast, the silicon analogue of **3a**, [(Me₃Si)₂NLi(pmdta)], displays a wide Si–N–Si angle (125.3(2)°).^[9, 10] Although this wide angle may be caused by repulsive interactions of the silyl groups, the different electronic properties of the Si–N and Sn–N bonds need to be taken into consideration when discussing the molecular structures of these compounds. The nitrogen atom in **3a** resides in a trigonal-planar environment.

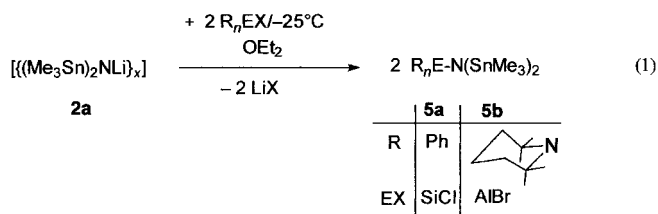
In the dimer **4a**, the lithium atoms are three-coordinate, and the N₂Li₂ ring is planar. The Li–N distances are longer than those in **3a**, but slightly shorter than those in the dimer [{(Me₃Si)₂NLi(Et₂O)}₂].^[12] The planes around the lithium and the oxygen atoms in **4a** form an angle of about 40°. The N–Li–N angle of 103.9(2)° as well as the Li–N–Li angle of 76.1(2)° are similar to that in the corresponding bis(silyl) derivative. However, the small Sn–N–Sn angle (111.0(1)°) in **4a** (which is even smaller than in **3a**) is again remarkably different from the Si–N–Si angle (121.9(4)°).

The relationship between solid-state structures of lithium amides^[13] and their state of association in solution^[14] can be assessed best by studying ⁶Li- and ¹⁵N-labeled material. Therefore, the reactions of ¹⁵N-labeled (ca. 12%) **1a** and **1b** with ⁶Li-labeled (99%) MeLi in *t*BuOMe were carried out and studied by ⁶Li, ¹⁵N, and ¹¹⁹Sn NMR spectroscopy. After complete reaction of **1a** with MeLi in *t*BuOMe, **4a** was formed as a single species together with Me₄Sn. The dimer **4a** was identified by the typical pattern in the ¹⁵N NMR spectrum (coupling of ¹⁵N nucleus with two equivalent ⁶Li nuclei) and by the intensities of the ¹⁵N satellites in the ⁶Li NMR spectrum (according to the degree of ¹⁵N labeling). Furthermore, the ¹¹⁹Sn NMR spectrum of **4a** shows a single resonance, accompanied by ¹⁵N satellites and ¹¹⁷Sn satellites with correct intensities. Therefore, the structure of the dimer **4a** at low temperature is retained in solution. When pmdta was added to the solution of **4a** in *t*BuOMe, the presence of mainly two species, **3a** and **4a**, and a minor unidentified compound, became apparent.

The monomeric structure of **3a** (–40 °C) follows from the 1:1:1 triplet (¹*J*(¹⁵N,⁶Li) = 7.5 Hz) in the ¹⁵N NMR spectrum. This confirms that only one ⁶Li atom is linked to the central nitrogen atom. The ⁶Li NMR spectrum (–40 °C) displays a singlet, which is accompanied by satellite signals with coupling constants of ¹*J*(¹⁵N,⁶Li) ≈ 7.5 Hz and ²*J*(^{119/117}Sn,⁶Li) ≈ 7.5 Hz. The monomer **3a** exhibits a broad ¹¹⁹Sn NMR signal at room temperature which splits below –20 °C into two broadened signals of equal intensity. Again there are ¹⁵N satellites, as well as ¹¹⁹Sn (AB spin system) and ¹¹⁷Sn satellites (AX spin system), indicating that the two ¹¹⁹Sn NMR signals belong to tin atoms in the same molecule. Apparently there is restricted rotation about the Li–N(SnMe₃)₂ bond (Δ*G*[‡] = 43.1 ± 1.0 kJ mol^{–1}), a feature observed here for the first time.

Thus, the molecular structure in solution must be very similar to that “frozen” in the solid state. Similar findings can be deduced from the NMR data available for **3b** and **4b**; in this case additional data arises from the presence of the ^{29}Si isotope. The low-temperature NMR spectra are further complicated by the presence of two isomers in the case of **4b** and two rotamers in the case of **3b**.^[15]

The application of the new building blocks has been documented with two extreme examples. The reactions of the bulky substituted silicon and aluminum halides with **2a** [Eq. (1)] proceed under mild conditions (-25°C) providing



the products in good yield. These reactions can be carried out with crude **2a**, isolated as a powdery substrate, redissolved in Et_2O . The reaction of the halides with **1a** instead of **2a** was unsuccessful even at elevated temperatures. To our knowledge there is no other convenient route for the introduction of the distannylamine moiety.^[15]

Experimental Section

N-lithiois(trimethylstannyl)amine **2a**, $[(\text{Me}_3\text{Sn})_2\text{NLi}(\text{pmdta})]$ (**3a**), and $[(\text{Me}_3\text{Sn})_2\text{NLi}(\text{MeO}t\text{Bu})_2]$ (**4a**): A solution of **1a** (2.53 g, 5 mmol) in hexane (20 mL) was cooled at -50°C , and BuLi (3.1 mL, 1.6 M in hexane) was added dropwise over 15 min under vigorous stirring. After the reaction mixture had been allowed to slowly attain ambient temperature (15 h), the white precipitate was filtered off, and volatile material was removed in vacuo (10^{-3} Torr, 4 h). The colorless, extremely air- and moisture-sensitive powder **2a** was suspended in hexane (20 mL), cooled at -40°C , and pmdta (1.0 mL, 0.87 g, 5.0 mmol) was added. The colorless liquid was cooled in a freezer at -78°C for 30 days, from which **3a** (1.80 g, 69%; m.p. $>128^\circ\text{C}$ (decomp)) crystallized as colorless prisms. Dissolution of **2a** in *t*BuOMe (15 mL), followed by storage of the solution at -20°C for 10 days gave crystals of the dimer **4a** (1.66 g, 76%; m.p. $>260^\circ\text{C}$ (decomp)). The compounds **2b**, **3b**, and **4b** could be obtained in the same way.^[15]

3a: ^1H , ^{13}C , ^6Li , ^{15}N , and ^{119}Sn NMR (C_6D_6 or $[\text{D}_8]\text{toluene}$): $\delta(^1\text{H}) = 0.37$ (s, $^2J(^{119}\text{Sn}, ^1\text{H}) = 48.8$ Hz, 18H; SnMe_3), 1.68–1.72 (brs, 15H; NMe and NMe₂), 2.01–2.18 (brs, 8H; CH_2); $\delta(^{13}\text{C}) = -0.2$ ($^1J(^{119}\text{Sn}, ^{13}\text{C}) = 297.3$ Hz, SnMe_3), 46.2 (NMe₂), 53.7 (NMe), 57.1, 58.4 (CH_2); at -40°C : $\delta(^6\text{Li}) = 2.7$ ($^1J(^{15}\text{N}, ^6\text{Li}) = 7.5$, $^2J(^{119}\text{Sn}, ^6\text{Li}) = 7.5$ Hz); $\delta(^{15}\text{N}) = -359.6$ (1:1:1 t, $^1J(^{15}\text{N}, ^6\text{Li}) = 7.5$ Hz); $\delta(^{119}\text{Sn}) = 49.3$ (brs, $^1J(^{119}\text{Sn}, ^{15}\text{N}) = 148$, $^2J(^{119}\text{Sn}, ^{117}\text{Sn}) = 470$ Hz), 54.4 (brs, $^1J(^{119}\text{Sn}, ^{15}\text{N}) = 146$, $^2J(^{119}\text{Sn}, ^{117}\text{Sn}) = 470$ Hz).

4a: ^1H , ^{13}C , ^6Li , ^{15}N , and ^{119}Sn NMR ($[\text{D}_8]\text{toluene}$): $\delta(^1\text{H}) = -0.69$ (s, $^2J(^{119}\text{Sn}, ^1\text{H}) = 49.2$ Hz, 36H; SnMe_3), 1.02 (s, 18H; *t*Bu), 3.05 (s, 6H; Me); $\delta(^{13}\text{C}) = -1.83$ ($^1J(^{119}\text{Sn}, ^{13}\text{C}) = 320.6$ Hz, SnMe_3), 27.2 ($(\text{CH}_3)_3$), 50.2 (OMe), 75.0 (OC); at -40°C : $\delta(^6\text{Li}) = 3.1$ ($^1J(^{15}\text{N}, ^6\text{Li}) = 4.5$ Hz); $\delta(^{15}\text{N}) = -366.2$ (1:2:3:2:1 q, $^1J(^{15}\text{N}, ^6\text{Li}) = 4.5$ Hz), $\delta(^{119}\text{Sn}) = 63.0$ (brs, $^1J(^{119}\text{Sn}, ^{15}\text{N}) = 143.5$, $^2J(^{119}\text{Sn}, ^{117}\text{Sn}) = 467$ Hz).

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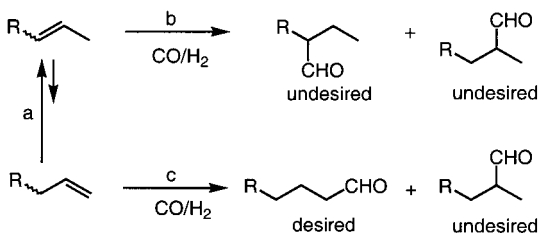
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- [7] a) Crystal structure analysis: Siemens/Bruker P4 diffractometer, CCD area detector with Siemens LT 2 device; MoK_α radiation, $\lambda = 0.71063$ Å, graphite monochromator; single crystals, coated with perfluoroether oil, were mounted on a glass fiber. Crystal data were determined and intensity data recorded at 193 K. The data reduction was carried out by using the program SAINT, the structure solution by the Patterson method, and the refinement by using the SHELXTL system;^[16] final refinement was performed by using the SHELX-97 programs.^[17] All atoms except the hydrogen atoms are described with anisotropic temperature factors, all hydrogen positions were geometrically placed ($d(\text{C-H}) = 0.96$ Å) and refined by using the riding model and fixed U_{ij} . b) Crystal structure data of **3a**: $M_r = 521.84$; colorless rhombs; size $0.20 \times 0.20 \times 0.30$ mm, monoclinic; space group $P2(1)/n$, $Z = 4$, $a = 9.671(1)$, $b = 15.367(3)$, $c = 17.007$ Å, $\beta = 101.73(1)^\circ$, $V = 2474.82(5)$ Å³, $\rho_{\text{calcd}} = 1.401$ Mg m⁻³, $\mu = 2.020$ mm⁻¹, $F(000) = 1048$. Data collection: 10248 reflections in $-10 \leq h \leq 10$, $-17 \leq k \leq 12$, $-18 \leq l \leq 18$, 2θ range 13.64 – 46.50° ; 3078 independent reflections; $R_{\text{int}} = 0.0366$, 2331 reflections with $F_o > 4\sigma(F_o)$, semiempirical absorption correction, max/min transmission: 1.000/0.787; $\text{GOOF} = 1.117$; 210 variables, $R = 0.040$, $wR^2 = 0.0919$, largest difference peak: 0.921 e Å⁻³. c) 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-159571 (**3a**) and CCDC-159570 (**4a**). 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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Highly Selective Catalyst Systems for the Hydroformylation of Internal Olefins to Linear Aldehydes**

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Dedicated to Professor Manfred Michalik on the occasion of his 60th birthday

The rhodium-catalyzed hydroformylation of aliphatic olefins to give linear aldehydes constitutes the most important homogeneously catalyzed process in industry today in terms of volume produced.^[1] More than seven million tons of various aldehydes and alcohols are produced in this way each year. The hydroformylation of propene is especially important for the production of *n*-butyraldehyde, used as a valuable starting material for 2-ethylhexanol, which is currently the most important plasticizer alcohol in use. Recently there has been an increasing interest in the production of alternative plasticizer alcohols due to environmental concern about current products.^[2] For economic reasons internal olefins or mixtures of internal and terminal olefins are the starting materials of choice for new plasticizer alcohols, yet preferably unbranched alcohols are the desired products due to better physical properties of the resulting plasticizers. However, the selective hydroformylation of internal olefins to give linear aldehydes (and subsequently linear alcohols) is difficult (Scheme 1). To obtain linear aldehydes from internal olefins



Scheme 1. Selective hydroformylation of internal olefins to give linear aldehydes: a) Isomerization, b) hydroformylation of internal olefin, c) hydroformylation of terminal olefin.

the catalyst has to perform a fast isomerization between the internal and terminal olefin (Scheme 1: reaction a). Unfortunately the thermodynamic equilibrium mixture contains in general less than 5 % of the terminal olefin (for this reason isomerization should be avoided if terminal olefins are used as starting material). In addition the hydroformylation of the

terminal olefin (Scheme 1, reaction c) must occur many times faster (and with high *n* selectivity) than that of the internal olefin (Scheme 1, reaction b).

To date only a limited number of rhodium catalysts^[3] with sterically hindered phosphites^[4] have been shown to be suitable for this purpose. Owing to the limited thermal stability and the ease of hydrolysis of these ligands there is considerable interest in new hydroformylation catalysts for the selective conversion of internal olefins.^[5] Clearly phosphanes would offer advantages in terms of stability compared to phosphite ligands.^[6] Regarding phosphane catalyst systems there has been only one recent development, which was described by van Leeuwen and co-workers, where modified xanthene ligands were used for the hydroformylation of 2- and 4-octene.^[7] With these ligands linear:branched (*n*:*i*) ratios up to 90:10 and catalyst turnover frequencies (TOF)^[8] of 112 h⁻¹ were achieved for the hydroformylation of 2-octene.

Herein we report the development of new rhodium catalyst systems that enable the hydroformylation of different terminal and internal olefins in excellent selectivities, which represents a substantial improvement in efficiency of this industrially important reaction.

While studying the homogeneous and biphasic^[9] hydroformylation of 1- and 2-pentene in the presence of phosphane-modified cobalt catalysts,^[10] we became interested in similar reactions with more active rhodium catalysts. Among the various phosphanes tested for this reaction 2,2'-bis(diphenylphosphanylmethyl)-1,1'-binaphthyl (NAPHOS,^[11] a structural variation of the 2,2'-bis(diphenylphosphanylmethyl)biphenyl (BISBI) ligand), which resembles the backbone of the sterically demanding phosphites, gave the best results (Table 1).

Table 1. Hydroformylation of 1- and 2-pentene with NAPHOS.^[a]

Entry	Olefin	<i>p</i> [bar]	<i>T</i> [°C]	Yield ^[b] [%]	<i>n</i> : <i>i</i>	TOF [h ⁻¹]
1	1-pentene	10	120	76	99:1	475
2	1-pentene	50	120	88	97:3	550
3	2-pentene	10	120	22	89:11	138
4	2-pentene	50	120	7	55:45	44

[a] Reaction conditions: olefin (70.0 mmol; 40 mL solution), [Rh(acac)-(CO)₂] (0.01 mol %; 20.7 ppm Rh), NAPHOS:Rh = 5:1, *t* = 16 h. [b] No significant amounts (> 1 %) of other products apart from isomerized olefin were detected.

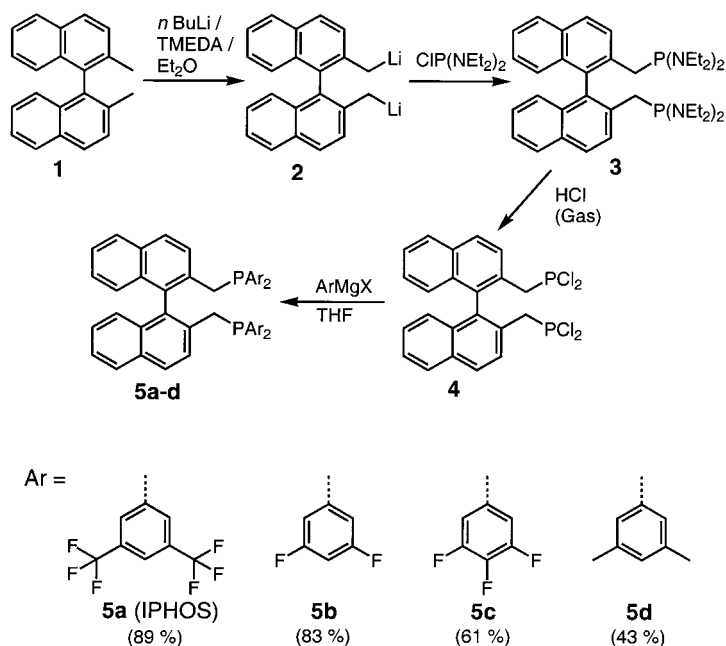
As expected the rhodium-catalyzed hydroformylation of the terminal olefin proceeds in the presence of NAPHOS in good yields (76–88 %) and excellent selectivities (*n*:*i* = 97:3–99:1). However, much to our surprise NAPHOS also gives a highly selective (*n*:*i* = 89:11) catalyst system for the reaction of 2-pentene at a low CO/H₂ pressure (10 bar). Although the yield (22 %) is modest after 16 h (TOF = 138 h⁻¹),^[12] it is important to note that this is only the second known phosphane that allows a selective synthesis of linear aldehydes from internal olefins. The even lower yield at 50 bar, can be explained by the fact that the isomerization step, which is necessary for the formation of the linear aldehyde from an internal olefin, is faster at a lower CO pressure.

In view of this promising result, we were interested in the synthesis of new substituted NAPHOS derivatives, especially

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those with electron-withdrawing substituents on the aryl rings. After considerable experimentation we developed a simple and general synthesis for NAPHOS derivatives, which can be performed without problems on a multigram scale.^[13] 2,2'-Dimethyl-1,1'-binaphthyl (**1**) is lithiated^[14] with 2.5 equivalents of *n*-butyllithium to give the double lithiated derivative **2** (Scheme 2). Reaction of **2** with chlorobis(diethylamino)phosphane^[15] in *n*-hexane at -70°C yields 2,2'-bis[bis(diethylamino)phosphanylmethyl]-1,1'-binaphthyl (**3**), which is converted directly to 2,2'-bis(dichlorophosphanylmethyl)-1,1'-binaphthyl (**4**) with gaseous hydrogen chloride in 44% overall yield from **1**.



Scheme 2. Synthesis of substituted NAPHOS derivatives. The yield of the final step of **4**→**5** is given in parentheses below the Ar groups of **5a–d**.

Reaction of the new central building block **4** with Grignard reagents provides an efficient synthesis of a number of new NAPHOS derivatives. As shown in Scheme 2 mainly fluorinated ligands were synthesized due to the electron-withdrawing nature of the substituents. In general such electron-deficient substituents in aryl diphosphanes (with large bite angles) have a positive effect on the activity and the linear:branched selectivity of hydroformylation catalysts in case of primary olefins.^[16] This can be explained by a preference for the diequatorial over the equatorial–apical binding mode of the ligand.^[17]

The best yields in the Grignard coupling were obtained with 3,5-bis(trifluoromethyl)-1-bromobenzene and 1-bromo-3,5-difluorobenzene (89 and 83 %, respectively), while 1-bromo-3,4,5-trifluorobenzene and 1-bromo-3,5-dimethylbenzene gave only moderate coupling yields (61 and 43 %, respectively; non-optimized).

The rhodium-catalyzed hydroformylation with the new ligands was performed at a synthesis gas pressure of 10 bar and 120 as well as 100°C .^[18] Apart from 1- and 2-pentene other internal olefins such as 2-butene, 2-octene, and 4-octene

were used as starting materials. As shown in Table 2 ligands with electron-withdrawing substituents (**5a–c**) led to much more active and slightly more selective catalysts for the hydroformylation of internal olefins compared to NAPHOS and **5d**.

Table 2. Rhodium-catalyzed hydroformylation in the presence of substituted NAPHOS derivatives.^[a]

Entry	Olefin	<i>p</i> [bar]	<i>T</i> [°C]	Yield ^[b] [%]	<i>n</i> : <i>i</i>	TOF [h ^{−1}]
1	5a	1-pentene	120	82	96:4	512
2	5a	2-pentene	120	68	91:9	425
3	5a	2-pentene	100	52	89:11	325
4	5a	2-butene ^[b]	120	66	91:9	825
5	5a	2-octene ^[c]	120	51	86:14	319
6	5b	1-pentene	120	78	97:3	488
7	5b	2-pentene	120	59	94:6	369
8	5b	2-pentene	100	21	95:5	131
9	5c	1-pentene	120	83	97:3	519
10	5c	2-pentene	120	61	93:7	381
11	5c	2-pentene	100	24	94:6	150
12	5c	2-butene ^[b]	120	74	95:5	925
13	5c	2-octene ^[c]	120	48	91:9	300
				(64)	88:12	(114) ^[d]
14	5c	4-octene ^[e]	120	14	66:34	88
				(41)	70:30	(43) ^[f]
15	5d	1-pentene	120	76	81:19	475
16	5d	2-pentene	120	11	78:22	69

[a] Reaction conditions: pentene (73 mmol), solution (40 mL anisole + isooctane as internal standard), *p* = 10 bar, [Rh(acac)(CO)₂] (0.01 mol %; 20.7 ppm Rh), ligand:Rh = 5:1, *t* = 16 h. [b] 2-Butene (146 mmol), anisole (30 mL), [Rh(acac)(CO)₂] (0.005 mol %). [c] 2-Octene (73 mmol), solvent (28.5 mL; anisole + toluene). [d] After a reaction time of 56 h. [e] 4-Octene (73 mmol), solvent (10 mL; anisole + toluene). [f] After a reaction time of 96 h.

In the case of 2-pentene the ligand **5a** (IPHOS) led to the most active catalyst system, especially at 100°C (Table 2, entry 3; TOF = 325 h^{−1}). The largest amount of *n*-aldehydes (Table 2, entries 7, 8; *n*:*i* = 94:6 (95:5); 120 (100°C)) was obtained in the presence of **5b**. Significantly higher catalyst activities but similar selectivities (up to 95:5) (Table 2, entries 4 and 12; TOF = 825 and 925 h^{−1}, respectively) were observed with the industrially important olefin 2-butene. These are the highest selectivities known so far for the hydroformylation of internal olefins to give linear aldehydes with phosphane ligands. Interestingly, the catalyst activities in the presence of **5a–c** do not significantly differ for 1- and 2-pentene (compare entries 1,2 or 6,7 or 9,10 in Table 2). Thus the isomerization of 1-pentene is most likely similar in rate or faster than the hydroformylation step.

In addition the new ligands **5a–c** also allow a selective hydroformylation of higher internal olefins. With 2-octene a *n*:*i* ratio of 91:9 was obtained in the presence of **5c** (Table 2, entry 13; TOF = 300 h^{−1}). However in the case of 4-octene the selectivity is comparably low (Table 2, entry 14; *n*:*i* = 66:34) after 16 h. To understand this different behavior the reactions of 2- and 4-octene in the presence of **5c** (Table 2, entries 13 and 14, respectively) were monitored by removing samples from the autoclave, which were taken during the reaction. The results are displayed in Figure 1 and 2. The conversion of 2- and 4-octene reveals significant differences. Utilizing 2-octene

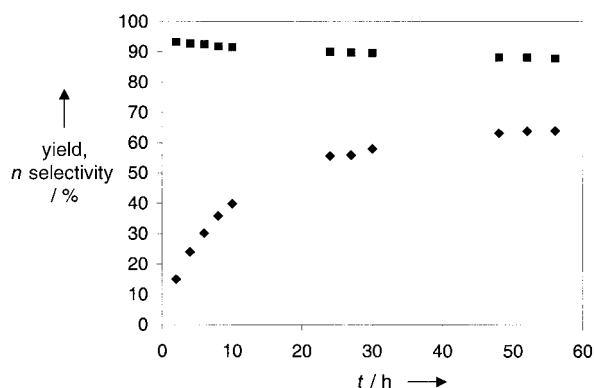


Figure 1. Yield (♦) and *n* selectivity (■) of the rhodium-catalyzed hydroformylation of 2-octene with **5c**.

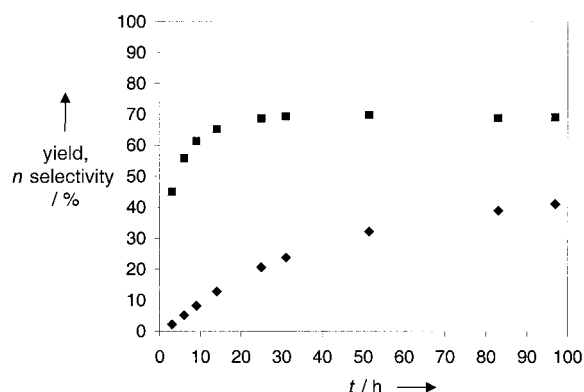


Figure 2. Yield (♦) and *n* selectivity (■) of the rhodium-catalyzed hydroformylation of 4-octene with **5c**.

the *n*:*i* ratio decreases from 94:6 after 2 h to 88:12 after 56 h. In contrast, with 4-octene the *n*:*i* ratio increases from 45:55 after 3 h to 70:30 after 31 h and then remains approximately constant. It seems that in case of 2-octene the isomerization towards the 1-octene is significantly faster than that to give 3-octene. The isomerization is much slower for 4-octene, hence hydroformylation of the internal double bond occurs preferentially. Interestingly, the hydroformylation of 4-octene still proceeds after 80 h, which underlines the high catalyst stability.

In conclusion we have shown that NAPHOS-type ligands induce excellent selectivities for the conversion of internal olefins to linear aldehydes. Based on our newly developed synthesis of **4**, variously substituted NAPHOS derivatives have been synthesized on a multigram scale. We believe the interesting features and the easy synthesis of this ligand class will lead to further applications for industrially important hydroformylation reactions, although for a practical application the activity and long-time stability still have to be improved.

Experimental Section

General: All reactions were carried out under an argon atmosphere. For the hydroformylation experiments a 100-mL stainless steel autoclave (Parr Co.) equipped with a magnet-driven propeller stirrer or a 160-mL stainless steel autoclave (Parr Co.) stirred magnetically were used.

Example of a typical hydroformylation experiment (Table 2, entry 1): A solution of $[\text{Rh}(\text{acac})(\text{CO})_2]$ (7.3 μmol , 1.88 mg; acac = acetylacetonate)

and ligand **5a** (M_r = 1194.73; 44 mg, 36.5 μmol) in anisole (30 mL) was treated with isooctane (2 mL as internal standard) and 1-pentene (8 mL, 73.0 mmol). This mixture was added to the autoclave, which was subsequently pressurized to 5 bar with synthesis gas ($\text{CO}:\text{H}_2$ = 1:1). The autoclave was then heated to 120 °C and the pressure adjusted to 10 bar. After 16 h the autoclave was cooled in an ice bath and the pressure was released. A sample was taken immediately and analyzed by GC.

All hydroformylation experiments were conducted under isobaric conditions. Synthesis gas (purity 99.97–99.997%) was purchased from Aga Gas GmbH, Berlin (Germany).

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–30 °C. The resulting crystals were collected, washed with a minimum amount of cold hexane, and dried in vacuum. Yield of **4**: 2.9 g (44% related to **1**). Mg turnings (302 mg, 12.4 mmol) were placed in a three-necked flask (100 mL) and treated with diethyl ether (10 mL). A solution of 3,5-bis(trifluoromethyl)-1-bromobenzene (3.6 g, 12.4 mmol) in diethyl ether (10 mL) was added dropwise to the magnetically stirred mixture. After 1 h the solution was transferred into a dropping funnel and slowly added to a solution of **4** (1.5 g, 3.1 mmol) in THF (25 mL). The mixture was then heated at reflux until the reaction was complete (~2 h). After removal of the solvents in vacuum the residue was dissolved in toluene. The solution was washed with degassed water and dried with anhydrous Na₂SO₄. The toluene was removed in vacuum, and the residue was recrystallized from acetone/methanol. Yield of **5a**: 3.3 g (89%).

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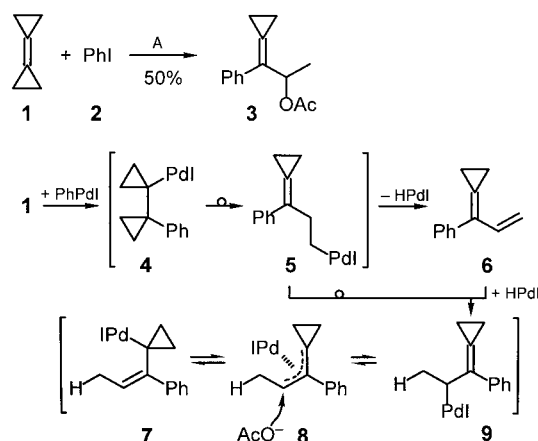
Nucleophilic Trapping of π -Allylpalladium Intermediates Generated by Carbopalladation of Bicyclopropylidene: A Novel Three-Component Reaction**

Hanno Nüske, Mathias Noltemeyer, and
Armin de Meijere*

*Dedicated to Professor Barry M. Trost
on the occasion of his 60th birthday*

The construction of complex molecules from simple starting materials is a challenging task for chemists. One of the most elegant ways to achieve this is by utilizing so-called domino reactions.^[1] Multicomponent reactions such as the Mannich and the Ugi reaction are examples of all-intermolecular domino reactions. Recently, our group has published a new all-carbon-coupling three-component reaction, the dom-

ino Heck–Diels–Alder reaction of bicyclopropylidene (**1**).^[2] This sequence involves the Heck-type coupling of bicyclopropylidene (**1**)^[3] with haloarenes and -alkenes to form 1-substituted allylidene-cyclopropanes which then undergo [4+2] cycloadditions with dienophiles yielding 7-substituted spiro[2.5]oct-7-enes in a one-pot operation. In the course of a more extensive study of this methodology we became aware of a new reaction mode through the isolation of the side product **3**, the formation of which can only be rationalized by an intermolecular nucleophilic attack of an acetate anion stemming from the catalyst precursor on an intermediate π -allylpalladium species **8** (Scheme 1).^[4]



Scheme 1. Mechanism of the nucleophilic trapping of π -allylintermediate **8**. A) 5 mol % Pd(OAc)₂, 10 mol % TFP, 5.0 equiv LiOAc, K₂CO₃, Et₄NCl, MeCN, 80 °C, 24 h.

The π -allylpalladium intermediate **8** must be formed after the initial carbopalladation and β -hydride elimination by readdition of the hydridopalladium species onto the newly formed double bond^[5] via a σ -allylpalladium complex **9**. The ligand tris-(α -furyl)phosphane (TFP), which is known to retard β -hydride elimination^[6] and thus favor the readdition of the hydridopalladium complex onto the double bond, proved to be best for this reaction, and the yield of the allyl acetate **3** could be raised up to 50% by using LiOAc as an additional source of acetate.

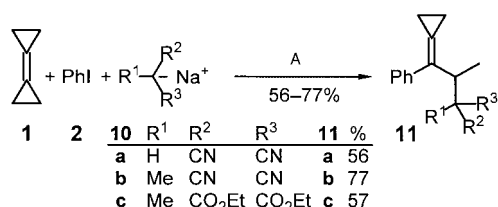
In view of previous observations on the nucleophilic substitution reactions on 1,1-dimethylenallylpalladium intermediates,^[7] stabilized enolates and other carbon nucleophiles were tested. These were generated by separately deprotonating malononitriles and diethyl malonates with sodium hydride and then adding to the reaction mixture with **1**, the palladium precatalyst, and iodobenzene (**2**) to give the malonic acid derivatives **11a–c** in up to 77% yield.^[8] In accordance with earlier findings,^[7] only products of the nucleophilic attack at the sterically less encumbered terminus of the allyl moiety were obtained (Scheme 2).

Especially interesting is the possible preparation of amino acid derivatives by this method. With O'Donnell's nucleophilic glycine equivalent **12**^[9] the methylenecyclopropane derivative **13**, a substituted isomer of hypoglycin A in a protected form,^[10] was obtained in 76% yield (Scheme 3). Glycine methyl ester **14a**, in the presence of triethylamine in

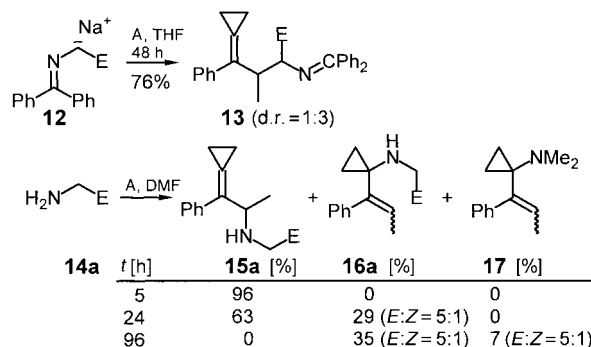
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Scheme 2. Malonic ester derivatives as nucleophiles. A) 5 mol % Pd(OAc)₂, 10 mol % TFP, Et₃N, THF, 80 °C, 24–96 h.



Scheme 3. Preparation of C- and N-substituted amino acids. A) 1.0 equiv **1**, 0.5 equiv **2**, 5 mol % Pd(OAc)₂, 10 mol % TFP, Et₃N, 80 °C. E = CO₂Me.

this one-pot transformation, gave the substitution product **15a** after 5 h in nearly quantitative yield (96%). Interestingly, upon prolonged heating (24 h) of the reaction mixture, the yield of **15a** decreased to 63%, and in addition 29% of the regioisomer **16a** was isolated as a mixture of *E* and *Z* isomers (5:1). This indicates that the nucleophilic substitution of **8** with **14a** is reversible and that **16a** is the thermodynamically more stable product.

The successful application of the glycine ester as a nitrogen nucleophile led to the evaluation of a number of primary and secondary amines (Table 1). Very good results were achieved with primary amines, and the products were formed in good to excellent yields. No twofold substitution of the amine, which is a common side reaction of primary amines,^[11] was detected.

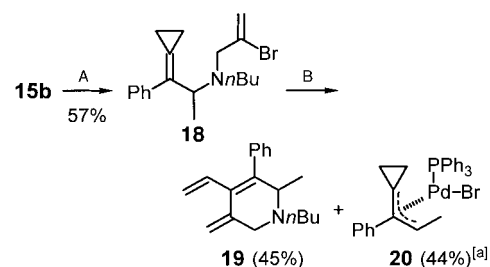
Table 1. The use of amines as nucleophiles in the new three-component reaction.^[a]

Entry	Amine	t [h]	Product (E/Z)	Yield [%]
1	H ₂ N <i>n</i> Bu (14b)	48	15b	73 ^[a]
2	H ₂ N <i>n</i> Bu (14b)	24	16b (6:1) 17 (5:1)	15 19
3	H ₂ N <i>i</i> Bu (14c)	48	15c	73
4	H ₂ N <i>n</i> Bu (14d)	48	15d	95
5	H ₂ NBn (14e)	1	15e	98
6	H ₂ NBn (14e)	48	16e (10:1) 17 (5:1)	60 28
7	(3-methylbut-1-yn-3-yl)amine (14f)	24	15f	41
8	HNEt ₂ (14g)	48	15g	75
9	piperidine (14h)	1	15h	79
10	piperidine (14h)	24	16h (5:1)	67
11	morpholine (14i)	1.5	15i	99
12	morpholine (14i)	20	16i (>20:1)	42
13	morpholine (14i)	48	15i	70 ^[b]

[a] Conditions: 2.00 equiv **1**, 1.00 equiv **4**, 3.00 equiv amine, 5 mol % Pd(OAc)₂, 10 mol % TFP, DMF, 80 °C. [b] 10 mol % P(*o*Tol)₃.

Secondary amines should not be too bulky, as diethylamine (**14g**) (Table 1, entry 6) gave **15f** in 75% yield, while dibenzylamine and diisobutylamine gave no coupling products. Piperidine (**14h**) and morpholine (**14i**) afforded compounds **15h** and **15i** in 79 and 99% yield, respectively, after only 1 h. In these cases prolonged heating also led to the formation of the thermodynamically favored products, for example after 48 h the products from **14h** and **14i** were completely converted to **16h** and **16i**. Interestingly, in the presence of tris(*o*-tolyl)phosphane, products of type **15** were formed exclusively, even after 48 h (Table 1, entry 13).

The allylamine **15b** from bicyclopropylidene (**1**), iodobenzene (**2**), and *n*-butylamine (**14b**) was further elaborated (Scheme 4). Alkylation of **15b** with 2,3-dibromopropene gave



Scheme 4. Intramolecular cross-coupling of the 4-aza-2-bromo-1,6-diene **18**. A) 1) K₂CO₃, CH₂Cl₂; 2) 2,3-dibromopropene, 0–25 °C, 20 h. B) 5 mol % Pd(OAc)₂, 10 mol % PPh₃, Et₃N, DMF, 80 °C, 24 h. [a] Yield based on amount of Pd(OAc)₂ used.

the 4-aza-2-bromo-1,6-diene **18**, which underwent an intramolecular Heck-type cross coupling with opening of the cyclopropane ring to yield the cross-conjugated 5-methylene-4-ethenyl-1,2,5,6-tetrahydropyridine **19** in 45% yield and, in addition, the 1,1-dimethyleneallylpalladium complex **20** (44% yield based on Pd(OAc)₂ used) could be isolated.^[12]

The X-ray structure analysis of **20** (Figure 1) discloses that all three allyl carbon atoms are located at nearly the same distance^[13] from the metal atom, with C(5) just barely closer to the palladium than C(3).^[14] Thus, the preferred initial attack of any nucleophile at C(5) of a π-allylpalladium complex of type **20** must be attributed to the less pronounced

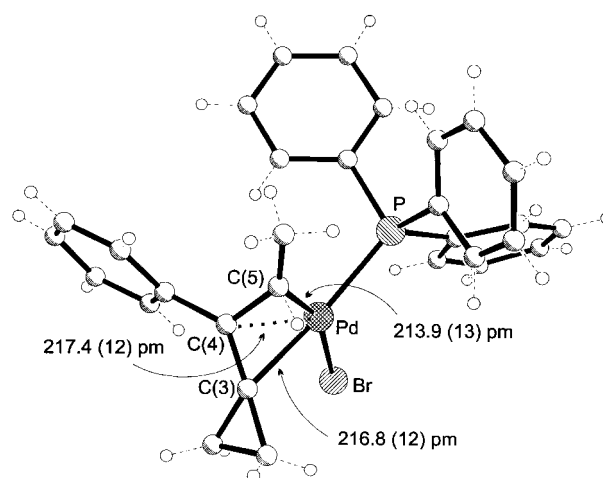


Figure 1. Structure of the π-allylpalladium complex **20** in the crystal.^[12]

steric encumbrance at this site and the fact that an S_N2 -type attack on the cyclopropyl carbon C(3) has to pass through a highly strained transition structure.^[15] The formation of complex **20** from **18** also shows that the attack at least of nitrogen nucleophiles on π -allylpalladium intermediates of type **8** must be reversible.

In conclusion, this new three-component reaction bears a significant combinatorial potential in that all three components may be varied just as in the previously described domino Heck–Diels–Alder sequence,^[2] leading to a three-dimensional library of small molecules.

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- [8] Representative procedure: 1-Cyclopropylidene-1-phenyl-2-morpholinopropane (**15i**): Morpholine (**14i**) (261 mg, 3.00 mmol) was added to a solution containing $\text{Pd}(\text{OAc})_2$ (11.2 mg, 50.0 μmol ; 5 mol %), TFP (23.2 mg, 100 μmol ; 10 mol %), iodobenzene (**2**) (204 mg, 1.00 mmol), and bicyclopopylidene (**1**) (160 mg, 2.00 mmol) in DMF (0.5 mL), and the mixture was stirred for 1.5 h at 80 °C. After cooling to room temperature, the solution was added to water (10 mL), the mixture extracted with diethyl ether (5 $\times$ 20 mL), and the combined organic phases dried (MgSO_4). After removal of the solvent in a rotatory evaporator the residue was chromatographed on silica gel (25 g, column 2 $\times$ 20 cm, pentane/diethyl ether 5/1) to yield **15i** (240 mg, 99 %) as a yellowish oil, n_D^{20} = 0.55. IR (film): $\tilde{\nu}$ = 3052, 2971, 2851, 2805 (C–N), 1598, 1494, 1447, 1373, 1261, 1118, 1071, 926, 762, 698 cm^{-1} ; ^1H NMR (250 MHz, CDCl_3): δ = 1.21–1.43 (m, 4H; cPr-H), 1.28 (d, ^3J = 6.7 Hz, 3H; 3-H), 2.42–2.63 (m, 4H; CH_2NCH_2), 3.58 (q, ^3J = 6.7 Hz, 1H; 2-H), 3.69–3.78 (m, 4H; CH_2OCH_2), 7.23 (dd, ^3J = 7.0, ^4J = 1.1 Hz, 1H; 4'-H), 7.32 (dd, ^3J = 7.0, ^3J = 7.2 Hz, 2H; 3'-H, 5'-H), 7.82 (dd, ^3J = 7.2, ^4J = 1.1 Hz, 2H; 2'-H, 6'-H); ^{13}C NMR (62.9 MHz, CDCl_3 , DEPT): δ = 2.74 (–, cPr-C), 3.74 (–, cPr-C), 15.77 (+, C-3), 50.73 (–, C-2'', C-6''), 64.99 (+, C-2), 67.36 (–, C-3'', C-5''), 123.76 (C_{quat} , cPr-C), 126.35 (+, C-4'), 127.23 (+, Ar-C), 127.77 (+, Ar-C), 128.59 (C_{quat} , Ar-C), 139.99 (C_{quat} , C-1); MS (70 eV): m/z (%): 243 (12) [M^+], 228 (5) [M^+ – CH_3], 198 (1) [M^+ – $\text{C}_2\text{H}_4\text{O}$], 156 (1) [M^+ – morpholine – H], 128 (6) [M^+ – morpholine – C_2H_4], 114 (100) [M^+ – C_6H_5 – C_4H_4]; elemental analysis calcd for (%) $\text{C}_{16}\text{H}_{21}\text{NO}$ (243.4) calcd: C 78.97, H 8.70, N 5.76; found: C 79.20, H 8.69, N 5.92.
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Insertion Reactions of Nitriles into the P–C Bond of $[(\eta^1\text{-C}_5\text{Me}_5)\text{P}\{\text{W}(\text{CO})_5\}_2]$ —A Novel Approach to Phosphorus-Containing Heterocycles**

Michael Schiffer and Manfred Scheer*

Dedicated to Professor Dieter Sellmann
in occasion of his 60th birthday

In the thermal activation of $[\text{Cp}^*\text{P}\{\text{W}(\text{CO})_5\}_2]$ (**1**; $\text{Cp}^* = \eta^1\text{-C}_5\text{Me}_5$), a Cp^* migration from the P atom to the transition metal atom occurs to form the highly reactive intermediate $[\text{Cp}^*(\text{CO})_2\text{W}=\text{P} \rightarrow \text{W}(\text{CO})_5]$ **A**.^[1] The chemistry of this highly reactive intermediate **A** offers promising synthetic routes to a large variety of new phosphametallaheterocycles. Thus, the trapping reaction of **A** with phosphalkynes^[1] and alkynes^[2] proceeds by formal [2+2] cycloaddition reactions to form novel main group element transition metal cage compounds. In continuation of these reactivity studies we attempted to employ nitriles for trapping reactions of intermediate **A**. Surprisingly, however, we observed insertion reactions into the P–C bond of the starting material.

Insertion reactions of organonitriles into metal–hydrogen and metal–carbon bonds are established processes.^[3] Furthermore, it is known that nitriles insert into the Mo–Cl bond of MoCl_5 ,^[4] into the Zr–O bond of $[\text{Cp}_2^*\text{Zr}=\text{O}]$,^[5] and into E–N bonds (E = B,^[6] Al,^[7] P,^[8] Pt^[9]). Recently, Neumüller et al. reported on CsX-catalyzed trimerization reactions of acetonitrile with EMe_3 (E = element of Group 13) under elimination of CH_4 and formation of $[\text{Me}_2\text{E}\{\text{HNC}(\text{Me})\}_2]$ –

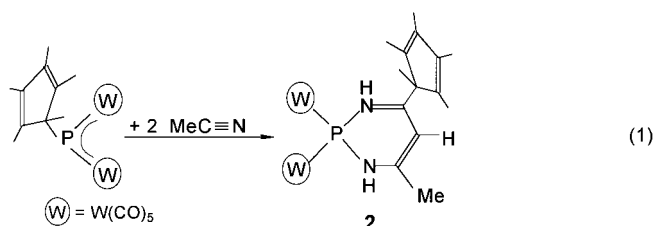
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CCN] ($E = \text{Al},^{[10]} \text{Ga},^{[11]} \text{In}^{[12]}$). However, in contrast, nitriles insert into a P–C bond only in the case of strained three-membered rings of the 2*H*-azaphosphirene at higher temperatures as has been shown only recently by Streubel et al.^[13]

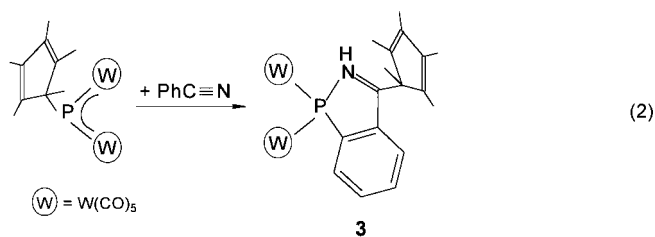
We report herein on a smooth insertion of nitriles into the unstrained P–C bond of $[\text{Cp}^*\text{P}\{\text{W}(\text{CO})_5\}_2]$ (**1**), which already proceeds at ambient temperatures to give dihydro-1,3,2-diazaphosphinine and benzo-1,2-azaphosphole derivatives. The synthesis of the latter compound is accompanied by an additional C–H bond activation at room temperature and offers a novel and straightforward synthetic approach to this class of compounds.

Stirring the dark blue solution of **1** in toluene with an excess of acetonitrile [Eq. (1)] results in a color change to red at room temperature.^[14] After workup and crystallization from

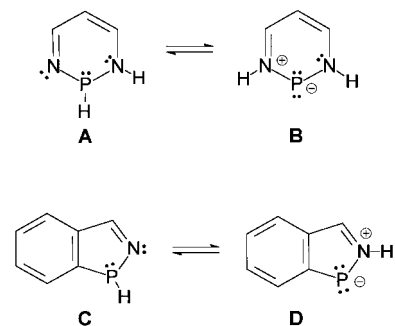


dichloromethane, the 1,2-dihydro-1,3,2-diazaphosphinine derivative **2** was obtained as red crystals in 88 % yield. Complex **2** shows that two acetonitrile molecules have been inserted into the P–C bond of **1**, under C–C bond formation and subsequent migration of the β -H atoms of one acetonitrile to the N atoms. Thereby one molecule of acetonitrile loses two hydrogen atoms of its methyl group. To check this hypothesis of the 1,3-H shift of acetonitrile to the N atoms, reaction (1) was carried out with CD_3CN . Indeed, the obtained product **2a** contains deuterium at the N atoms, which confirms the 1,3-D shift.

To avoid the observed insertion reaction and to obtain a [2+2] cycloaddition product from the intermediate $[\text{Cp}^*(\text{CO})_2\text{W}=\text{P} \rightarrow \text{W}(\text{CO})_5]$ **A** and the $\text{C}\equiv\text{N}$ triple bond of a nitrile, we used exclusively organonitriles without β -hydrogen atoms. However, when $t\text{BuC}\equiv\text{N}$ was used as the trapping reagent in the thermolysis reaction, it was found not to react with **1**. No product, even an insertion or addition product, could be detected by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy even after refluxing in toluene for 48 h. However, the use of benzonitrile in the reaction with **1** at ambient temperature^[14] leads to a dark red toluene solution which affords after workup dark red crystals of 3,4-benzo-1*H*-1,2-azaphosphole **3** in 43 % yield [Eq. (2)]. The formation of the **3** indicates that after insertion of the nitrile into the P–C bond, a C–H bond activation occurs.



The synthesis of 1,3,2-diazaphosphinines^[15] and benzo-1,2-azaphospholes^[16] is so far restricted to the use of dimethyltitanocene and diphenylzirconocene complexes. Thus, our approach appears to provide an alternative synthesis for such derivatives. Furthermore, it should be mentioned that alkylation of benzo-2*H*-1,2-azaphospholes was observed at the P atom of the heterocycle^[16] and 1,3,2-diazaphosphinines react readily at room temperature with protic reagents to give 1,2-dihydro-1,3,2-diazaphosphinines.^[15] Complexes **2** and **3** are in principle the betaine-type tautomers **B** and **D** of 1,2-dihydro-1,3,2-diazaphosphinine **A** and benzo-2*H*-1,2-azaphosphole **C**, respectively, stabilized by $[\text{W}(\text{CO})_5]$ moieties at the P atoms.



Compounds **2** and **3** are red crystalline solids, readily soluble in hexane, toluene, and CH_2Cl_2 and are stable in air for a short period of time. The IR spectra of both compounds display CO stretching frequencies for terminal CO ligands. The mass spectra of **2** and **3** show the molecular ion peaks as well as additional fragments revealing the subsequent loss of CO.

The ^{31}P NMR spectrum of **2** reveals a pseudotriplet (doublet of doublets) at $\delta = 176.2$ with one pair of ^{183}W satellites, which are also split into pseudotriplets due to the coupling of the phosphorus atom to the two almost identical hydrogen atoms bound to the nitrogen atoms ($^2J(\text{P},\text{H}) = 22$ Hz). The coupling constant $^1J(\text{W},\text{P})$ of 202 Hz is comparable to those of other terminally bound $[\text{W}(\text{CO})_5]$ groups.^[17] For **2** the existence of a methine proton at the C(13) atom is confirmed by its ^1H NMR spectrum as well as by a $^{13}\text{C}\{\text{DEPT}\}$ spectrum. Furthermore, for the D-containing complex $[\text{Cp}^*\text{CC}(\text{D})\text{C}(\text{CD}_3)\text{N}(\text{D})\text{P}\{\text{W}(\text{CO})_5\}_2\text{N}(\text{D})]$ **2a**, the ^{31}P NMR spectrum reveals only one singlet at $\delta = 175.2$ with a $^1J(\text{W},\text{P})$ coupling of 201 Hz and the ^1H NMR spectrum shows only the protons of the Cp^* group. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **3** reveals a singlet at $\delta = 90.6$ with a $^1J(\text{W},\text{P})$ coupling of 162 Hz. The coupling of the P atom to the NH proton could not be resolved, even in the ^1H NMR spectrum.

The X-ray crystal structure analysis of **2**^[18] (Figure 1) reveals the six-membered ring of a 1,2-dihydro-1,3,2-diazaphosphinine derivative. Thus, the tetrahedrally coordinated phosphorus atom is bent by $29.6(2)^\circ$ out of the plane of the remaining ring formed by the atoms N(1), C(11), C(13), C(14), and N(2). The phosphorus atom is further connected to two $[\text{W}(\text{CO})_5]$ moieties. In comparison to the W–P bond lengths in the starting phosphinidene complex **1** (2.445(2), 2.428(2) Å), which are indicative of multiple-bond character, the W–P bond lengths in **2** (2.5409(12), 2.5311(14) Å) are

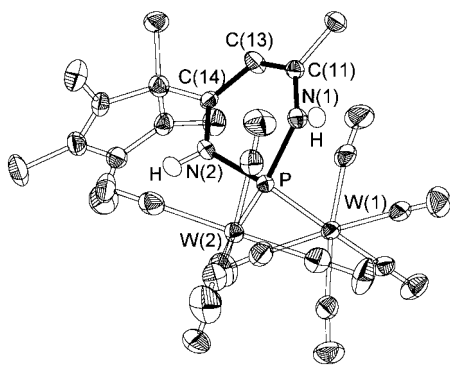


Figure 1. Molecular structure of **2** in the crystal; thermal ellipsoids are drawn at the 30% probability level (hydrogen atoms (except the NH protons) are omitted for clarity); selected bond lengths [Å] and angles [°]: W(1)–P 2.5409(12), W(2)–P 2.5311(14), P–N(1) 1.763(4), P–N(2) 1.765(4), N(1)–C(11) 1.331(6), N(2)–C(14) 1.342(6), C(11)–C(12) 1.495(6), C(11)–C(13) 1.396(7), C(13)–C(14) 1.392(6); N(1)–P–N(2) 92.48(18), N(1)–P–W(2) 107.37(14), N(2)–P–W(2) 111.07(14), N(1)–P–W(1) 107.26(13), N(2)–P–W(1) 111.06(14), W(2)–P–W(1) 123.04(5), C(11)–N(1)–P 126.1(3), C(14)–N(2)–P 125.2(3), N(1)–C(11)–C(13) 121.4(4), N(1)–C(11)–C(12) 118.5(4), C(14)–C(13)–C(11) 121.1(4), N(2)–C(14)–C(13) 121.4(4).

significantly longer. The slightly longer P–N single bond lengths in **2** are almost identical (1.763(4), 1.765(4) Å) and are in good agreement with the apical P–N single bond length in 5,6-benzo-1,3-dimethyl-2-dimethylamino-8,8,9,9-tetrakis(trifluoromethyl)-1,3-diaza-7,10-dioxo-2- λ^5 -phosphaspiro[4,5]decan-4-one (1.767(2) Å).^[19] All other N–C and C–C bond lengths of the six-membered ring of **2** are in good agreement with those of cyclic conjugated ring systems.^[20]

The X-ray crystal structure analysis of **3** (Figure 2)^[18] reveals a planar benzo-1*H*-1,2-azaphosphole derivative, in which the phosphorus atom is coordinated by two [W(CO)₅]

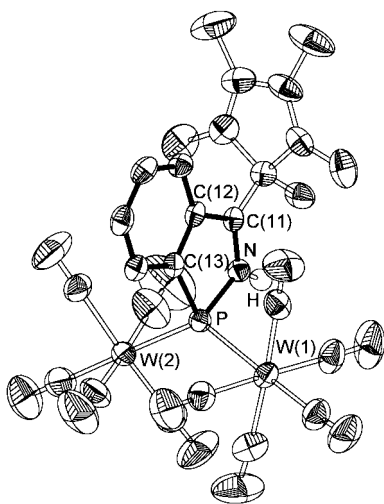


Figure 2. Molecular structure of **3** in the crystal; thermal ellipsoids are drawn at the 30% probability level (hydrogen atoms (except the NH protons) are omitted for clarity); selected bond lengths [Å] and angles [°]: W(1)–P 2.5494(17), W(2)–P 2.5396(18), P–N 1.775(5), P–C(13) 1.802(6), N–C(11) 1.311(7), C(11)–C(12) 1.467(7), C(12)–C(13) 1.398(8), C(13)–C(14) 1.400(8), C(14)–C(15) 1.375(9), C(15)–C(16) 1.379(10), C(16)–C(17) 1.373(8), C(12)–C(17) 1.415(8); N–P–C(13) 86.4(2), N–P–W(2) 107.68(17), C(13)–P–W(2) 108.63(19), N–P–W(1) 108.39(16), C(13)–P–W(1) 107.05(19), W(2)–P–W(1) 130.02(6), C(11)–N–P 118.3(4), N–C(11)–C(12) 110.7(5), C(13)–C(12)–C(11) 111.7(5), C(12)–C(13)–P 112.9(4), C(14)–C(13)–C(11) 121.1(4), N(2)–C(14)–C(13) 121.4(4).

moieties in a distorted tetrahedral geometry. The W(1)–P (2.5494(17) Å) and W(2)–P bond lengths (2.5396(18) Å) are comparable to the W–P bond lengths in complex **2**. The P–N (1.775(5) Å) bond length corresponds to a P–N single bond, whereas the N–C(11) bond length (1.311(7) Å) corresponds to a N–C double bond. All other C–C distances are in good agreement with those of cyclic conjugated benzo-1,2-azaphosphole systems.

To shed light onto the pathway of the insertion, we carried out reaction (1) with the starting materials in a 1:1 ratio at room temperature. Even after 48 h, there was no change of color, and no additional signal was observed in the ³¹P NMR spectrum. Titration of the solution with additional CH₃CN to a **1**:CH₃CN ratio of 1:1.4 led to color change, which was accompanied by the appearance of an additional signal at $\delta = 119.3$ in the ³¹P NMR spectrum. Unfortunately it was not possible to isolate any compound from this solution. This signal disappears when the color of the solution turns to red at a **1**:CH₃CN ratio of 1:5. For the transient ³¹P NMR signal, we propose the formation of an adduct of the formula [Cp*P(W(CO)₅)₂(NCMe)], since it is known that phosphinidene complexes [RP(W(CO)₅)₂] behave as Lewis acids. Owing to the electron deficiency at the phosphorus atom, Lewis bases can add at the central P atom leading to complexes with a tetrahedrally coordinated phosphorus atom.^[21] Such initially formed compounds seem to be plausible for reactions (1) and (2), a subsequent insertion reaction into the P–C σ -bond gives the formed products. While for reaction (2) a C–H bond activation occurs to form the benzo-1*H*-1,2-azaphosphole derivative, for reaction (1) presumably a second molecule of acetonitrile is subsequently added followed by a cyclization reaction under migration of two protons of the methyl group of the Cp*-substituted MeCN unit.

The reported insertion of nitriles into the C–P bond of the phosphinidene complex **1** is a novel reaction pattern for this compound. Besides the formation of the intermediate [Cp*(CO)₂W=P → W(CO)₅] **A** containing a W–P triple bond,^[1,2] and a Cp* elimination,^[22] also an insertion reaction into the unstrained P–C σ bond of **1** proceeds under very mild conditions. With benzonitrile as a reaction component an additional C–H bond activation occurs. The described reaction provides a straightforward route for the synthesis of novel phosphorus heterocycles.

Experimental Section

2: Me–C≡N (0.780 g, 0.019 mol) was added to a solution of **1**^[23] (0.630 g, 0.77 mmol) in toluene (40 mL) and stirred for two hours at room temperature (or refluxed for one hour). The solvent was completely removed in vacuo and the resulting residue was dissolved in dichloromethane (5 mL). Red crystals of **2** (0.609 g, 0.68 mmol, 88%) were obtained at –25 °C. ³¹P{¹H} NMR (101.256 MHz, [D₆]benzene, 298 K, 85% H₃PO₄ ext.): $\delta = 176.2$ (t, ¹J(P,H) = 22, ¹J(W,P) = 202 Hz); ¹H NMR (250.113 MHz, [D₆]benzene, 298 K, TMS): $\delta = 4.88$ (br, 1 H; NH), 4.26 (br, 1 H; NH), 1.58 (s, 6 H; CH₃(Cp*)), 1.51 (s, 6 H; CH₃(Cp*)), 0.98 (s, 3 H; CH₃), 0.84 (s, 3 H; CH₃), 0.31 (s, 1 H; CH); IR (KBr): $\tilde{\nu} = 2075$ (s), 2050 (s), 1990 (sh), 1920 (br) (4 × ν (CO)), 1544 cm^{–1} (w) (δ (NH)); MS (EI): m/z (%): 896 (27) [M^+], 868 (17) [$M^+ - CO$], 812 (28) [$M^+ - 3 CO$], 728 (26) [$M^+ - 6 CO$], 672 (29) [$M^+ - 8 CO$], 616 (100) [$M^+ - 10 CO$], 571 (25) [$M^+ - (CO)_{10}(CH_3)_3$]; elemental analysis (%) calcd for C₂₄H₂₁N₂O₁₀PW₂ (896.11): C 32.17, H 2.36, N 3.13; found: C 31.86, H 3.01, N 2.81.

2a: The corresponding reaction of **1** (0.104 g, 0.13 mmol) with $\text{CD}_3\text{C}\equiv\text{N}$ (1.0 mmol) in toluene (40 mL) at room temperature led after stirring for 2 h to a red solution of **2a**. After complete removal of the solvent, the residue was recrystallized from dichloromethane to give **2a** (0.100 g, 0.11 mmol, 85%) at -25°C . $^{31}\text{P}\{^1\text{H}\}$ NMR (101.256 MHz, $[\text{D}_6]\text{benzene}$, 298 K, 85% H_3PO_4 ext.): $\delta = 175.2$ (s, $^1J(\text{W,P}) = 201$ Hz); ^1H NMR (250.113 MHz, $[\text{D}_6]\text{benzene}$, 298 K, TMS): $\delta = 1.58$ (s, 6H; $\text{CH}_3(\text{Cp}^*)$), 1.51 (s, 6H; $\text{CH}_3(\text{Cp}^*)$), 0.98 (s, 3H; $\text{CH}_3(\text{Cp}^*)$).

3: $\text{PhC}\equiv\text{N}$ (0.028 g, 0.27 mmol) was added to a solution of **1** (0.20 g, 0.25 mmol) in toluene (40 mL) and stirred for 2 h at room temperature (or refluxed for one hour). The solvent of the orange-colored solution was completely removed in vacuo and the resulting residue was extracted with *n*-pentane to give red crystals of **3** (0.099 g, 0.108 mmol, 43%) at -25°C . $^{31}\text{P}\{^1\text{H}\}$ NMR (101.256 MHz, $[\text{D}_6]\text{benzene}$, 298 K, 85% H_3PO_4 ext.): $\delta = 90.6$ (s, $^1J(\text{W,P}) = 162$ Hz); ^1H NMR (250.113 MHz, $[\text{D}_6]\text{benzene}$, 298 K, TMS): $\delta = 7.92$ – 6.65 (aromatic protons), 3.55 (s, 1H; NH), 1.50 (s, 6H; $\text{CH}_3(\text{Cp}^*)$), 1.48 (s, 6H; $\text{CH}_3(\text{Cp}^*)$), 1.15 (s, 3H; $\text{CH}_3(\text{Cp}^*)$); IR (KBr): $\tilde{\nu} = 1985$ (w), 1980 (w), 1965 (sh), 1910 cm^{-1} (br) ($4 \times \nu(\text{CO})$); MS (EI): m/z (%): 917 (6) [M^+], 889 (10) [$\text{M}^+ - \text{CO}$], 861 (10) [$\text{M}^+ - 2\text{CO}$]; elemental analysis (%) calcd for $\text{C}_{27}\text{H}_{20}\text{NO}_{10}\text{PW}_2$ (917.13): C 35.36, H 2.20, N 1.53; found: C 35.39, H 2.29, N 1.58.

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- [18] Crystal structure analyses of **2** and **3** were performed on a STOE IPDS diffractometer with $\text{MoK}\alpha$ radiation ($\lambda = 0.71073\text{ \AA}$). The structures were solved by direct methods with the program SHELXS-93,^[24a] and full-matrix least-squares refinement on F^2 in SHELXL-97^[24b] was performed with anisotropic displacements for non-H atoms. Hydrogen atoms were located in idealized positions and refined isotropically according to the riding model. **2**: $\text{C}_{24}\text{H}_{21}\text{N}_2\text{O}_{10}\text{PW}_2$, $M_r = 896.10$, crystal dimensions $0.15 \times 0.15 \times 0.04\text{ mm}^3$, monoclinic, space group $P2_1/n$ (no. 14); $a = 10.753(2)$, $b = 11.986(2)$, $c = 22.052(4)\text{ \AA}$, $\beta = 94.65(3)^\circ$, $T = 200(1)\text{ K}$, $Z = 4$, $V = 2832.8(10)\text{ \AA}^3$, $\rho_{\text{calcd}} = 2.101\text{ Mg m}^{-3}$, $\mu(\text{MoK}\alpha) = 8.227\text{ mm}^{-1}$, 5448 independent reflections ($R_{\text{int}} = 0.0572$, $2\theta_{\text{max}} = 52^\circ$), 4449 observed with $F_o = 4\sigma(F_o)$; 358 parameters, $R_1 = 0.0316$, $wR_2 = 0.0798$. **3**: $\text{C}_{15}\text{H}_{20}\text{NO}_{10}\text{PW}_2$, $M_r = 917.11$, crystal dimensions $0.20 \times 0.12 \times 0.10\text{ mm}^3$, monoclinic, space group $P2_1/c$ (no. 14); $a = 15.643(3)$, $b = 15.738(3)$, $c = 13.126(3)\text{ \AA}$, $\beta = 110.86(3)^\circ$, $T = 253(1)\text{ K}$, $Z = 4$, $V = 3019.5(10)\text{ \AA}^3$, $\rho_{\text{calcd}} = 2.017\text{ Mg m}^{-3}$, $\mu(\text{MoK}\alpha) = 7.720\text{ mm}^{-1}$, 5799 independent reflections ($R_{\text{int}} = 0.0579$, $2\theta_{\text{max}} = 52^\circ$), 4751 observed with $F_o = 4\sigma(F_o)$; 375 parameters, $R_1 = 0.0356$, $wR_2 = 0.0914$. 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-161080 (**2**) and CCDC-161081 (**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).
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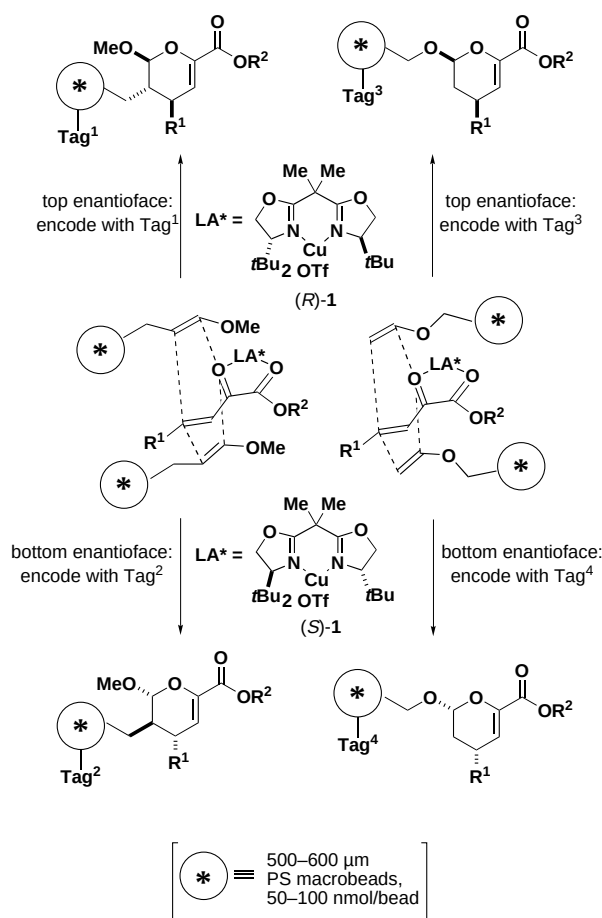
Asymmetric Catalysis in Diversity-Oriented Organic Synthesis: Enantioselective Synthesis of 4320 Encoded and Spatially Segregated Dihydropyrancarboxamides**

Robert A. Stavenger and Stuart L. Schreiber*

Small molecules have been used to explore many facets of biology for over a century. However, research in biology is not routinely performed using this approach, in the way that it is with biochemical, genetic, and increasingly, genomic approaches. Several problems limit the use of the former approach. Arguably, the primary one is the lack of routine access to structurally complex and diverse small molecules that can be used to modulate biological systems.^[1] Diversity-oriented organic synthesis, especially when coupled with an economical and efficient technology platform, offers the means to change this situation, as it aims to synthesize complex and diverse small molecules efficiently.^[2] Diversity-oriented synthesis is central to chemical genetics, which aims to explore biology with small molecules in a *systematic* way.^[3]

Although enantioselective catalysis is often used in target-oriented synthesis, it is still relatively underexplored in diversity-oriented synthesis.^[4, 5] We have been interested in reactions catalyzed by bis(oxazoline)metal Lewis acid complexes because of their high efficiency, selectivity, and broad substrate tolerance.^[6] We chose to concentrate on inverse electron demand heterocycloadditions of vinyl ethers and β,γ -unsaturated ketoesters (Scheme 1).^[7, 8] An account of related cycloadditions on solid support has been described;^[9] however, the reported reactions were performed in the presence of achiral catalysts and with the heterodiene bound to the PS solid support through the ester. We initially investigated this mode of cycloaddition and found it to be highly selective when the enantiomerically pure catalysts (*S*)- or (*R*)-**1** were used.^[5, 8] However, in the interest of effectively functionalizing the cycloadduct, we found an alternative mode using

support-bound vinyl ethers, linked to a macrobead through either carbon or oxygen, to be more effective.



Scheme 1. Encoded, catalytic, asymmetric heterocycloaddition. The polystyrene macrobead serves as a microreactor, an important element of the “one-bead, one-stock-solution” technology platform.^[10] LA* = the chiral Lewis acid, PS = polystyrene, Tf = triflate = trifluoromethanesulfonyl.

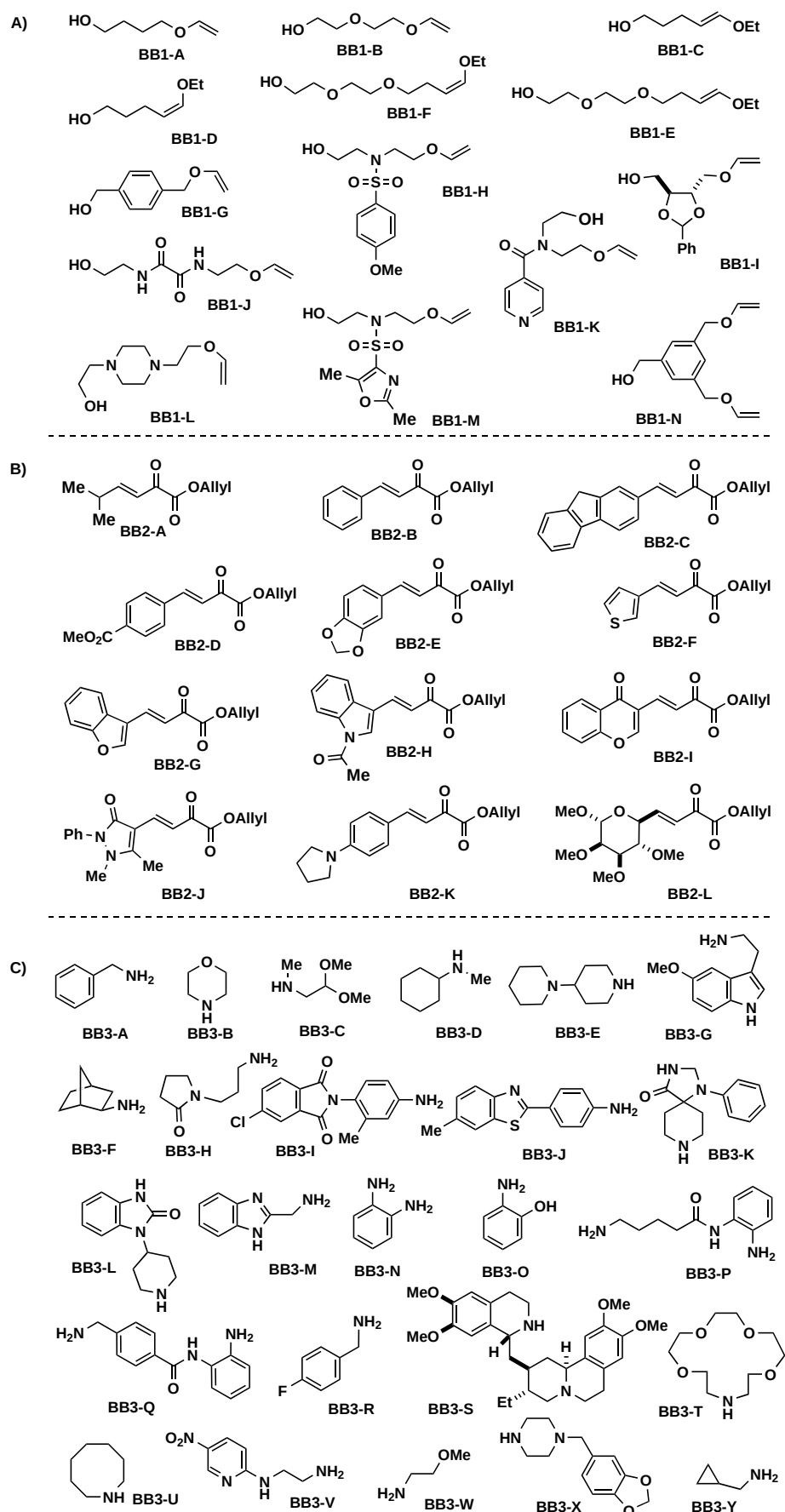
Here we report our application of this asymmetric cycloaddition reaction to the synthesis of dihydropyrancarboxamides on high-capacity, 500–600 μ m PS macrobeads, key elements in a one-bead, one-stock-solution technology platform.^[6, 10] The diversity pathway explored resulted in the highly diastereo- and enantioselective synthesis of 4320 encoded^[11] small molecules,^[12] which were arrayed as 5 mm stock solutions from individual beads,^[13] each containing predominantly a single dihydropyrancarboxamide. These stock solutions permit many phenotypic and proteomic assays to be performed.

We first synthesized collections of vinyl ethers (Scheme 2A) and unsaturated ketoesters (Scheme 2B) as candidate partners for the cycloaddition reaction. Each of the vinyl ethers **BB1-A–N** was loaded onto pools of PS macrobeads via the silyl triflate **3** which is generated in situ (method shown in Scheme 3).^[10] The support-bound vinyl ethers **4–6** were then treated with heterodienes (either **BB2-B** or **BB2-E**, R³ = phenyl and 4-piperonyl, respectively; 3 equivalents) in THF in the presence of 20 mol % of the [(*t*BuBOX)Cu(OTf)₂] complex ((*S*)- or (*R*)-**1**) and 4 Å molecular sieves^[14] to provide

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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

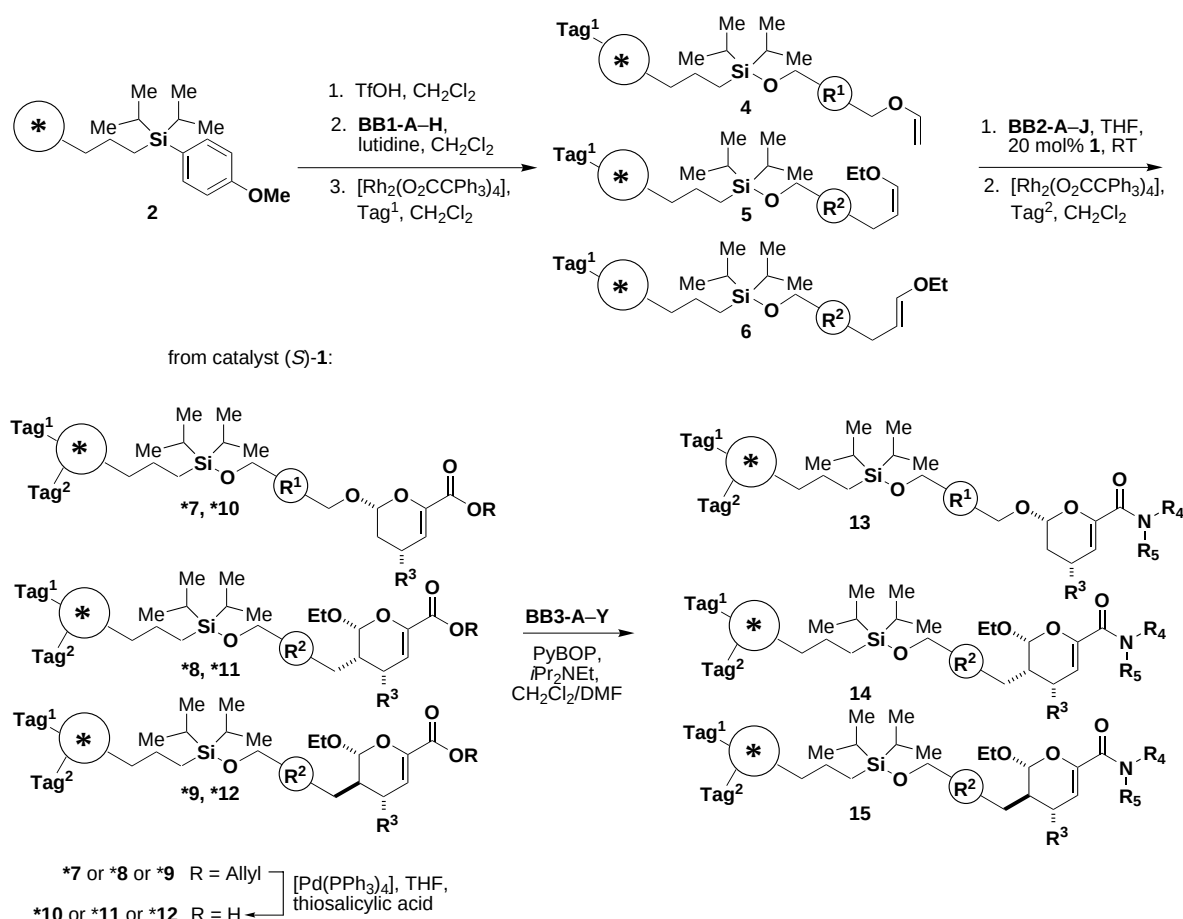


Scheme 2. A) Vinyl ether alcohol building blocks **BB1-A–N**. B) β,γ -Unsaturated ketoester building blocks **BB2-A–L**. C) Amine building blocks **BB3-A–Y**.

support-bound cycloadducts ***7–*9**. Both enantiomers of the ligand were used in separate reactions to obtain a duplicate result and to detect potential matched/mismatched pairs when chiral starting materials were used. After washing and drying steps, each of the cycloadducts was cleaved from the silyl ether linker with hydrogen fluoride/pyridine (HF–py) and analyzed for purity with ^1H NMR spectroscopy and liquid chromatography/mass spectrometry (LC–MS).

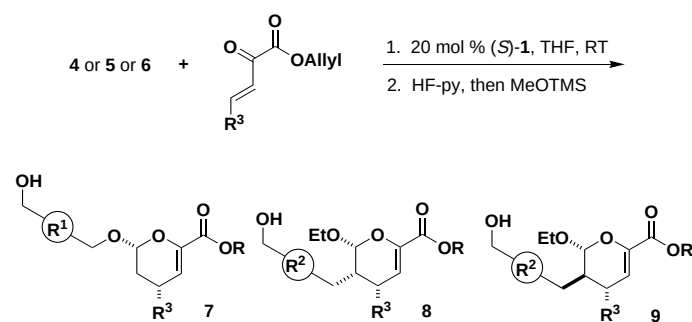
These studies showed that support-bound vinyl ethers with amino or amido functionality led to low conversion, and the support-bound form of bis(vinyl ether) **BB1-N** underwent a single, rather than the desired double, cycloaddition, even when a stoichiometric amount of the copper complex was used. The chiral vinyl ether derived from threitol (support-bound form of **BB1-I**) reacted efficiently with only the *S* enantiomer of the catalyst, which suggests that double diastereoselection was taking place (see below). Upon treatment of the corresponding PS macrobeads with HF–py, the remaining vinyl ethers studied provided high purity cycloadducts **7–9** (Table 1). The ethenyl ethers **BB1-A, B, G, H** also yielded dihydropyrans **7** in high diastereo- and enantioselectivity.^[15] Both configurations of substituted enol ethers **BB1-C–F** led to moderate to high diastereoselectivity of the tetrasubstituted dihydropyrans **8** and **9**. Although previous results^[8a,c] had shown high diastereoselectivity with cyclic vinyl ethers, we found that *Z*-configured enol ethers (**BB1-D, F**) provided only moderate diastereoselection, whereas the *E* enol ethers (**BB1-C, E**) resulted in high levels of diastereoselection. The lower diastereoselectivity in the *Z* enol ether cycloadditions may arise from an endo–exo switch in the transition structure for cycloaddition.^[16]

We next turned our attention to the substitution on the heterodiene partner. In most instances, treatment of the support-bound vinyl ether **BB1-H** with a heterodiene under the previous conditions again



Scheme 3. The encoded split-pool synthesis of dihydropyrancarboxamides, with the (*S*)-1 catalyst. The corresponding opposite enantiomers of compounds 7–15 are obtained when the (*R*)-1 catalyst is used. Encircled R¹ and R² symbols represent elements found in building blocks **BB1-A-H** in Scheme 2. PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate, DMF = *N,N*-dimethylformamide, THF = tetrahydrofuran.

Table 1. Asymmetric cycloadditions of resin-bound vinyl ethers **BB1**.^[a]



BB1-	BB2-	Product	Purity [%] ^[b]	d.r. ^[c]	e.r. ^[d]
A	E	7-A-E	≥ 95	≥ 15:1	≥ 49:1
B	E	7-B-E	≥ 95	≥ 15:1	≥ 24:1
C	B	9-C-B	≥ 95	≥ 20:1	≥ 49:1
D	B	8-D-B	≥ 95	≥ 5:1	≥ 30:1
E	B	9-E-B	≥ 95	≥ 20:1	≥ 49:1
F	B	8-D-B	≥ 95	≥ 10:1	≥ 30:1
G	E	7-G-E	≥ 95	≥ 30:1	≥ 49:1
H	E	7-H-E	≥ 95	≥ 20:1	≥ 24:1

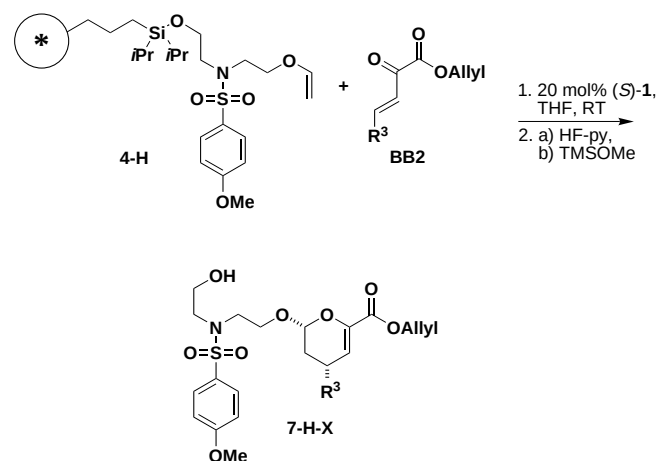
[a] Reactions were performed with 20 mol % of (*S*)-1 or (*R*)-1; the results presented are an average of the two runs. [b] Estimated based on ¹H NMR analysis and HPLC–ESI MS. [c] Determined by ¹H NMR analysis and/or CSP HPLC or CSP SFC. [d] Determined by CSP HPLC or CSP SFC. HPLC–ESI MS = high-pressure liquid chromatography/electron spray ionization MS, CSP = chiral stationary phase, SFC = supercritical fluid chromatography, TMS = trimethylsilyl.

led to highly pure cycloadduct following HF–py cleavage from the PS macrobeads (Table 2), though again amine functionality (**BB2-K**) was incompatible. Similar to the case above with the threitol-derived vinyl ether, only the *S* enantiomer of the catalyst efficiently provided cycloadduct with the mannose-derived heterodiene **BB2-L**. Overall, ten heterodienes (**BB2-A–J**) resulted in somewhat variable, but uniformly high, diastereo- and enantioselectivities and high purities based on ¹H NMR spectroscopy and LC–MS analyses. These building blocks were chosen for subsequent incorporation into the library synthesis.

Further functionalization of the cycloadducts was then pursued. Conversion of the support-bound cycloadduct **7-H-E**, upon treatment with [Pd(PPh₃)₄] and thiosalicylic acid, into the corresponding acid **10-H-E** was achieved in high purity (Scheme 3). Treatment of the support-bound acid **10-H-E** with 20 equivalents of benzylamine, PyBOP, and diisopropylethylamine in CH₂Cl₂:DMF (3:1) led to the desired benzylamide. These conditions were applied to a diverse collection of amines with support-bound acid **10-H-E** to select 25 amines for use in the library synthesis (Scheme 2C).

These pathway development studies were necessary to select the reactions and building blocks for a library realization that would result in single-compound stock solutions from individual macrobeads. The library synthesis was

Table 2. Asymmetric cycloadditions of resin-bound vinyl ether **4-H** with various heterodienes.^[a]



BB2-	Products	Purity [%] ^[b]	d.r. ^[c]	e.r. ^[d]
A	7-H-A	≥ 95	≥ 16:1	≥ 16:1
B	7-H-B	≥ 95	≥ 20:1	≥ 24:1
C	7-H-C	≥ 95	≥ 9:1	≥ 24:1
D	7-H-D	≥ 95	≥ 9:1	≥ 9:1
E	7-H-E	≥ 95	≥ 20:1	≥ 24:1
F	7-H-F	≥ 95	≥ 25:1	≥ 24:1
G	7-H-G	≥ 95	≥ 9:1	≥ 49:1
H	7-H-H	≥ 95	≥ 15:1	≥ 24:1
I	7-H-I	≥ 95	≥ 12:1	≥ 49:1
J	7-H-J	≥ 95	≥ 9:1	≥ 49:1

[a]–[d] See footnotes for Table 1.

initiated with sufficient PS macrobeads (13000) to produce, on average, three beads containing each theoretical compound. The chosen vinyl ethers were attached to the supports and following the initial cycloaddition step, the two enantiomeric sets of cycloadducts were not pooled. (Each set includes cycloadduct attached to either the C1 oxygen or C2 carbon of the dihydropyran ring.) Instead, the two sets were carried through the remaining steps in parallel in order to provide an independent means (when coupled to mass spectrometry) to assess the ability of tags to infer the absolute configuration of library members (Scheme 1). The supports were not repooled following the amide coupling, thereby reducing the number of chemical encoding steps to which the macrobeads were subjected and simplifying the decoding of library members.^[3c] In the end, 54 separate portions of macrobeads were produced (50 portions containing dihydropyran carboxamides, 2 containing dihydropyran carboxylic acids, and 2 containing dihydropyran carboxylic esters, see the Supporting Information for details), each containing, theoretically, three copies of 80 compounds for a total of 4320 distinct, spatially-segregated, and stereochemically-defined dihydropyran derivatives.

In order to analyze the purity of members of the library, two macrobeads from each of the above 54 pools were removed, arrayed, and treated with HF-py, and fractions of the eluted products (10 µL of 5 mM stock solutions) were assayed by LC-MS.^[12, 17] In summary, 78 samples (72 %) were ≥ 95 % pure, 93 samples (86 %) were ≥ 90 % pure, 104 samples (96 %) were

≥ 75 % pure, and the remaining 4 samples were of roughly 50 % purity. Direct structure determination by MS was successful in 83 of the 108 cases,^[18] and indirect structure inference by decoding of the chloroaromatic diazoketone tags^[11b, 12] was successful in all cases. Full details of this procedure are described in the following Communication.^[12]

Although this library synthesis succeeded in using stereochemistry as a diversity element and extended the asymmetric heterocycloaddition reaction to solid phase,^[19] only one of two potential diastereomers (for the unsubstituted vinyl ethers) was accessed. Catalyst systems with truly complete external control over enantio- and diastereoselectivity are required to realize fully the potential of stereoselective catalysis in diversity-oriented organic synthesis. The generation of spatially-segregated stock solutions from individual macrobeads guarantees that the compounds are amenable to both phenotypic and protein-binding assays, and their common primary hydroxy group ensures that every compound can be robotically arrayed onto a glass microscope slide for protein-binding assays.^[20] Indeed, small molecule microarrays of the dihydropyran carboxamides have already been manufactured and screened, which led to the discovery of a small molecule that binds to a protein of interest.^[21, 22]

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- There are examples of simple, achiral modulators of biological systems, notably “drug-like” molecules, although in these cases the smaller size and complexity of the species have more to do with delivery and pharmacokinetic parameters than with affinity and selectivity for a protein target. It is our hypothesis that structurally complex and diverse collections of “natural product like”, rather than “drug-like”, molecules will be better suited as biological probes and this report is part of a collective effort to address this very hypothesis.
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- [13] A fully automated procedure for deriving and arraying stock solutions from the dihydropyranocarboxamide-containing macrobeads described herein has been developed and will be reported elsewhere; ref. [10c].
- [14] After surveying several loading/ligand/metal/solvent combinations, 20 mol % of **1** in THF was found to provide the best combination of kinetics and selectivity.
- [15] The configurations of the cycloadducts were assigned by analogy. See refs. [8a, c].
- [16] We have ruled out isomerization of the alkenyl ether and epimerization of the acetal center as reasons for the lower selectivity.
- [17] The details of this procedure and the relationship between the 108 cleaved compounds and their associated electrophoretic tags are reported in ref. [12].
- [18] For 25 of the 108 samples, the molecular ion observed upon ionization corresponded to a fragment of the compound.
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Decoding Products of Diversity Pathways from Stock Solutions Derived from Single Polymeric Macrobeads**

Helen E. Blackwell, Lucy Pérez, and
Stuart L. Schreiber*

Efficient phenotypic and proteomic screening of small molecules derived from diversity-oriented organic syntheses^[1] requires a library realization platform^[2] that 1) produces a sufficient quantity of compound per bead to perform many hundreds of assays^[3] and 2) supports reliable compound structure identification. To facilitate the latter, solid-phase library-encoding strategies^[4] have been developed that allow the identity of the compounds to be inferred postsynthesis directly from individual beads.^[5] We recently adapted the chemical-encoding strategy introduced by Still and co-workers^[6] to a high-capacity (1.4 mequiv g^{−1} ≈ 100 nmol/bead), 500–600 μm polystyrene (PS) solid support (Scheme 1),^[3] a key element of a “one-bead, one-stock-solution” technology platform.^[7] We have now discovered that the stock solutions of compounds cleaved from individual beads contain sufficient tags to allow the structures of their corresponding small molecules to be inferred reliably. Two methods used for the decoding of the library of 4320 dihydropyranocarboxamides reported in the preceding Communication are described.^[8]

The encoding method features structurally related chloroaromatic diazoketone “tags”,^[6b] which are introduced through an acylcarbene insertion into the phenyl rings of PS catalyzed by [Rh₂(O₂CCPh₃)₄] (**1**) to yield cycloheptatrienes (Scheme 1).^[9] To decode a library compound, the tags are cleaved oxidatively from the solid support with ceric ammonium nitrate (CAN) to yield free alcohols,^[10] which are then silylated (with *N,O*-bis-(trimethylsilyl)acetamide, BSA) and injected directly onto a gas chromatograph equipped with electron-capture detection (GC/ECD) for analysis (each tag trimethylsilyl ether has a unique GC retention time). For low-loaded solid support (≈ 100 pmol/bead) it has been postulated that the carbene inserts predominantly into the support due to

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the greater proportion of the support relative to compound.^[11, 12] However, because our PS macrobead supports contain considerably more bound compound than standard solid-phase resin (that is, $\approx 50\text{--}100\text{ nmol/bead}$),^[3] and we had to use higher relative tag concentrations to encode the supports effectively,^[11] we decided to investigate the amount of tag attached directly to the support-bound synthetic compounds. On one hand, we were concerned that acylcarbene insertion could cause significant compound contamination, which would defeat the goal of a “one-bead, one-compound” approach.^[7] On the other hand, we were hopeful that the level of compound tagging would be sufficient to allow effective decoding without complicating analyses of the properties of the compounds ($< 0.1\%$).

(Scheme 1) did not concurrently cleave the attached tags,^[13] therefore, we surmised the difference in relative peak area could be explained by significant incorporation of the diazoketone tags into the support-bound compound. These compound-associated tags were being cleaved simultaneously with those inserted into the PS macrobead and were thus inflating the relative tag peak intensities.

Model compound

with HF–py to effect cleavage of the model compound and to generate an unadulterated compound sample, before any encoding took place. Another 20 mg sample was encoded with four chloroaromatic diazoketone tags following our optimized procedure^[7a] and subsequently treated with HF–py to release the attached compound. The two compound samples were characterized by ¹H NMR spectroscopy and liquid chromatography/mass spectrometry (LC–MS), and both techniques failed to show any detectable tag incorporation into the compound (see Supporting Information). However, these results did not preclude direct compound decoding by GC/ECD because the level of sensitivity of the EC detector (subpicomolar) is far greater than that of ¹H NMR spectroscopy and LC–MS detection techniques.

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(20 μ L of a $\approx$ 5 mM solution per bead) encoded at this level remains analytically pure ($>99.9\%$) and anticipate that the trace impurities introduced by tag insertion should not compromise a “one-bead, one-compound” approach.

The ability to decode directly from a compound's stock solution should be advantageous in many circumstances, including those where GC signals from bead decoding are not sufficiently strong or clear to assign a chemical structure to a “hit” or if the parent bead cannot be located (a reality in solid-phase synthesis, for example, due to static electricity). As direct “stock-solution decoding” gives GC signals approximately 100 times greater than those obtained from bead alone, we postulated it should be possible to decode a compound, or “hit”, by decoding only a small portion of its respective stock solution. To this end, fractions (that is, 1%, 5%, 10%, 50%, and 100%) of a stock solution of the model compound of **2** cleaved from a single encoded bead were subjected to the decoding procedure. This analysis demonstrated that submitting just 1% of the compound's stock solution was sufficient for clear and reproducible decoding, equivalent or superior to bead-decoding data (Figure 1).

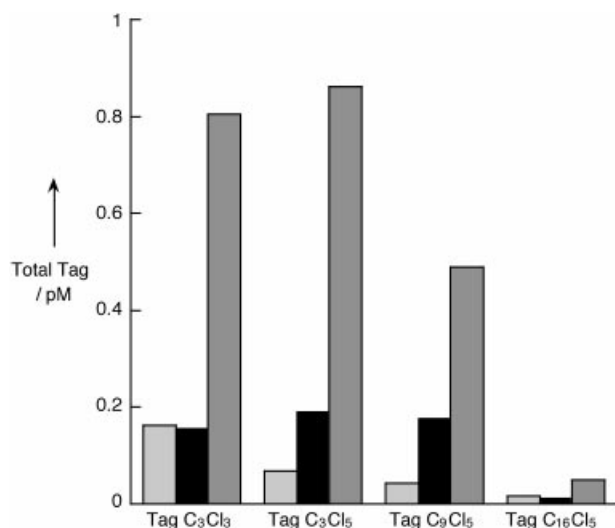


Figure 1. Graph of quantitative GC data for decoding from either an individual macrobead of test support **2** (light gray) or from 1 or 5% (black and dark gray, respectively) compound stock solutions prepared from a single macrobead of **2**. Bead and stock-solution decoding procedure: 0.25 M CAN (1:1 tetrahydrofuran/H₂O), 2 h, 25 °C, 1 min sonication, BSA:decane (1:1). See the Supporting Information for further details.

We now have three ways to determine the chemical history of a bead relying solely on encoding: the bead can be decoded either 1) before compound cleavage, 2) after compound cleavage, or 3) from a fraction of its respective compound's stock solution. To the best of our knowledge, the latter is the first example of decoding chemical tags covalently attached to compounds as a strategy for structure elucidation after split-pool synthesis.^[11]

In order to test the reliability of stock-solution decoding to determine the chemical history of an actual library member, this method was applied to selected samples from the dihydropyranocarboxamide library described in the preceding

paper.^[8] This synthesis resulted in 54 (27×2 enantiomers) separate portions of PS support containing 4320 stereochemically and structurally distinct compounds. Two beads were taken from each of the 54 final library pools, arrayed into individual tubes, and treated with HF-py to release the compounds from the beads. Samples from each of the stock solutions for the 108 compounds (diluted to 5 μ M in acetonitrile) were subjected to MS (atmospheric pressure chemical ionization, APCI) analysis, and the corresponding beads and/or aliquots of stock solution were subjected to GC/ECD decoding to compare the two results and check for agreement between mass spectral and chemical encoding data.

We were gratified to observe that, for 107 of the 108 samples, the structure assignments made by GC bead and/or stock-solution decoding were in complete agreement with the MS data. The majority of the samples could be decoded from their respective beads; however, the bead-decoding GC traces for 10 of the samples were not easily interpreted, so a fraction ($\approx 5\%$) of the corresponding stock solutions was subjected to direct decoding as described above. All 10 of these samples yielded clean GC traces that decoded for a compound identical to that assigned by MS, which thus demonstrates the feasibility of direct stock-solution decoding with real library compounds. Finally, there were two cases in which the parent bead was lost, so it was necessary to resort to stock-solution decoding. Once more, stock-solution decoding agreed with the MS data for these missing beads. Of note, there was only one example of the 108 samples analyzed where the compound identity assigned by GC decoding could not be confirmed by MS: In this case, both bead and stock-solution GC decoding were identical but neither a parent molecular ion nor a compound fragment was observed in the MS spectrum which was in agreement with this structure assignment.^[17] Representative GC, LC, and MS traces and chemical structure assignments, which demonstrate bead and stock-solution decoding, respectively, are shown in Figure 2 for samples 12 and 48.^[18]

The successful partial decoding of the dihydropyranocarboxamide library validates not only our encoding/decoding protocol, but also the use of direct stock-solution decoding as a reliable decoding strategy. While we decoded only 2.5% of the theoretical total library members for this study, stock-solution decoding has been done on an ad hoc basis during the biological screening of this library (data not shown), and to date all of the 25 “hits” have been successfully identified. Currently, the “hits” that were difficult to decode previously from a split-pool library on PS macrobeads that used a completely different set of reactions and substrates are being decoded directly from the stock solutions with the compound-decoding approach reported herein. Preliminary results of four different diversity-oriented synthetic pathways suggest stock-solution decoding will be feasible with many different chemistries. Obtaining the chemical history of small molecules synthesized through diversity-oriented syntheses by direct stock-solution decoding should expedite compound structure elucidation, minimize storage requirements (parent beads can be discarded), and be amenable to automation with a laboratory liquid-handling robot; this will potentially make

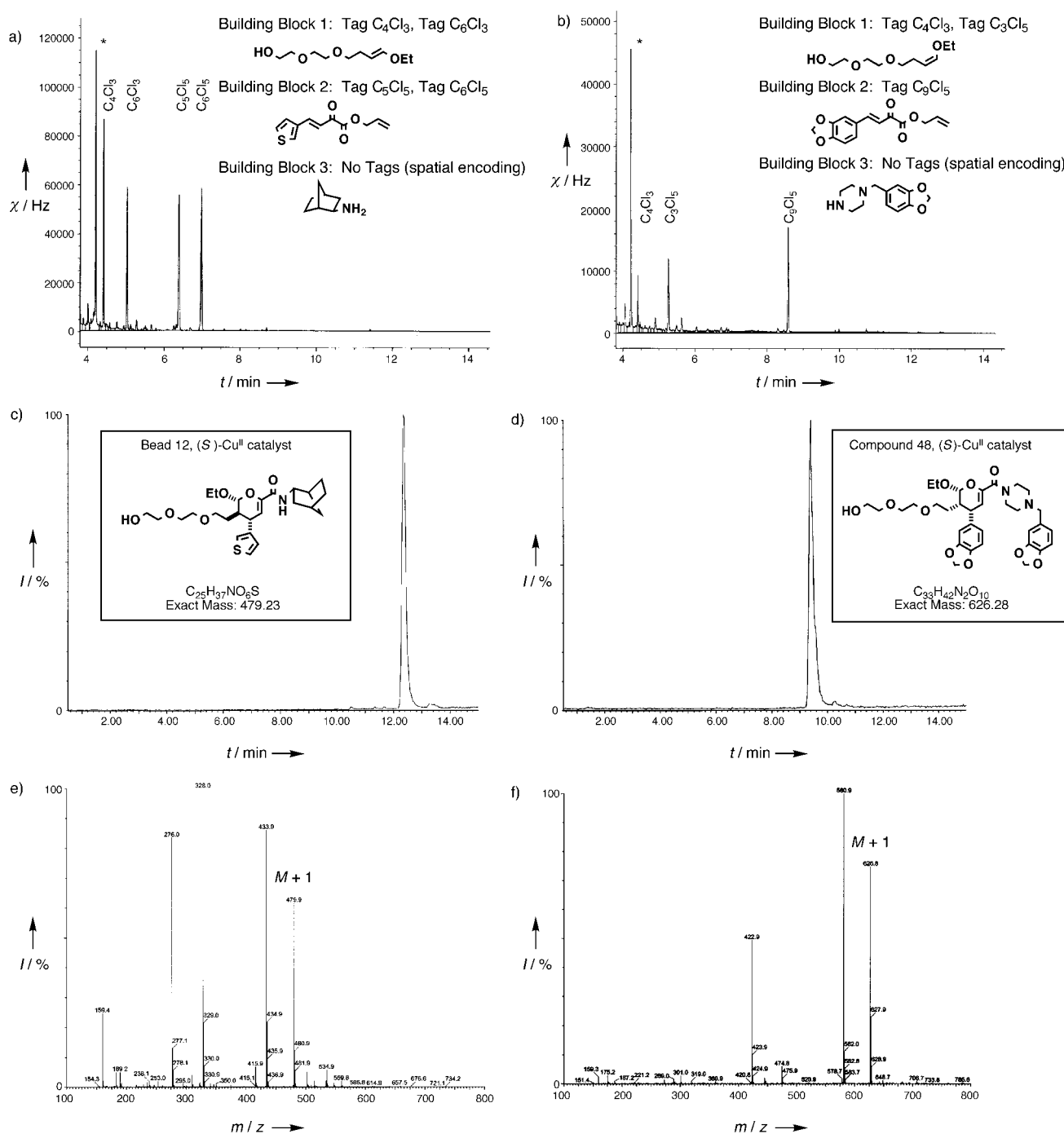


Figure 2. Representative examples of GC (a, b), LC (c, d), and MS (e, f) spectra from bead and stock-solution decoding (samples 12 and 48, respectively). The *bead-decoding* GC trace for sample 12 (a) decodes for a library compound with an exact mass identical to that obtained by MS (e) of the compound cleaved from that bead (APCI, observed mass = 479.9 [$M+1$]). The *stock-solution-decoding* GC trace for sample 48 (b) decodes for a library compound with an exact mass identical to that obtained by MS (f) of the compound stock solution (APCI, observed mass = 626.8 [$M+1$]). The single peaks in the LC spectra (c, d) correspond to these molecular ions. [The starred peak (*) in the GC traces (a, b) is an impurity frequently present with the electrophoric tags.]

the “global” decoding of entire split-pool libraries a reality in the future.^[19]

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- [16] Given the different efficiencies of the homogeneous and heterogeneous tag-cleavage reactions, we could not determine quantitatively if more tag was inserting into the compound rather than the bead, or vice versa.
- [17] This discrepancy can be attributed to either unsuccessful chemical reaction in one or more of the library steps and/or poor ionization by MS, and serves as a reminder that encoding records the sequence of chemical reactions to which a bead is subjected, not the structure of the compound.
- [18] The structures assigned to the 108 samples can be found in the Supporting Information.
- [19] Supporting Information available: Representative encoding and decoding protocols, ^1H NMR spectroscopy, LC, and GC data, and full details of the partial library decoding.

Highly Enantioselective Hydrogenation of Acyclic Imines Catalyzed by Ir–f-Binaphane Complexes**

Denming Xiao and Xumu Zhang*

Dedicated to Professor K. Barry Sharpless on the occasion of his 60th birthday

Although great accomplishments have been achieved in the highly enantioselective hydrogenation of prochiral alkenes and ketones in the last few decades,^[1] limited progress has been made in the asymmetric hydrogenation of imines.^[2] A number of efficient asymmetric catalysts for the reduction of alkenes and ketones are ineffective for the hydrogenation of related imine compounds. Since chiral amines are important functionalities in many biologically active molecules, the development of practical methods for the hydrogenation of imines is extremely important in organic synthesis.

Among some notable achievements in the field, Buchwald and co-workers^[3] have developed a chiral titanocene catalyst for the reduction of imines, and the system is highly effective for the hydrogenation of cyclic imines. A number of group VIII transition metal catalysts that bear chiral chelating phosphanes have been explored for the hydrogenation of imines, and these systems generally have a very limited substrate scope.^[4, 5] The highly enantioselective hydrogenation of *N*-(phenylethylidene)aniline (89% *ee*) and related imines by using an Ir–phosphaneoxazoline complex as the catalyst have been reported by Pfaltz and co-workers.^[6] A significant industrial process that uses the Ir-catalyzed asymmetric hydrogenation of an *N*-arylimine (10⁶ turnovers and 80% *ee*) has been developed.^[7] Despite these impressive advances, the lack of highly enantioselective and reactive catalysts with broad substrate scopes for the hydrogenation of imines is still a major problem in asymmetric catalysis. Herein we report a highly enantioselective hydrogenation of *N*-arylimines catalyzed by Ir complexes with a novel chiral ferrocene phosphane group and an important additive effect for the Ir-catalyzed asymmetric hydrogenation of imines.

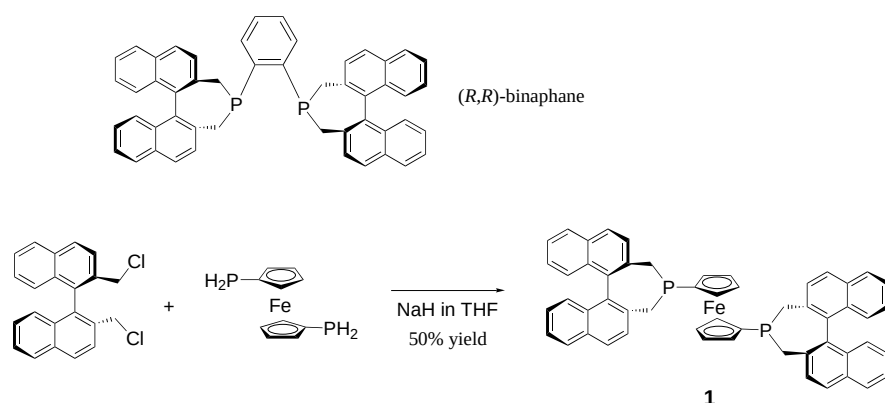
Recently we made a chiral phosphane ligand, binaphane, and used it for the Rh-catalyzed highly enantioselective hydrogenation of enamides.^[8] However, Rh and Ir complexes of chiral binaphane are not effective for the hydrogenation of imines. To alter the steric and electronic properties of the binaphane ligand, we envisioned that the introduction of a ferrocene backbone would be helpful. The new air-stable, chiral 1,1'-bisphosphanoferrrocene (**1**; abbreviated as f-binaphane) was then prepared by using a similar route used in the synthesis of binaphane (Scheme 1).

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Scheme 1. (R,R)-Binaphthane and (R,R)-f-binaphane (**1**).

Prior to our investigation, a number of effective chiral ligands with a ferrocene backbone were prepared and used for many asymmetric catalytic reactions.^[9, 10] Based on these studies, we reason that there are many advantages of f-binaphthane over the binaphthane ligand: a) the low rotation barrier of the ferrocene backbone in f-binaphthane offers flexibility, b) the bite angle of P-M-P is larger with the f-binaphthane ligand than with the binaphthane, c) the f-binaphthane ligand is more electron donating than the binaphthane ligand. We proposed that a strong electron back-donating ability from an Ir complex to an imine substrate is increased by the electron-donating f-binaphthane ligand with a large P-Ir-P bite angle. The flexible ferrocene backbone of the f-binaphthane can facilitate the binding of sterically demanding imines to the Ir center.

During the last two decades, several Ir-phosphane complexes were found to be active towards the hydrogenation of *N*-arylimines.^[4, 6, 11] An advantage of many of these substrates is that the *E* isomers of the imines can be formed exclusively. If the *N*-aryl group can be removed at the end of reaction, it represents an attractive method for making chiral amines. The asymmetric hydrogenation of *N*-(1-phenylethylidene)aniline (**2a**) by using the Ir-f-binaphthane complex as the catalyst showed promising results (Table 1, entry 1, 84 % *ee*). Optimal

conditions were found when **2a** was used as the substrate by screening catalyst precursors, solvents, and hydrogen pressures. The enantioselectivity with a neutral precursor [Ir(cod)Cl]₂ (cod = cycloocta-1,5-diene) was higher than that achieved with a cationic precursor [Ir(cod)₂]PF₆. The weak coordinating solvent CH₂Cl₂ was more desirable than other solvents such as THF, toluene, and methanol. A change in hydrogen pressure has no clear effect on the enantioselectivity, but the conversion was increased under high H₂ pressure. Interestingly, high enantioselectivity (> 99 % *ee*)

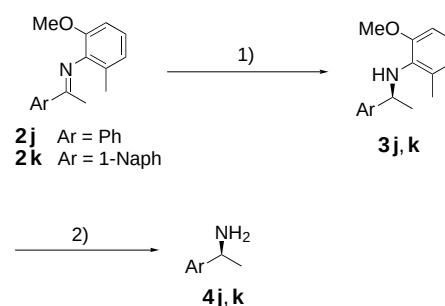
was achieved when a new substrate **2b** with a 2,6-dimethyl-*N*-phenyl group was employed (Table 1, entry 2). With the sterically hindered 2,6-dimethyl-*N*-phenyl group, several *N*-arylimines were hydrogenated with high enantioselectivities (Table 1, entries 3 and 4). When the aryl group at the carbon terminal of the C=N bond was replaced with an alkyl group, low enantioselectivities and reactivities were observed (Table 1, entries 5–7). Several other *N*-arylimines with a substituent at the *ortho* or *para* positions were hydrogenated with high conversions, albeit with low enantioselectivities (Table 1, entries 8 and 9).

Although high enantioselectivities were achieved in the asymmetric hydrogenation of **2b**, the removal of the 2,6-dimethylphenyl group in the end product was difficult. Since the *ortho*-methoxy-substituted *N*-phenyl group can be cleaved with CAN (cerium ammonium nitrate), we selected *N*-arylimines with a 2-methyl-6-methoxyphenyl group as the preferred substrate for achieving high enantioselectivities and reactivities. Two substrates (**2j** and **2k**) were reduced smoothly and with high enantioselectivities to afford amines (Scheme 2). Based on this strategy, an efficient method of synthesizing chiral primary amines from *N*-arylimines has been realized.

Table 1. Enantioselective hydrogenation of acyclic imines catalyzed by an Ir-f-binaphthane complex.^[a]

Entry	Substrate	R	Ar'	<i>t</i> [h]	Conversion [%]	<i>ee</i> [%] ^[b]
1	2a	Ph	Ph	40	100	84
2	2b	Ph	2,6-dimethyl-C ₆ H ₃	44	77	> 99
3	2c	4-MeO-C ₆ H ₄	2,6-dimethyl-C ₆ H ₃	44	77	98
4	2d	4-CF ₃ -C ₆ H ₄	2,6-dimethyl-C ₆ H ₃	44	80	99
5	2e	<i>t</i> Bu	2,6-dimethyl-C ₆ H ₃	44	15	8
6	2f	<i>i</i> Pr	2,6-dimethyl-C ₆ H ₃	44	29	23
7	2g	Cy	2,6-dimethyl-C ₆ H ₃	44	24	31
8	2h	Ph	4-MeO-C ₆ H ₄	12	100	77
9	2i	Ph	2-MeO-C ₆ H ₄	14	100	81

[a] See Experimental Section for details. [b] Absolute configurations were not determined.



Scheme 2. Synthesis of chiral amines. Reagents and conditions: 1) Ir-f-binaphthane (1 mol %), CH₂Cl₂, RT, H₂ (1000 psi); 2) CAN, MeOH, H₂O; (S)-**4j** (72 %, 98 % *ee*), (S)-**4k** (75 %, 96 % *ee*).

The study of additive effects is important to achieve high reactivities and enantioselectivities in asymmetric catalysis. Inspired by the work of the Novartis group,^[7] we investigated the additive effect in the asymmetric hydrogenation of acyclic imines (Table 2). Interestingly, different *N*-arylimines showed

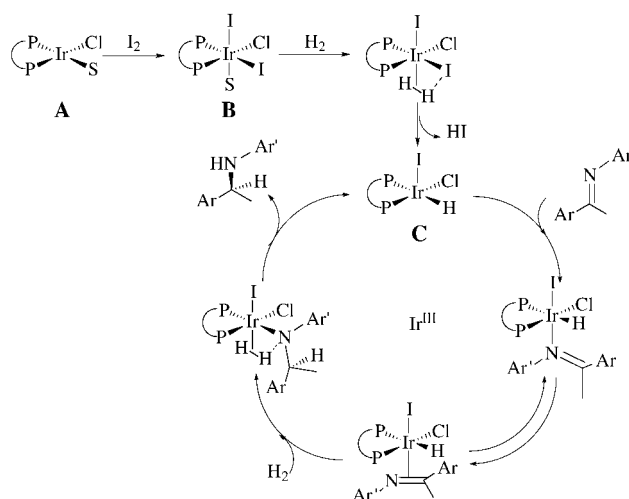
Table 2. Additive effect in Ir–f-binaphane-catalyzed hydrogenation of imines.^[a]

Entry	Substrate	Additive	T [°C]	t [h]	Conversion [%]	ee [%] ^[b]
1	2a	none	RT	40	100	84
2	2a	I ₂ 10 %	RT	24	100	89
3	2a	I ₂ 10 %	–5 °C	24	100	94
4	2h	none	RT	24	100	77
5	2h	I ₂ 10 %	RT	24	100	94
6	2h	I ₂ 10 %	–5 °C	24	100	95
7	2b	none	RT	24	64	98
8	2b	I ₂ 10 %	RT	24	8	69

[a] See Experimental Section for details. [b] Absolute configurations were not determined.

different behaviors when I₂ was used as an additive. For *N*-(1-phenylethylidene)aniline (**2a**), the enantioselectivity increased from 84 % *ee* (Table 2, entry 1) to 89 % *ee* (Table 2, entry 2) when I₂ (10 mol %) was introduced as an additive, and an *ee* value of 94 % was obtained at a lower temperature (Table 2, entry 3; –5 °C). However, other common additives such as phthalimide, tetrabutylammonium iodide, and benzylamine have no effect on the enantioselectivity of the hydrogenation reaction of imines. A significant change in the enantioselectivity was observed in the hydrogenation of **2h** in the presence of I₂ (Table 2, entries 4 and 5; 77 to 94 % *ee*). Since *p*-methoxyaniline is an inexpensive starting material for making *N*-*p*-methoxyphenyl-substituted aryl **2h**, and this group can be removed from the product by oxidation with CAN, the high yielding and enantioselective hydrogenation of **2h** is potentially a practical way of making chiral amines. Interestingly, the enantioselectivity of the hydrogenation reaction of a sterically hindered imine **2b** decreased from 98 to 69 % *ee* and the conversion changed from 64 to 8 % (Table 2, entries 7 and 8). Based on these observations, we conclude that the catalytic process with I₂ as an additive must differ from that without the additive.

Although the oxidative addition of H₂ to Ir^I to give Ir^{III} is a normal process for the hydrogenation of imines, Osborn and co-workers^[11b] demonstrated that the Ir complex $[\{\text{Ir}^{\text{III}}(\text{P}-\text{P})\text{-HI}_2\}_2]$ facilitates the hydrogenation of *N*-arylimines, and that the Ir^{III} species remains in the same oxidation state in the catalytic cycle. They proposed that the heterolytic cleavage of H₂ by the Ir–N species can generate an amine and an Ir^{III}–H species. In the Novartis process for the synthesis of metolachlor, the addition of I₂ is critical in the hydrogenation of the imine. Through their mechanistic studies, investigators believe that the addition of I₂ leads to an Ir^{III}-catalyzed pathway.^[7, 12] Although the situation is not exactly the same with our system, we speculate that the Ir^{III}-catalyzed hydrogenation of imines in the presence of I₂ as the additive is also possible (Scheme 3). The oxidative addition of I₂ to the Ir^I precursor **A** generates the Ir^{III} complex **B**. The heterolytic cleavage of H₂ can occur in the presence of an amine to form the Ir^{III}–H species **C**. An imine substrate can coordinate with **C** in a η^1 or η^2 fashion. Migratory insertion of the η^2 -imine into the Ir^{III}–H bond forms an Ir^{III}–amide complex. The heterolytic cleavage of H₂ by the Ir^{III}–amide intermediate gives an amine and regenerates the Ir^{III}–H species **C**.^[13]



Scheme 3. Proposed mechanism for the Ir–f-binaphane-catalyzed hydrogenation of imines. S = substrate or solvent.

In conclusion, a novel chiral ligand (f-binaphane) has been developed and high enantioselectivities (up to 99 % *ee*) were achieved in the asymmetric hydrogenation of some acyclic *N*-arylimines. A mechanistic rationale was provided for the hydrogenation of imines with I₂ as the additive. Future work will focus on exploring the substrate scope of the imine hydrogenation with the Ir–f-binaphane complex and on the investigation of reaction mechanism.

Experimental Section

1: 1,1'-Bis(phosphano)ferrocene (1.32 g, 5.28 mmol) was added to a solution of (*R*)-2,2'-dichloromethyl-1,1'-binaphthyl^[8] (3.71 g, 10.6 mmol) and NaH (2 g, 83 mmol) in THF (125 mL) at –78 °C under nitrogen. The mixture was stirred at room temperature for 24 h, and then heated at reflux for 48 h. After the reaction was completed (monitored by ³¹P NMR spectroscopy), the solvent was removed under vacuum, and the residue was washed with CH₂Cl₂ (3 × 25 mL). The organic phase was filtered through a silica-gel plug to give the crude product. Further purification by recrystallization from CH₂Cl₂/hexanes afforded **1** as an air-stable yellow solid (2.2 g, 50 %). $[\alpha]_D^{25} = 160$ (*c* = 0.2, CHCl₃); ¹H NMR (CD₂Cl₂, 360 MHz): δ = 8.07–7.99 (6H, m; Ar-H), 7.87–7.85 (2H, d, *J* = 8.34 Hz), 7.80–7.78 (2H, d, *J* = 8.31 Hz), 7.53–7.50 (4H, m; Ar-H), 7.31–7.28 (8H, m; Ar-H), 7.07–7.04 (2H, d, *J* = 8.37 Hz), 4.49 (2H, s; Cp-H), 4.45 (2H, s; Cp-H), 4.26 (2H, s; Cp-H), 3.56 (2H, s; Cp-H), 3.10–3.06 (2H, m; ArCH₂), 2.82–2.72 (4H, m; ArCH₂), 2.63–2.59 (2H, m; ArCH₂); ¹³C NMR (CD₂Cl₂, 90 MHz): δ = 135.79, 135.18, 134.28, 133.57, 133.21, 133.05, 132.96, 132.67, 129.28, 129.00, 128.94, 128.10, 128.04, 127.29, 127.19, 126.58, 126.45, 125.73, 125.46, 76.22 (d, *J*_{CP} = 21.92 Hz), 75.62 (d, *J*_{CP} = 32.00 Hz), 72.43, 71.63, 71.14, 34.60 (d, *J*_{CP} = 20.90 Hz), 31.36 (d, *J*_{CP} = 11.74 Hz); ³¹P NMR (CD₂Cl₂): δ = –1.26; MS: *m/z*: 807 [*M*+H⁺].

Hydrogenation of imines: The Ir–f-binaphane complex was made in situ by mixing $[\{\text{Ir}(\text{cod})\text{Cl}\}_2]$ (6.7 mg, 0.01 mmol) and **1** (17.7 mg, 0.022 mmol) in CH₂Cl₂ (20 mL). The mixture was stirred for 30 min and then part of this solution (5 mL) was transferred into a 10-mL vial with an imine substrate (0.5 mmol). The hydrogenation was performed at room temperature under 1000 psi of hydrogen. After the reaction was finished, hydrogen was released carefully, and the reaction mixture was passed through a silica-gel plug with CH₂Cl₂ as eluent. Without further purification of the product, the enantiomeric excess was measured by using chiral GC with a Supelco chiral select 1000 column.

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Porous Polymer Films and Honeycomb Structures Made by the Self-Organization of Well-Defined Macromolecular Structures Created by Living Radical Polymerization Techniques**

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Anthony G. Fane, and Vicki Chen

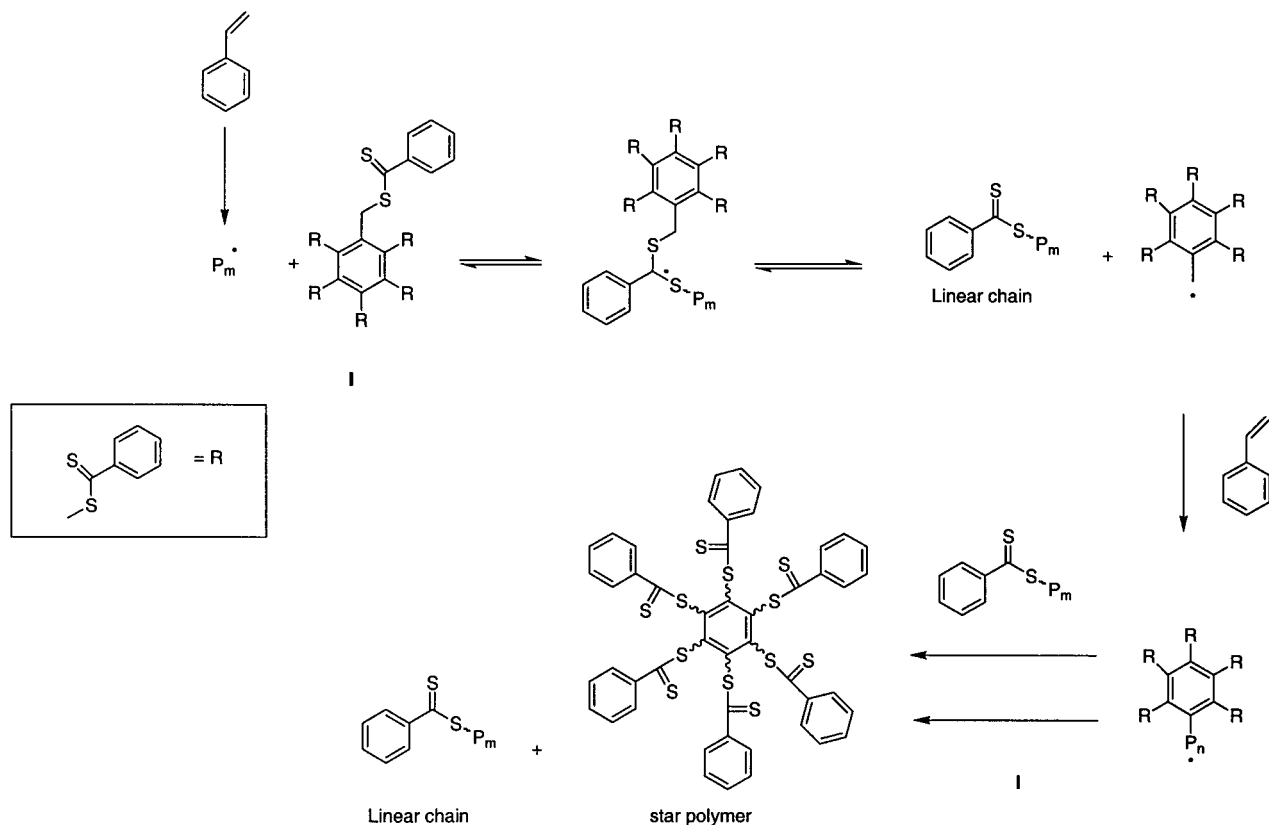
Macroporous polymers have become materials of high interest in recent years, because of their potential applications in diverse areas from membranes to medical devices.^[1] Star and block polymers are known to template around water droplets to form isoporous arrays.^[2–5] However, the utilization of this discovery has been severely restricted because the synthetic route used, anionic polymerization, is difficult and limited to a small number of (generally nonfunctional) monomers. A number of other synthetic routes to star polymers have become available in recent years,^[6] and the development of living radical polymerization provides a great opportunity to significantly broaden the range of functional materials. We have adopted both metal-mediated radical polymerization and reversible addition–fragmentation transfer (RAFT) polymerization to synthesize star polymers for application in isoporous film and honeycomb-structure production. The porous structures are created from casting solutions of star polymers in an organic solvent onto a glass substrate under a humid atmosphere as described by Francois and co-workers.^[4, 7, 8]

The star polymers were synthesized either by metal-mediated polymerization using a copper (atom-transfer radical polymerization, ATRP) or an iron catalyst system or by RAFT polymerization. Both types of polymerization require a multifunctional initiator, from which arm growth occurs. The preparation of 6-arm polystyrene stars by the RAFT process utilized hexakis(thiobenzoylthiomethyl) benzene (**1**) as an initiator.^[9] The polymerization process is shown in Scheme 1.^[10] The pseudo first-order reaction kinetics and molecular-weight development were both found to be consistent with a living radical polymerization process. This RAFT method yielded narrow polydispersity polystyrene stars with molecular weights of up to 400 000, and six arms, each with a thioester end group. One complication in this process is the parallel synthesis of a linear chain along with the star structure (Scheme 1).

ATRP/metal-mediated polymerization was also utilized to synthesize stars. Two different approaches were taken, both based on the application of sugar initiators. The first approach we took was based on the work of Haddleton and co-workers

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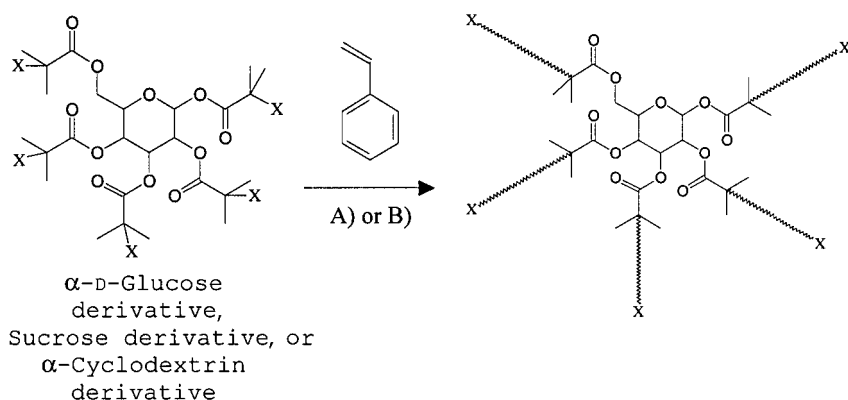


Scheme 1. Star production using the RAFT process. The star synthesis is accompanied by a parallel linear arm synthesis. This is not necessarily a disadvantage in the production of porous films, as some linear-polymer inclusion is often found to be beneficial (see main text); P_m = linear polymer; P_n = single polymer star arm.

who developed a bromide containing initiator based on α -D-glucose^[11] for the polymerization of styrene or methyl methacrylate in the presence of CuBr and *N*-propyl-2-pyridylmethanime (Scheme 2). The second approach was to utilize iron-mediated free-radical polymerization as pioneered by Sawamoto and co-workers^[12–14] together with a multifunctional iodine-containing initiator based on sugar cores. Utilizing glucose, sucrose, and α -cyclodextrin in this way we prepared star polymers with 5, 8, and 18 arms, respectively,^[15] (Scheme 2). In both approaches the kinetics and molecular-weight development were consistent with a living radical mechanism. The copper-based ATRP system

proved more versatile and robust. However, we found the porous-film formation was very sensitive to residual copper/ligand and extensive purification was required to ensure reproducible film formation. In contrast, the iron-mediated system, whilst requiring more rigorous reaction conditions (it is also rather slow), necessitated much less purification of the star polymer product. The stars produced by an ATRP route have the advantage of facile end-group functionalization allowing us to produce functional porous films.

The preparation of the honeycomb-structured films seems to require a star polymer which is largely free of impurities (such as residual catalyst) to avoid disturbance of the film formation. The films were cast on a glass slide from a solution of the star polymer in carbon disulfide ($10\text{--}20\text{ mg L}^{-1}$) under humid conditions. The type of morphology we attained from a film cast from a polystyrene star polymer made by iron-catalyzed ATRP is shown in Figure 1. The five-arm polystyrene star had a molecular weight of 28000 and a polydispersity index (PDI) of 1.2. The film contains pores organized in a hexagonal array with a monodisperse pore size of ~ 1 micron. The surface of the porous film can easily be removed with adhesive tape to expose the honeycomb structure underneath (Figure 2). The pores are thought to originate by templating of the polymer stars



Scheme 2. Typical star structures made by ATRP techniques. A) $X = \text{Br}$, CuBr, *N*-propyl-2-pyridylmethanime, bulk, 90°C . B) $X = \text{I}$, $\text{Ti}(\text{O}-i\text{Pr})_4$, $[\text{CpFeI}(\text{CO})_2]$, toluene, 80°C .

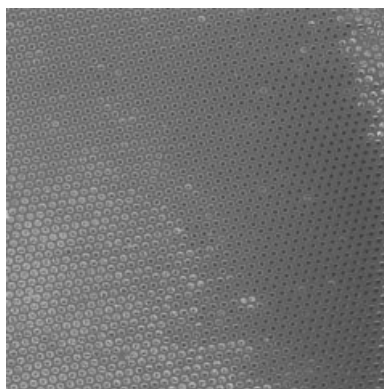


Figure 1. Scanning electron microscope (SEM) image of a porous film made from polystyrene stars. The pore size is approximately 1 micron in diameter.

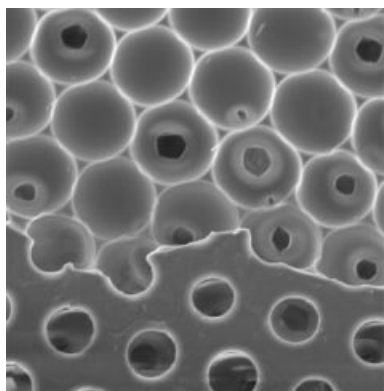


Figure 2. SEM image of the honeycomb structure of the porous film. The structure is revealed when the top layer is removed with adhesive tape.

around condensed water droplets. The precipitating star polymers stabilize the water droplets by forming a solid envelope allowing the self-organization of the stable water droplets in a hexagonal close packed array.^[7, 8] It is clear that the molecular dimensions of the stars can directly affect the pore structure (Figure 3). The pore diameter can be altered in the range from 0.4–3 microns. The pore size is not only

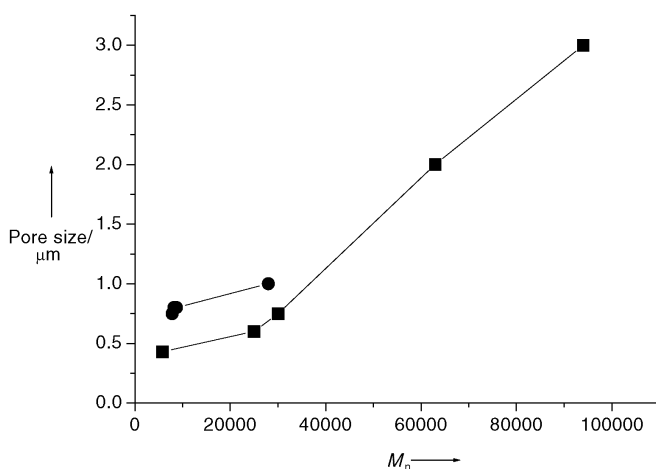


Figure 3. A plot showing the relationship between pore size and the molecular weight of the star arms. The ATRP stars have five arms, and the RAFT stars have six arms; ● ATRP, ■ RAFT.

controlled by the molecular weight of the polymer, but also by the number of arms and the nature of the end groups. This is a remarkable result as it clearly indicates that structure on the micron scale can be controlled by very small changes at the molecular level. The data in Figure 3 indicate that the polystyrene stars made by the RAFT process, with six arms and a thioester end group, form smaller pores than the five-arm polystyrene star of the same molecular weight made with iron-catalyst ATRP (iodine end groups). Confirmation that this observation cannot be solely attributed to the different arm configuration comes from a direct comparison of stars (10000 molecular weight, five arms) made by ATRP utilizing iodine and bromine initiators. Changing the end group from iodide to bromide decreased the film pore size from 750 nm to 650 nm. We also altered the bromine end groups by post-polymerization reaction with sodium pentadecafluoro-1-octanolate or sodium salicylate. The film pore diameters were again found to change to 450 nm ($-\text{OC}_8\text{H}_2\text{F}_{15}$) and 550 nm ($-\text{salicyl}$). The hexagonal honeycomb structure was not disrupted by these structural modifications to the star molecules. We have also observed that we can attain control over the porous morphology by controlling the number of arms grown from the star core, with an 18-arm star (molecular weight 10000, PDI 1.2) produced even smaller diameter pores of 250 nm. One major advantage of living radical polymerization is that it is very simple to synthesize block structures and thus broaden porous-film production to a whole range of functional substrates. To exemplify this we synthesized star-block copolymers of styrene and ethyl acrylate. We prepared stars starting with a polystyrene block, with the subsequent addition of an ethyl acrylate block (and vice versa). The porous-film structure cast from these star-block molecules is shown in Figure 4.

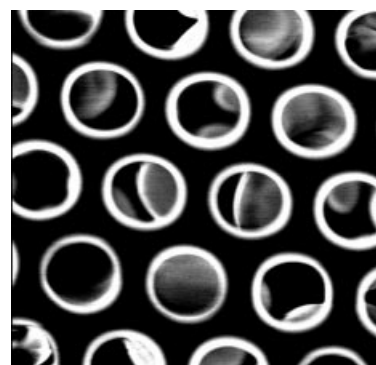


Figure 4. SEM image of a porous film cast from a polystyrene-ethyl acrylate star block. The molecular weight of the star block was measured to be 30000, with a polydispersity of 1.17. The arms consist of (approximately) a block of 50 styrene units followed by 20 ethyl acrylate units.

In our work we also found that it is possible to blend the star polymer with linear polymers, cast down the blend, and maintain highly structured films. We found that up to 30 wt % of narrow dispersity linear polystyrene can be added to the casting solution without any disturbance of the hexagonal orientation. If too much linear polymer is added then the organization of the pores is disrupted, ultimately leading to the disappearance of the pore structure altogether (Figure 5).

The blending of star polymers with linear chains is in fact beneficial, we found that the addition of 20 wt % of linear polystyrene added to a polystyrene star leads to less

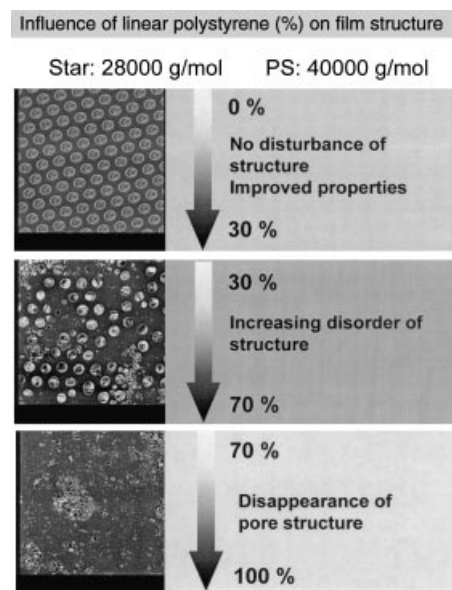


Figure 5. SEM micrographs of porous films made by adding linear polystyrene (PS) to a polystyrene star (five arm).

brittle films and fewer defects during the film formation. The precise relationship between the star and linear polymers and the influence on the porous-film formation is highly dependent on the molecular weight and the chemical composition of the components. The blending process can be extended to

mixtures of different polymers as a method of introducing functionality to the films. All of the polymer architectures we utilized and the nature of the resulting cast films are detailed in Table 1.

In summary, we have shown, that living radical polymerization techniques can be easily applied to the preparation of different star polymers. This is a facile approach to highly ordered porous materials with a monodisperse pore size, honeycomb morphology. We have demonstrated strong correlations between arm length, arm number, and end-group functionality with pore diameter. We have also shown that polymer blends can be used to improve the integrity of the films and as a way of introducing functionality into the materials.

Experimental Section

Initiators were prepared according to literature procedures for RAFT^[9, 10] and ATRP^[11, 13, 15]

ATRP: A schlenk flask was charged with styrene (10 mL; with inhibitor removed), penta-*O*-isobutyryl bromide- α -D-glucose (173 mg), *N*-propyl-2-pyridylmethanone (291 mg), and CuBr (135 mg). The mixture was immediately degassed by three freeze–pump–thaw cycles and then purged with N₂ under a nitrogen atmosphere. The mixture was stirred continuously and kept in an isothermal oil bath at 90 °C. After 8 h, the polymerization reached a conversion of 50 %. The reaction mixture was passed through a short alumina column and then precipitated in methanol or hexane.

A typical iron-catalyzed ATRP experiment was carried out with penta-*O*-isobutyryl iodo- α -D-glucose (100 mg), [CpFeI(CO)₂] (26.1 mg), titanium isopropoxide (245 mg), styrene (6 mL), and toluene (3 mL). A typical conversion after 50 h at 80 °C was 20 %. The workup procedure was as described above. The star-block copolymers were synthesized by the same method. After the required reaction time, the mixture was cooled and excess styrene was distilled off under vacuum. The styrene star polymer was

Table 1. A summary of synthetic methods and physical properties.

Synthetic approach	Star structure/synthetic method	M_n	End group	Number of arms	Concentration of casting solution [g L ⁻¹]	Pore diameter [μm]
metal-mediated/Fe styrene	ref. [15]	4000	I	5	10	no pores
80 or 100 °C in toluene	all based on a glucose core	7800	I	5	10	0.75
		8200	I	5	10	0.8
		9000	I	5	10	0.8
		28000	I	5	10	1.0
ATRP/Cu styrene	glucose core	8000	Br	5	10	0.65
90 °C in bulk	cyclodextrin core	10000	Br	18	10	0.25
	cyclodextrin core	15000	Br	18	10	0.34
ATRP/Cu styrene	end-group modification of polymer with $M_n = 8000$ and Br end group					
90 °C in bulk	reaction with HOC ₈ H ₂ F ₁₅	8500	-OC ₈ H ₂ F ₁₅	5	10	0.45
	reaction Na-salicylate	8500	-salicylate	5	10	0.53
RAFT styrene star	all based on a hexakis(thiobenzoyl thiomethyl)benzene core	6000	-S(C=S)Ph	6	10	0.43
styrene		25000	-S(C=S)Ph	6	10	0.6
100 °C in bulk		30000	-S(C=S)Ph	6	10	0.75
		68000	-S(C=S)Ph	6	10	2
		94000	-S(C=S)Ph	6	10	3
ATRP/Cu styrene/ethyl acrylate	glucose core	23000	Br	5	25	1.0
star-block copolymers	50 styrene/5 ethyl acrylate units					
styrene star	50 styrene/12 ethyl acrylate units	27000	Br	5	20	1.4
90 °C in bulk, isolated then diluted with toluene and dissolved in ethyl acrylate	50 styrene/20 ethyl acrylate units	30000	Br	5	23	1.0

dissolved in toluene (5 mL) and ethyl acrylate (2.5 mL, inhibitor-free), followed by three freeze–pump–thaw cycles, prior to further chain-extension polymerization.

RAFT: RAFT polymerization was carried out in glass bottles. Hexakis-(thiobenzoylthiomethyl)benzene (50 mg) and styrene (10 mL) were mixed together and degassed by purging with nitrogen. The bottles were sealed and placed in an oil bath at 100 °C. In typical reaction conditions a conversion of 50% was attained after 75 h. The polymer was isolated by evaporation of the styrene.

Analyses: The stars were characterized by size exclusion chromatography and NMR spectroscopy. The porous films were analyzed by SEM. The molecular weights quoted in the paper were determined against linear polystyrene standards and are provided as an approximate guide to the true molecular weight.

Film casting: The films were prepared^[4] from carbon disulfide solutions with a polymer concentration of 10 g L⁻¹ (unless specified differently in Table 1). The solution was cast on a glass support and dried with a humid air (or nitrogen) flow at 22 °C. The casting process was carried out in a box at a humidity of 85%. The saturation of the air flow was achieved by bubbling the gas through a water reservoir. The air flow was measured with a flow meter to ensure reproducibility.

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Rapid Access to Diverse Arrays of Chiral 3,4-Diazaphospholanes**

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Chiral phosphine ligands are central to many developments in transition metal catalyzed enantioselective transformations.^[1] Recent demonstrations of high enantioselectivity for a wide range of hydrogenation reactions with Rh complexes of DuPHOS,^[2] PennPHOS,^[3] RoPHOS,^[4] BASPHOS,^[5] CnrPHOS,^[6] and related ligands highlight the unusual efficacy of rigid phosphacycles.^[7] Other emerging trends in ligand design include the use of heterofunctional bidentate ligands,^[8] implementation of attractive “secondary interactions” between complementary functional groups of the substrate and the catalyst periphery,^[9] and the construction of large libraries of chiral phosphines for high-throughput catalyst screening.^[10] All these strategies for the discovery of enantioselective catalysts can be accelerated by new synthetic methods for constructing cyclic phosphanes. We report a versatile synthetic route to a large number of new chiral diazaphospholanes that uses aldehydes, hydrazines, and primary phosphines as starting materials.

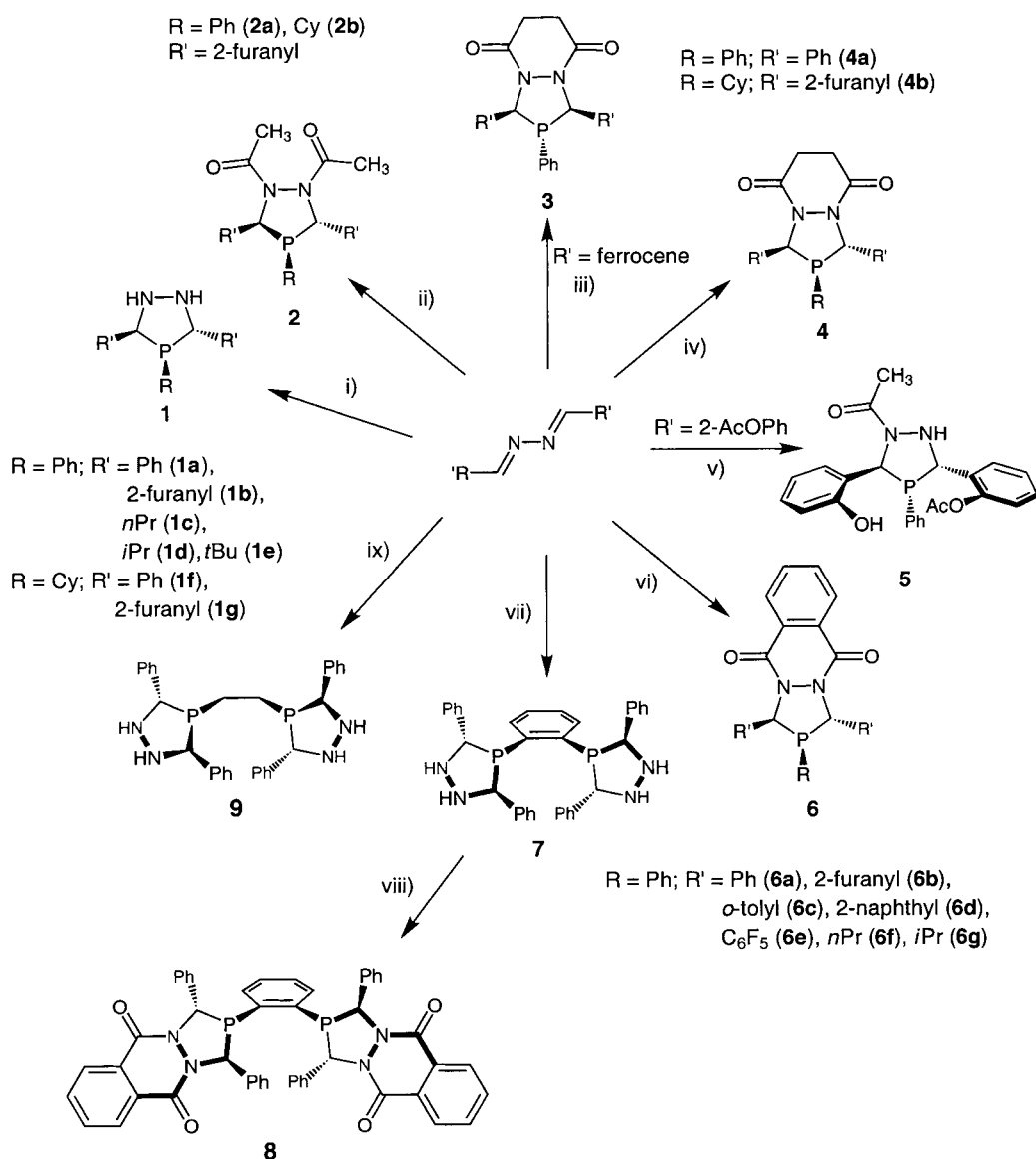
By analogy with P–C bond formation by means of the classic Mannich condensation^[11] reaction, we reasoned that the condensation of primary phosphines with azines (R–CH=N–N=CH–R), prepared by the treatment of hydrazine with two equivalents of the corresponding aldehyde, would yield diazaphospholanes such as **1** (Scheme 1). This procedure yields a variety of 3,4-diazaphospholanes in good yields (25–95%) with *rac* selectivity under mild reaction conditions. All compounds were thoroughly characterized by using ¹H, ¹³C, and ³¹P NMR spectroscopy, elemental analysis, and in most cases, X-ray crystallography (Figure 1). To the best of our knowledge, the only previous report of 3,4-diazaphospholanes involved the stepwise condensation of formaldehyde and phenylphosphane, followed by ring closure with *N,N'*-dialkylhydrazines.^[12]

The condensation of azines and primary phosphines with one equivalent of dry HCl as acid promoter renders simple 3,4-diazaphospholanes (**1**, **7**, **9**) upon workup. Acid chlorides function as both promoters and N-functionalization reagents to yield *N,N'*-dicarboxy-3,4-diazaphospholanes (**2**, **3**, **4**, **6**) directly in a one-step synthesis. The reaction of phenylphosphane with the azine derived from acetyl salicylaldehyde yields **5**, in which one of the salicyl acetyl groups is transferred to the hydrazine moiety. As exemplified by the transforma-

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Scheme 1. i) HCl, RPH₂; ii) CH₃COCl, RPH₂; iii) succinyl chloride, PhPH₂; iv) succinyl chloride, RPH₂; v) HCl, PhPH₂; vi) phthaloyl chloride, PhPH₂; vii) HCl, 1,2-(PH₂)₂C₆H₄; viii) phthaloyl chloride in THF; ix) HCl, PH₂CH₂CH₂PH₂. All the reactions were worked up with aqueous K₂CO₃ (10%).

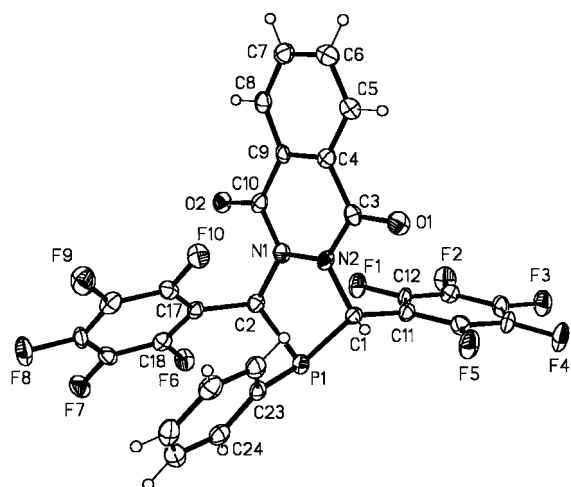


Figure 1. The X-ray crystal structure of *rac*-**6e**. The displacement ellipsoids are drawn at the 50% probability level.

tion of **7** into **8**, 3,4-diazaphospholanes and acid chlorides cleanly react to give *N,N'*-dicarboxy-3,4-diazaphospholanes. The *N,N'*-dicarboxy-3,4-diazaphospholanes exhibit substantially higher thermal and chemical stability than the simple 3,4-diazaphospholanes. For example, **3** persists for hours in refluxing toluene, whereas the ferrocenyl derivative of **1** decomposes upon warming at 80° for three hours; presumably functionalization at N inhibits the retro-addition of the phosphine to the azine.

Acid-promoted additions of primary phosphines to azines are generally *rac* selective, but sensitive to the substituents at the phosphorus center or at the 2- and 5-positions of the heterocyclic ring. For example, whereas phenylphosphane affords *rac/ meso* ratios (0.6–30:1) that are dependent on the substituents at the 2- and 5-positions, only the *rac* isomers are observed for cyclohexylphosphane. Azines derived from bulky, electron-withdrawing substituents such as pentafluorophenyl and ferrocenyl result in low *rac/ meso* ratios (**6e** 2:1; **30.6**:1). For most

diazaphospholanes, a simple recrystallization at room temperature effects the separation of diastereomers (e.g. *rac/ meso* 30:1 for **1a**).

The racemic diazaphospholanes **1a**, **1e**, and **9** are resolved by N-functionalization with di-*O*-methyl-L-tartaric acid dichloride to form bicyclic diastereomers, followed by chromatographic separation on silica gel. The exploration of other resolution protocols is an active line of our research.

Crystallographic analysis reveals interesting structural details of the 3,4-diazaphospholanes. The X-ray crystal structure determination of *rac*-**8** (Figure 2) exemplifies the approximate C₂ symmetry of bis(diazaphospholanes) (**7–9**). Clearly there is a remarkable communication of stereochemical information between the two phospholane rings during the condensation. 3,4-Diazaphospholanes are bulky ligands; for example, the cone angle^[13] of **1a** (172°) is comparable to that of P(Cy)₃ (170°) (Cy = cyclohexyl).

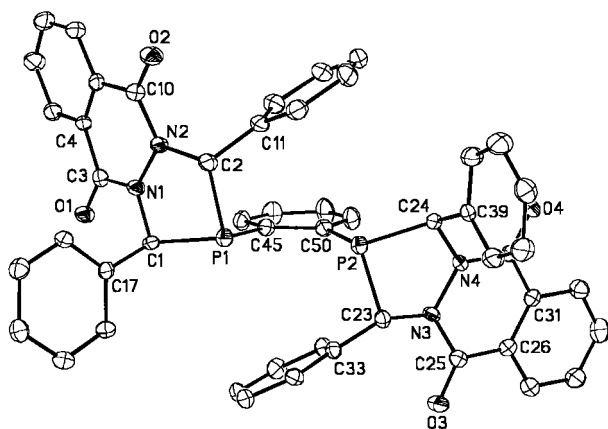


Figure 2. The X-ray crystal structure of *rac*-**8**. The ORTEP diagram is drawn at the 30% probability level. Solvent molecules and hydrogen atoms have been omitted for clarity.

Preliminary data indicate that *N,N'*-dicarboxy-3,4-diazaphospholanes ligate to transition metals in a manner similar to other phospholanes. For example, excess **6a** and **6b** react with $1/2[\text{Rh}(\text{nbd})\text{Cl}]_2$ (nbd = norbornadiene) to afford adducts with the formula $[(\text{6})\text{Rh}(\text{nbd})\text{Cl}]$ in quantitative yield. Similarly, the reaction of the *N,N'*-phthaloyl derivative of **9** with $[(\text{cod})\text{Pt}(\text{CH}_3)_2]$ (cod = cyclooctadiene) in solution yields $[(\text{9-phthaloyl})\text{Pt}(\text{CH}_3)_2]$ in quantitative yield, as determined by NMR spectroscopic and X-ray crystallographic analysis. Preliminary studies revealed that Rh complexes of 3,4-diazaphospholanes are active hydrogenation catalysts.

In summary, we have report herein an unprecedented, simple synthesis of a wide variety of 3,4-diazaphospholanes based on the acid-promoted addition of primary phosphines to azines. Functionalization of the diazaphospholanes at N stabilizes and rigidifies the 3,4-diazaphospholanes. Future work concerns the application of these functionalized, bulky phosphines to reactions catalyzed by transition metal complexes.

Experimental Section

Full experimental details can be found in the Supporting Information. The crystal structures of the following compounds are available^[14]: *rac*-**1a**, *rac*-**1c**, *rac*-**1d**, *rac*-**2a**, *meso*-**3**, *rac*-**4a**, *rac*-**4b**, *rac*-**5**, *rac*-**6a**, *rac*-**6b**, *rac*-**6c**, *rac*-**6e**, *rac*-**7**, *rac*-**8**.

General Procedure: A solution of the azine derivative (4.55 mmol) in diethyl ether (20 mL) was treated with HCl (ca. 4.75 mL, 4.75 mmol, 1.0M in Et₂O solution) at 0 °C. A white solid precipitated immediately. Phenyl- or cyclohexylphosphane (4.55 mmol) was added to this suspension at 0 °C and the reaction mixture was stirred for 4 h (or overnight) at room temperature. A degassed aqueous solution of K₂CO₃ (≈ 30 mL, 10%) was added to the resultant white slurry at 0 °C. The ether layer was separated by means of a cannula, dried over MgSO₄, and filtered through a cannula to obtain a colorless solution. The diethyl ether was evaporated under vacuum to yield the corresponding diazaphospholanes.

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Acentric Extended Solids by Self Assembly of 4,4'-Bipyrzolyls**

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Design of solid-state structure and therefore functionality by using the principles of crystal engineering represents a new and growing area of chemistry which offers intriguing possibilities for the development of novel functional materials.^[1] Solids that adopt noncentrosymmetric structures are predisposed to exhibit a range of useful bulk physical properties, including second-order nonlinear optical (NLO), piezoelectric, and pyroelectric effects.^[2] Elaboration of such materials that combine acentric structure with chemical and thermal stability presents a significant challenge.^[2, 3] A particularly promising tool in the control of overall polarity is supramolecular retro-synthesis, that is, to design the molecular building blocks (tectons), their size, and conformations and thus to program at the molecular level the pathways of self-assembly processes for the formation of a certain desired inherently polar topology. For instance, diamondoid^[1, 4] and helical^[5] architectures were recently investigated and represent the most common and obvious types of acentric arrays.

Novel insights into the development of strategies for designing polar solids without the need for chiral building blocks may be offered by a number of three-connected topologies, particularly due to the lack of inversion symmetry in three-connected units. An especially attractive target in this context is a family of three-dimensional (3D) three-connected decagonal nets,^[6] three types of which are exemplified by the structures of hydrogen peroxide, ThSi₂, and B₂O₃ (Figure 1).^[7]

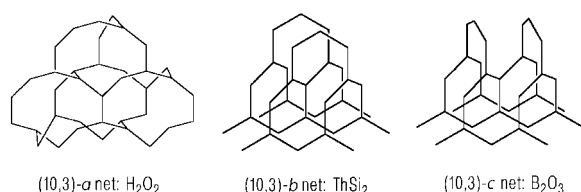
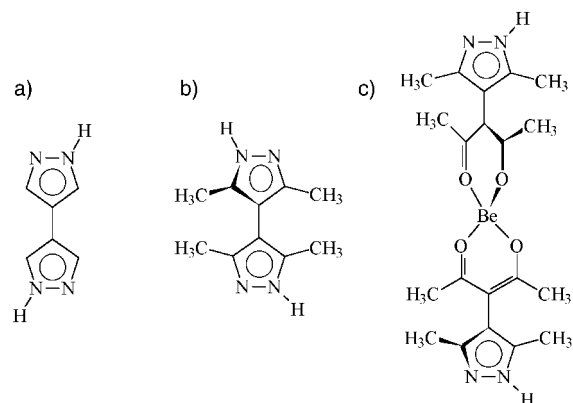


Figure 1. Three-connected decagonal nets.

The (10,3)-*b* and (10,3)-*c* topologies can be viewed as a system of zigzag polymeric chains,^[6] and they appear to be realistic models for the assembly of molecular species capable of generating two noncoplanar zigzag chains by self-complementary donor–acceptor interactions. The corresponding tecton is a tetrafunctional molecule containing two pairs of coplanar self-complementary binding sites that are rotated with respect to one another (e.g., hydrogen-bond donors and acceptors).^[8]

As a suitable molecular system for the design of (10,3) topologies we examined 4,4'-bipyrzolyls (Scheme 1). They have a set of effective self-complementary hydrogen-bond



Scheme 1. Bipyrzoly species used in the present study: a) 4,4'-bipyrzoly (4,4'-bpz (**1**)); b) 3,3',5,5'-tetramethyl-4,4'-bipyrzoly (4,4'-Me₄bpz; α -form: **2**, β -form: **3**); 3(4,4'-Me₄bpz) · H₂O · Solv: **4** (solv = acetone: **4a**, ethyl acetate: **4b**, THF: **4c**); c) bis[3-(3,5-dimethylpyrazol-4-yl)pentane-2,4-dione]beryllium (Be(acacpz)₂; Be(acacpz)₂ · 2CH₃OH: **5**).

donor (NH) and acceptor (N) sites for the formation of hydrogen bonds, and the desired orientation of these binding sites can be controlled by varying the substituents in the 3,3'- and 5,5'-positions.^[9] Tetramethyl-substituted 4,4'-bipyrzoly (4,4'-Me₄bpz, Figure 2) exists in at least two polymorphic forms,^[10] both of which are acentric, and the entities may be best described in terms of 3D nets. The points of such nets in both structures are single pyrazoly rings. Each point is three-coordinate and is linked to two of its three closest neighbors by hydrogen bonding, and to the third by the 4,4' carbon–carbon bond within the 4,4'-Me₄bpz molecule. Hence two-thirds of the links in the resulting net are N–H···N hydrogen bonds, and one-third carbon–carbon bonds. The shortest circuits of these nets include ten points, and in accordance with Wells their topological symbol is (10,3).^[6] Two of the present nets correspond to ThSi₂ (10,3)-*b* (monoclinic α -form **2**) and B₂O₃ (10,3)-*c* topologies (hexagonal β -form **3**) and differ in the angle of rotation of successive sets of zigzag chains in the structure: 79° and 60°, respectively (Scheme 2).

The characteristic parameter of the structure that determines the above-mentioned angles of rotations and is responsible for such dramatic topological changes is clearly the interplanar angle φ of the bipyrzoly molecule. These values for two polymorphs (α : 75.6°, β : 56.2°) almost exactly correspond to the observed geometry of the whole nets and satisfy the demands of their topology (90° and 60°). This solid-state manifestation of conformational flexibility of the tecton

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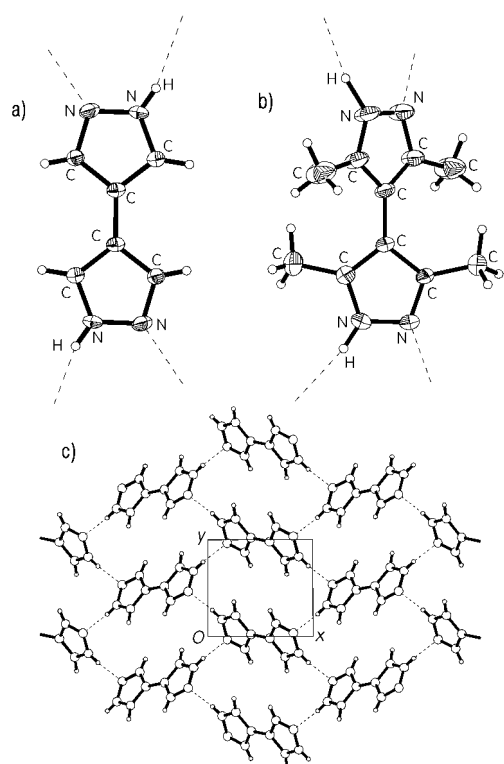
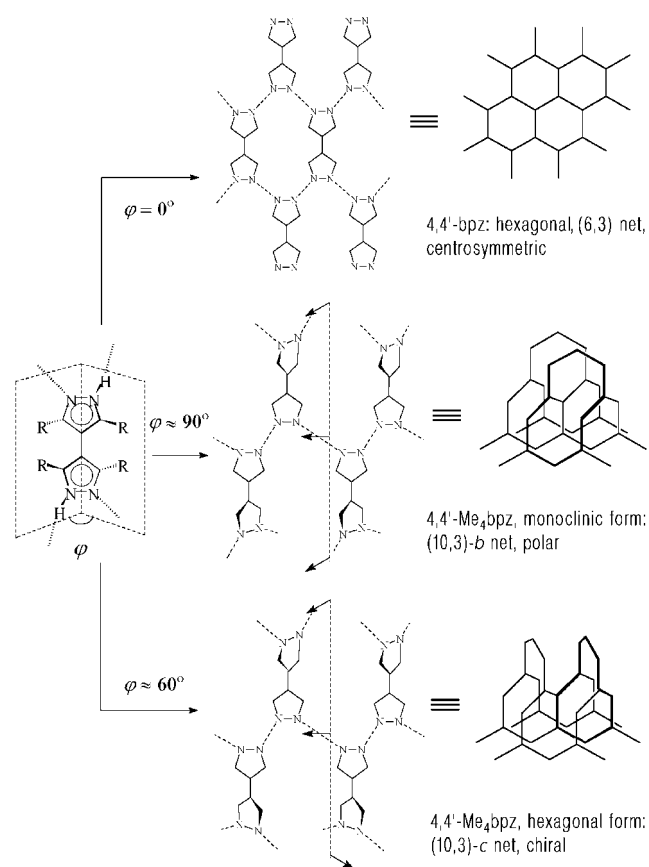


Figure 2. Molecular structures of planar tecton 4,4'-bpz (a) and angular tecton 4,4'-Me₄bpz (b); flat 2D hexagonal net by self assembly of 4,4'-bpz (c).



Scheme 2. Pathways for the self assembly of bipyrazolyls and nature of the supramolecular isomerism of 4,4'-Me₄bpz. Decagonal circuits of the resulting 3D nets are represented by bold lines.

agrees with a theoretical study:^[13] the optimal conformation of the 4,4'-Me₄bpz molecule has $\varphi = 87.5^\circ$, but in the broad range of $\varphi \approx 50-90^\circ$, the relative energies of the conformers are very similar (75° : $\Delta E = 0.92 \text{ kJ mol}^{-1}$; 60° : 3.89 ; 45° : 11.02 ; 30° : 27.17).

It seems likely that the desired topology of the 3D array, as well as the properties of the crystal in the context of inducing bulk polarity, can be designed at the molecular level by varying the angle φ of the molecular unit. Nonplanar species with corresponding interplanar angles of up to about 60° will tend to induce the chiral (10,3)-c net, while more highly substituted units and an interplanar angle near the maximum value of 90° will produce the (10,3)-b net. Related planar species with $\varphi \approx 0^\circ$ associate into a flat hexagonal net, that is, the centrosymmetric motif for self assembly of unsubstituted planar 4,4'-bpz tecton **1** (Figure 2).^[14]

Both the nets **2** and **3** are characterized by a helical motif of the aligned links. However, the (10,3)-b topology (Scheme 2) has an equal number of helices of opposite chirality, and the resulting network is achiral. In contrast, the (10,3)-c topology generates helices of the same chirality and hence is inherently chiral.^[6] The helical motif of the β -polymorph **3**, with a pitch of $26.35 \text{ \AA}$, is shown in Figure 3.

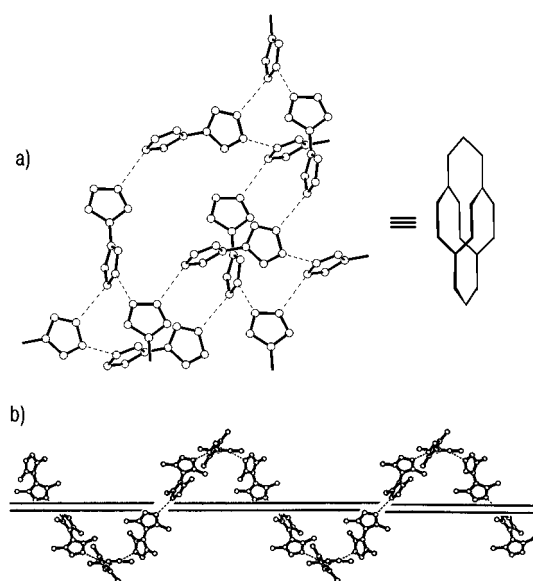


Figure 3. a) "Pseudoadamantoid" unit of the (10,3)-b net in the structure of the monoclinic polymorph and b) helicate motif in the structure of the hexagonal polymorph of 4,4'-Me₄bpz.

The 4,4'-Me₄bpz tecton also retains a tendency for helical self assembly in isomorphous compounds of composition $3(4,4'\text{-Me}_4\text{bpz}) \cdot \text{H}_2\text{O} \cdot \text{Solv } \mathbf{4}$ (Table 1).^[15] The 3D structures of these hydrogen-bonded adducts can also be clearly derived from the chiral (10,3)-c topology. In this idealized case $\frac{3}{4}$ of the net points are three-connected pyrazolyl cycles, and $\frac{1}{4}$ are water molecules to which three pyrazolyl cycles are bound. One NH group per three 4,4'-Me₄bpz molecules is not involved in the overall 3D linkage and forms a hydrogen bond with the molecule of solvent (Figure 4). This demon-

Table 1. Crystal data for the bipyrazolyl compounds **1**–**5**.

	1	2	3	4a	4b	4c	5
Formula	C ₆ H ₆ N ₄	C ₁₀ H ₁₄ N ₄	C ₁₀ H ₁₄ N ₄	C ₃₃ H ₅₀ N ₁₂ O ₂	C ₃₄ H ₅₂ N ₁₂ O ₃	C ₃₄ H ₅₂ N ₁₂ O ₂	C ₂₂ H ₃₄ BeN ₄ O ₆
<i>M_r</i>	134.14	190.25	190.25	646.85	676.88	660.88	459.54
Crystal size [mm]	0.6 × 0.3 × 0.15	0.4 × 0.25 × 0.2	0.4 × 0.3 × 0.3	0.4 × 0.2 × 0.1	0.4 × 0.3 × 0.3	0.3 × 0.2 × 0.1	0.3 × 0.3 × 0.07
Crystal system	monoclinic	monoclinic	hexagonal	orthorhombic	orthorhombic	orthorhombic	tetragonal
Space group	<i>P</i> 2 ₁ / <i>c</i>	<i>Cc</i>	<i>P</i> 6 ₅	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 4 ₁ 2 ₁ 2
<i>a</i> [Å]	5.9510(5)	12.544(3)	8.6522(9)	9.751(2)	9.8054(6)	9.754(2)	7.440(1)
<i>b</i> [Å]	5.4548(7)	11.443(2)		13.080(3)	13.0221(8)	13.200(3)	
<i>c</i> [Å]	9.6941(9)	8.301(2)	26.350(5)	30.346(7)	30.307(2)	30.197(6)	44.967(9)
β [°]	96.12(1)	96.81(3)					
<i>V</i> [Å ³]	312.89(6)	1183.1(5)	1708.5(4)	3870(2)	3869.9(4)	3888(1)	2489.1(7)
<i>Z</i>	2	4	6	4	4	4	4
Unique reflections	573	1730	1929	8388	6646	5087	2736
Observed reflections	516	1621	1866	4342	3875	3482	1519
Parameters	59	136	136	424	442	433	151
<i>R</i> , <i>wR</i> ₂ (obsd)	0.054, 0.147	0.048, 0.131	0.041, 0.113	0.064, 0.157	0.050, 0.117	0.081, 0.204	0.079, 0.206

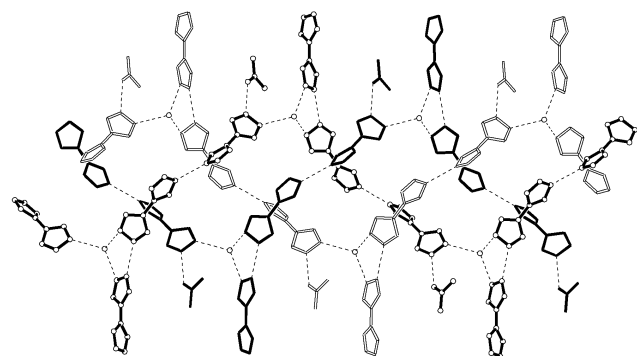


Figure 4. Triple-helicate motif of the structure of **4a**.

strates how molecules of small size may be immobilized in a chiral environment formed by self assembly of bipyrazolyls.

We were also interested in extending the bipyrazolyl frame into tectons of greater effective length, but which retain the ability for noncentrosymmetric self assembly.^[16] The desired angular configuration of hydrogen-bond donor/acceptor sites may exist in extended molecules containing connected planar fragments with an odd number of rotations between them (one rotation for each 4,4'-Me₄bpz core). We propose a flexible approach with species that combine the above features with structural simplicity and chemical accessibility. Beryllium-based β -diketonate **5**^[18] (Figure 5) exists as a molecular complex with the expected tetrahedral four-coordinate metal atom,^[6b] and the framework of the molecule includes four planes with three successive rotations between them. Two pyrazolyl groups are related by about 77.9°, and this “extended bipyrazolyl” retains the configuration of N/NH binding sites of the 4,4'-Me₄bpz core. Hydrogen-bonded methanol molecules appear to be simply bridging two pyrazolyl cycles: NH...OH...N, so that the overall linkage pattern in the structure is the same as for the α -polymorph of 4,4'-Me₄bpz, **2**, and the compound **5** adopts a chiral structure with a (10,3)-*b* net topology.

Thus, the angular 4,4'-bipyrazolyl moiety may be regarded as a tecton predisposed for self assembly into different variants of (10,3) nets with induced bulk chirality for the resulting solids. Our results provide many possibilities for the rational design of novel polar solids of (10,3) topology and

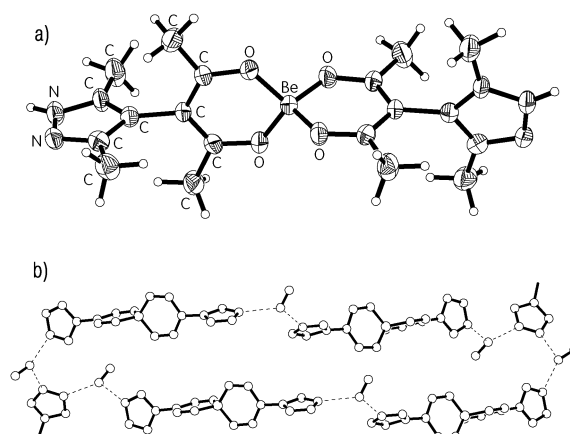


Figure 5. a) “Extended bipyrazolyl” Be(acacpz)₂ with angular alignment of pyrazolyl groups. b) The shortest circuits in the structure of **5** including ten pyrazolyl groups.

extended helicate arrays by 1) varying the substituents at the 3,3'- and 5,5'-positions of the bipyrazolyl core to tune the desired conformation of the tecton, 2) extension of the bipyrazolyl module without loss of the angular alignment of N/NH binding sites, and 3) elaboration of related systems with varying angular disposition of self-complementary donor/acceptor sites.^[8] Our study also contributes to the intriguing problem of polymorphism and supramolecular isomerism^[17] and illustrates how relatively slight rotational changes in the molecule may have pronounced chemical significance for the crystal packing. The approach used for the extension of the bipyrazolyl module may be considered as general and can be commonly applied in crystal engineering. We are currently studying self assembly of bipyrazolyls and their use for the rational design of solid-state functionality.^[22]

Experimental Section

1^[9a] was prepared in 82% yield by reaction of 1,1,2,2-ethanetetraaldehyde^[19] and hydrazine and was crystallized from H₂O/DMF (1/1). Sublimation of 4,4'-Me₄bpz^[20] (170 °C, 5 Torr) gave the monoclinic α -form **2** that was also prepared from hot water, while crystallization from acetonitrile provided the hexagonal β -form **3**. Both polymorphs melt above 300 °C; the melts, on cooling, afford the α -form **2**. Crystallization from different solvents at ambient conditions gave the isomorphous adducts (so-

called pseudopolymorphs)^[21] 3(4,4'-Me₂bpz)·H₂O·Solv (**4**; solv = acetone: **4a**, ethyl acetate: **4b**, THF: **4c**).^[15] They easily lose molecules of the solvent at room temperature with disintegration of the structure and formation of poorly hydrated noncrystalline products. The individual hydrate 2(4,4'-Me₂bpz)·H₂O was isolated from wet chloroform: tetragonal, *a* = 24.838(1), *c* = 14.9190(8) Å.

5: BeCO₃·4H₂O (0.070 g, 0.5 mmol) was dissolved in 5 mL of hot dilute HCl (2N), and combined under stirring with a hot solution of Hacacpz (0.388 g, 2.0 mmol)^[20] in aqueous ammonia (7 mL, 12 %). The solution was neutralized with HCl, and the resulting precipitate was recrystallized from aqueous methanol to give 0.075 g (30 %) of the complex as colorless plates.

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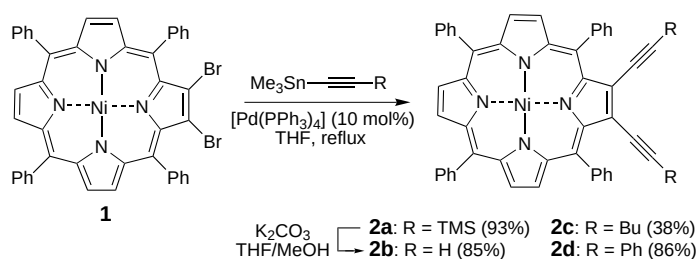
Multicarbocycle Formation Mediated by Arenoporphyrin 1,4-Diradicals: Synthesis of Picenoporphyrins**

Hidehiko Aihara, Laurent Jaquinod, Daniel J. Nurco, and Kevin M. Smith*

Porphyrin chromophores have received much attention, particularly as photoelectric devices and molecular wires^[1–4] in materials science, as photosensitive drugs for singlet oxygen formation in photodynamic therapy,^[5] and as models for the photosynthetic reaction center and antenna arrays.^[6] Efficient π -electronic communication between porphyrin macrocycles is pivotal in various complex functions. Our recent work has concentrated on the synthesis of novel porphyrin compounds bearing extended π systems, establishment of novel synthetic methods for such π -extended porphyrins, and fine-tuning of their electronic networks.^[7] In this connection we recently developed a novel synthesis of monobenzoporphyrins by the Diels–Alder reaction of a pyrroloporphyrin with dimethyl acetylenedicarboxylate.^[8] This led us to investigate the chemistry of 2,3-dialkynylporphyrins to seek more general methodologies to access such benzoporphyrin derivatives. Adjacent acetylenic units on porphyrins **2a–d** (see Scheme 1) might be expected to behave as components of an “enediyne”. Thus, they could undergo Bergman cyclization,^[9] that is, thermal benzoannulation via a 1,4-dehydrobenzene system, to fashion a fused benzo ring onto the porphyrin periphery.

Herein we report unprecedented multicarbocycle formation on the porphyrin periphery by Bergman aromatization of vicinal dialkynylporphyrins, to produce a new class of highly π -extended porphyrins. Precedents for the intramolecular cyclization of *meso*-aryl rings onto the porphyrin framework are limited to the acid-catalyzed condensations of 2-formyl- and 2-vinyl-tetra-arylporphyrins with a vicinal *meso*-aryl ring affording benzocyclohexenone porphyrins,^[10] or naphthochlorins.^[11]

Nickel(II) 2,3-dialkynyl-5,10,15,20-tetraphenylporphyrins **2a–d** were prepared by Pd⁰-catalyzed cross-coupling reactions^[12] of nickel(II) 2,3-dibromo-5,10,15,20-tetraphenylporphyrin (**1**)^[13] with the corresponding alkynyl trimethylstannanes in good to excellent yields (Scheme 1). Refluxing **2b** (R = H, Table 1) in 5% 1,4-cyclohexadiene (CHD, as a hydrogen source)/chlorobenzene solution at 190 °C gave a greenish compound as a single product; in the absence of CHD the same reaction only produced a complex mixture of



Scheme 1. Preparation of 2,3-dialkynylporphyrins **2**. TMS = trimethylsilyl.

Table 1. Thermal annulation of 2,3-dialkynylporphyrins **2**.

Entry	R	Conditions	2 (recovered)	3
1	TMS (2a)	190 °C, 12 h	quant.	–
2	H (2b)	190 °C, 8 h	–	89 %
3	Bu (2c)	190 °C, 60 h	44 %	50 %
4 ^[a]	Ph (2d)	280 °C, 18 h	–	86 %

[a] 5% CHD in 1,2,4-trichlorobenzene was used as solvent.

oligoporphyrins. Surprisingly, none of the expected monobenzoporphyrin **4** was obtained. The greenish compound was characterized by ¹H NMR spectroscopy, NOE experiments, and laser desorption/ionization time-of-flight (LDI-TOF) mass spectrometry (MS) as the highly π -extended porphyrin **3b**.^[14]

The ¹H NMR spectrum of **3b** clearly confirms the large structural and electronic changes of the porphyrin macrocycle as well as the disappearance of the characteristic terminal alkyne protons (δ = 3.49) observed in 2,3-diethynylporphyrin **2b**. The resonance signal from the aromatic protons on the benzo ring formed by the Bergman annulation appeared at δ = 8.48 as a sharp singlet. In addition, the absorption spectrum of **3b** is red-shifted (λ_{max} (CH₂Cl₂) = 450, 629 nm) relative to **2a**, a result of the extended π -conjugation in **3b**.

The new porphyrin obtained from this reaction is named as a “picenoporphyrin” after its [5]phenacene structure,^[15] and specifically nickel(II) 10,15-diphenyl-piceno[20,1,2,3,4,5-*fghij*]-porphyrin (**3b**).

The molecular structure of **3b** is shown in Figure 1.^[16] The porphyrin macrocycle exhibits a ruffled conformation with a 0.242 Å mean deviation of the 24 macrocyclic atoms from their least-squares plane. The piceno group is nearly planar with a 0.100 Å mean deviation of the 22 atoms from their least-squares plane. The Ni–N bond lengths are 1.988(4), 1.852(4), 1.970(4), and 1.934(4) Å for Ni(1) to N(1)–N(4) respectively. The Ni(1)–N(2) bond length is noteworthy because it is the shortest Ni–N bond length reported for any nickel(II) porphyrin crystal structure. This feature results from geometry imposed upon the porphyrin macrocycle by the

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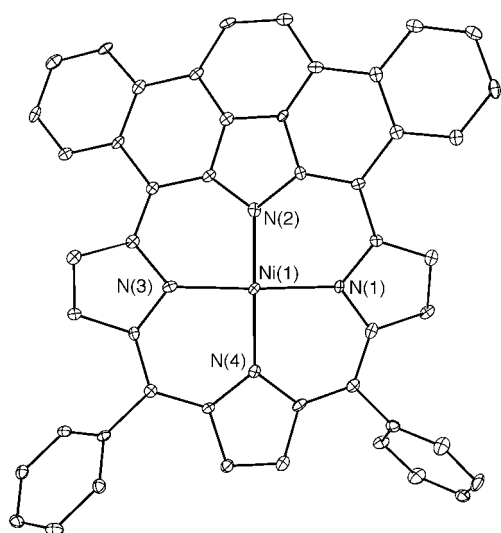


Figure 1. Molecular structure of picenoporphyrin **3b** (thermal ellipsoids at 30% probability). Hydrogen atoms have been omitted for clarity.

rigid piceno group. Of additional note in this regard were significant contractions of the fused C_{α} - C_{meso} - C_{α} bond angles and C_{α} - C_{meso} bond lengths along with expansions of the fused N - C_{α} - C_{meso} bond angles and C_{β} - C_{β} bond lengths in comparison to other reported nickel(II) porphyrin crystal structures.^[17]

Compounds **2c** and **2d**, which have internal alkynyl substituents ($R = Bu, Ph$), undergo the same multicarbocycle formation to give the corresponding dibutylpicenoporphyrin **3c** in 50% yield, and diphenylpicenoporphyrin **3d** (86%), (Table 1, entries 3, 4). These transformations required a longer reaction period (60 h, for **3c**) or a higher temperature (280 °C, for **3d**). Compound **2a** ($R = TMS$) gave none of the corresponding picenoporphyrin, and was recovered quantitatively after the reaction.

A tentative mechanistic pathway to the picenoporphyryns is shown in Scheme 2. Thermal annulation of the 2,3-alkynyl moieties would produce a 1,4-diradical species **5**. This diradical then attacks the vicinal *meso*-phenyl groups yielding

a planar diradical species **6**. Our experimental results indicate that the steric hindrance of the alkynyl substituents of **2** slows the thermal annulation. After two successive intermolecular hydrogen transfers from CHD, **6** affords tetrahydro species **7**, which is oxidized during the course of work-up to give picenoporphyryns **3b–d**. The absence of any monobenzoporphyrin **4** clearly indicates that intramolecular carbon–carbon bond formation via diradicals **5** is faster than intermolecular hydrogen transfer from CHD.

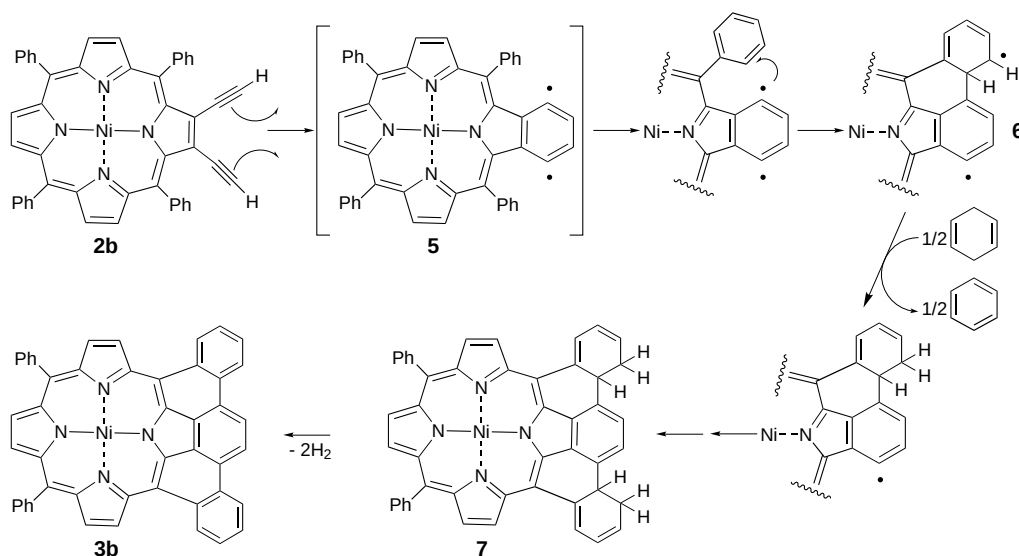
In conclusion, we have shown that neighboring acetylenic units on porphyrins provide a means for the efficient construction of aromatic superstructures triggered by Bergman reaction conditions, and give novel access to $[n]$ phen-acenoporphyryns.

Experimental Section

In a typical Bergman cyclization, 2,3-dialkynylporphyrins **3** (30 mg) was added to a Schlenk tube and dissolved in anhydrous 5% 1,4-cyclohexadiene (CHD) in chlorobenzene or 1,2,4-trichlorobenzene (20 mL). The reaction flask was sealed well and the mixture was then heated under reflux under appropriate conditions (see Table 1). After being cooled to room temperature, silica gel (ca. 1 g) was added to the flask, and the solvent was removed under vacuum. The resulting powder was loaded onto a silica gel column and eluted initially with 20% toluene in cyclohexane, and then with CH_2Cl_2 . The major green band was collected and recrystallized from CH_2Cl_2 /methanol. Compounds **3b–d** were soluble in common organic solvents despite their rigid piceno structures.

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Scheme 2. Proposed mechanistic pathway to picenoporphyryns **3**.

Olefin Metathesis with 1,1-Difluoroethylene**

Tina M. Trnka, Michael W. Day, and Robert H. Grubbs*

The recent development of ruthenium olefin-metathesis catalysts coordinated with N-heterocyclic carbene ligands is a significant advance because it extends the scope of the reaction to more challenging substrates, that is, those that are sterically hindered or that contain electronically deactivating groups, as well as monomers with low ring strain.^[1–4] One set of substrates that has received relatively little attention, however, is the halogenated olefins;^[5] the metathesis of allyl bromide, allyl chloride, and related substrates with the heterogeneous $\text{Re}_2\text{O}_7/\text{Al}_2\text{O}_3/\text{Me}_4\text{Sn}$ catalyst system are among the few examples.^[6] Most recently, the cross metathesis of 3,3,4,4,5,5,6,6-nonafluoro-1-hexene with terminal olefin^[4c] and the dimerization of vinyl *gem*-difluorocyclopropane derivatives^[7] have been achieved using [(H₂IMes)(PCy₃)(Cl)₂Ru=CHPh] (**1**) (H₂IMes = 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene; Cy = cyclohexyl). In these cases, the substrates are challenging because of the electron-withdrawing nature of the pendent halogens.

A particularly interesting situation arises when the olefin is directly halogenated, because then the metathesis reaction will involve a monohalo [M]=CXR or dihalo [M]=CX₂ carbene complex rather than the usual alkylidene [M]=CR₂. This possibility has been considered by Beauchamp and co-workers, who have speculated about the metathesis of directly fluorinated olefins with nickel or manganese complexes.^[8] A tungsten dichlorocarbene complex [W]=CCl₂ has also been proposed as an active species in the $\text{W}(\text{CO})_6/\text{CCl}_4/h\nu$ catalyst system.^[9] To our knowledge, there has been only one report of metathesis involving directly halogenated olefins, namely the cross metathesis of 1-chloro- and 1-bromoethylene with propylene using $\text{Re}_2\text{O}_7/\text{Al}_2\text{O}_3/\text{Me}_4\text{Sn}$.^[6c] Herein, we report the successful metathesis of 1,1-difluoroethylene with ruthenium catalyst **1**.

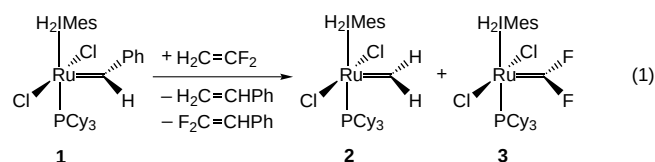
Under an atmosphere of 1,1-difluoroethylene, **1** reacts to form the corresponding methyldiene [(H₂IMes)(PCy₃)(Cl)₂Ru=CH₂] (**2**)^[10] and difluorocarbene [(H₂IMes)(PCy₃)(Cl)₂Ru=CF₂] (**3**) complexes [Eq. (1)].^[11] When the reaction is performed at room temperature, the product mixture contains approximately 40% methyldiene (**2**) and 60% difluorocarbene (**3**), as well as styrene and β,β -

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difluorostyrene. Remarkably, the amount of difluorocarbene complex formed increases to >98% when the reaction is carried out at 60 °C. This difference suggests that pathway A of Figure 1 is preferred over pathway B at elevated temper-

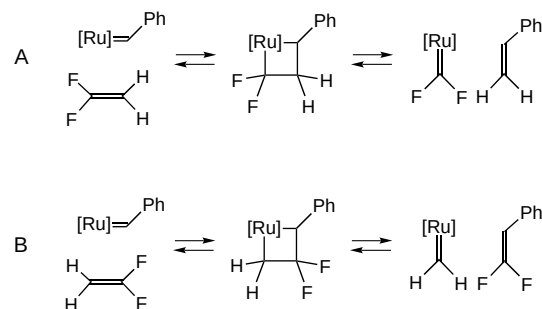


Figure 1. One turnover of olefin metathesis between the ruthenium benzylidene complex **1** and 1,1-difluoroethylene. Pathway A is favored at elevated temperatures.

atures. The organic products of a second turnover of olefin metathesis—ethylene and tetrafluoroethylene—were not observed in the product mixture, which indicates that the reaction is not catalytic. Nevertheless, the $\text{H}_2\text{C}=\text{CF}_2$ double bond is initially cleaved in a metathesis fashion, and as such, it is the first example of olefin metathesis involving a directly fluorinated olefin.

Moreover, this transformation provides access to 16-electron ruthenium difluorocarbene complexes, which have not been previously accessible.^[12] Complex **3** has been unambiguously identified as the difluorocarbene by NMR spectroscopy. It is characterized by a $^{31}\text{P}\{^1\text{H}\}$ NMR resonance signal at $\delta = 32.1$ and a ^{19}F NMR resonance at $\delta = 133.7$, both doublets with $J = 4.5$ Hz from $^{31}\text{P} - ^{19}\text{F}$ coupling. This downfield ^{19}F chemical shift is also diagnostic for metal fluorocarbenes, which resonate between $\delta = 80$ and 200.^[12, 13] The $^{13}\text{C}\{^1\text{H}\}$ resonance for the carbene carbon atom appears as a triplet of doublets at $\delta = 218.1$ ($^2J_{\text{CP}} = 12$ Hz, $^1J_{\text{CF}} = 432$ Hz). This resonance is shifted significantly upfield compared to those of related ruthenium alkylidenes; for example, the resonance signals of the benzylidene carbon of **1** and the methylidene carbon of **2** appear at $\delta = 295.1$ and 294.8, respectively.^[14]

The molecular structures of **2** and **3** were determined by X-ray diffraction (Figures 2 and 3). Notably, complex **2** is the first structurally characterized ruthenium methylidene (Figure 2). In both cases, the carbene is oriented in the [Cl–Ru–Cl] plane.^[15] The $d(\text{Ru}=\text{C})$ of 1.800(2) Å in **2** is shorter than that typical for ruthenium benzylidene complexes ($[\text{Ru}]=\text{CHPh}$) but similar to values observed for phenyl-substituted vinylidenes ($[\text{Ru}]=\text{C}=\text{CHPh}$).^[16]

Complex **3** (Figure 3), which is isostructural with **2**, exhibits an even shorter $\text{Ru}=\text{C}$ bond length of 1.775(3) Å.^[17] This value

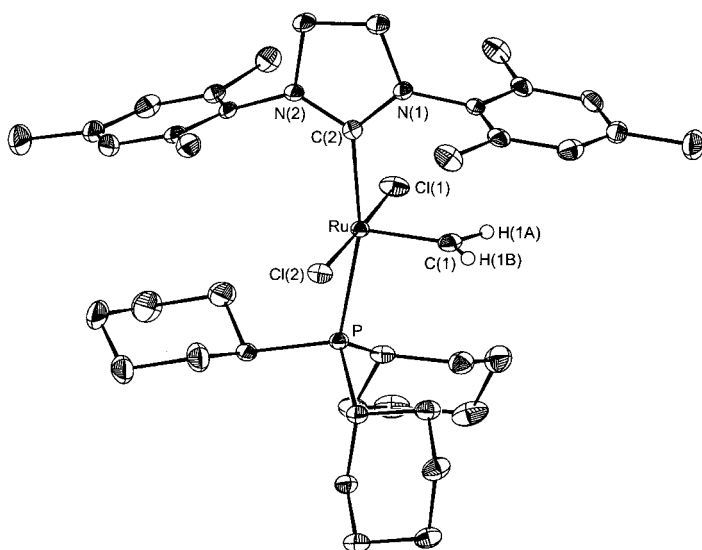


Figure 2. Molecular structure of **2**. For clarity, all hydrogen atoms except H(1A) and H(1B) have been omitted. Thermal ellipsoids are set at 50% probability; H(1A) and H(1B) are drawn at arbitrary scale. Selected bond lengths [Å] and angles [°]: Ru–C(1) 1.800(2), Ru–C(2) 2.065(2), Ru–Cl(1) 2.379(1), Ru–Cl(2) 2.393(1), Ru–P 2.427(1); Cl(1)–Ru–Cl(2) 177.05(2), C(2)–Ru–P 165.81(5), H(1A)–C(1)–H(1B) 108(2).

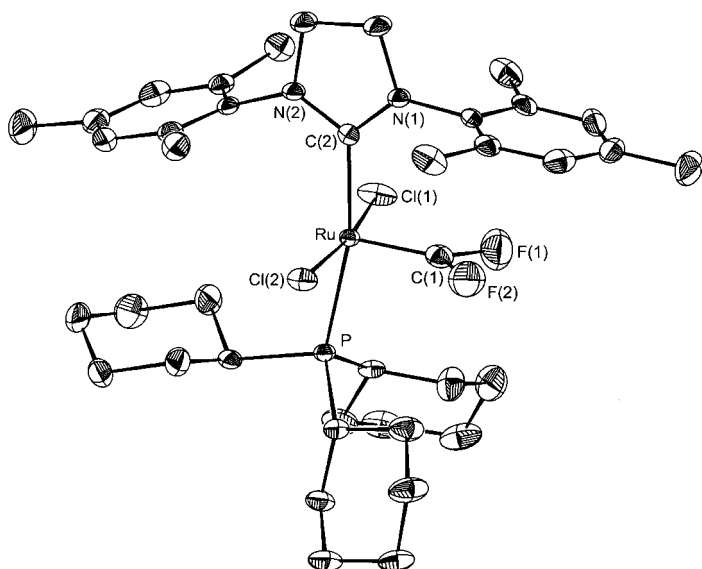


Figure 3. Molecular structure of **3**. For clarity, all hydrogen atoms have been omitted. Thermal ellipsoids are set at 50% probability. Selected bond lengths [Å] and angles [°]: Ru–C(1) 1.775(3), Ru–C(2) 2.076(2), Ru–Cl(1) 2.365(1), Ru–Cl(2) 2.385(1), Ru–P 2.433(1), F(1)–C(1) 1.335(3), F(2)–C(1) 1.305(3); Cl(1)–Ru–Cl(2) 170.90(3), C(2)–Ru–P 167.69(6), F(2)–C(1)–F(1) 103.4(2).

is also short compared to the $d(\text{Ru}=\text{C})$ of 1.83(1) Å for $[(\text{PPh}_3)_2(\text{CO})_2\text{Ru}=\text{CF}_2]$, a trigonal bipyramidal, 18-electron complex reported by Roper and co-workers.^[15a, 18] This difference between $[(\text{PPh}_3)_2(\text{CO})_2\text{Ru}=\text{CF}_2]$ and **3** may be rationalized by enhanced π back bonding from the more electron-rich metal center of **3** to the carbene carbon atom.

We are also interested in the olefin metathesis activity of **3** itself. The results in Table 1 provide a measure of the activities of **1–3** for the ring-opening metathesis polymerization (ROMP) of cycloocta-1,5-diene (COD).^[3c, 4d, 14] Compared to

Table 1. ROMP of COD with catalysts **1**–**3**.^[a]

Catalyst	<i>T</i> [°C]	Additive ^[b]	<i>t</i> [h]	Product ^[c] [%]
1 ^[d]	20	–	0.03	100
2 ^[d]	20	–	1.25	62
3	25	–	1.25	9
3	50	–	5	72
3	50	CuCl	5	81
3	50	HCl	5	92
3	50	AlCl ₃	5	21

[a] Conditions: 0.005 M [catalyst] and 300 equivalents COD in CD₂Cl₂. [b] 5 equivalents of additive; HCl used as a 1 M solution in Et₂O. [c] Percent conversion determined by ¹H NMR integration. [d] Ref. [14].

the superior activity of **1**, complex **3** is a poor catalyst. We suspected that the problem was catalyst initiation because no NMR signals for CF₂ end groups in the polymer were visible. Recent studies have shown that the rate-limiting step for the initiation of [(L)(PR₃)(X)₂Ru=CHR'] catalysts is phosphane dissociation.^[14, 19] For **3**, ³¹P{¹H} NMR magnetization-transfer experiments revealed no detectable phosphane dissociation up to 100 °C (*k* < 0.01 s^{−1}).^[20, 21]

Higher temperatures and additives that promote phosphane dissociation helped improve the activity of **3** (Table 1).^[22] The best results were obtained with HCl, which is capable of reversible phosphane protonation. In contrast, addition of AlCl₃ resulted in an immediate and irreversible decomposition reaction.

The reaction of 1,1-difluoroethylene with ruthenium catalyst **1** is significant for several reasons. Most importantly and of general interest, it demonstrates that olefin metathesis with directly fluorinated substrates is possible. Encouraged by these results, ongoing work is directed toward the application of olefin metathesis as a mild and selective method for the synthesis of halogenated molecules. Of greater interest to the organometallic chemist, this reaction provides a new method for the synthesis of electronically unsaturated halocarbene complexes. In addition, the structural data for a matched pair of ruthenium carbene complexes will aid our understanding of the bonding in this important catalyst family.

Experimental Section

Synthesis of 3: A solution of **1** (0.32 g 0.37 mmol) in dry, degassed benzene (15 mL) in a thick-walled glass ampule was put under ~1.5 atm of 1,1-difluoroethylene. The mixture was heated at 60 °C for 12 h, during which time it changed from reddish to brown. The solution was then concentrated to 5 mL and purified by column chromatography in air (silica gel, 5:1 pentane:THF). The orange fraction was stripped of solvent and dried under vacuum: yield 0.26 g (86%). ¹H NMR (499.852 MHz, 25 °C, CD₂Cl₂): δ = 1.118 (br, 15H, PCy₃), 1.626 (br, 15H, PCy₃), 2.248 (s, 3H, *p*-CH₃ of Mes), 2.285 (s, 3H, *p*-CH₃ of Mes), 2.385 (m, 3H, PCy₃), 2.480 (s, 6H, *o*-CH₃ of Mes), 2.551 (s, 6H, *o*-CH₃ of Mes), 4.003 (s, 4H, NCH₂CH₂N), 6.921 (s, 4H, *m*-H of Mes); ¹³C{¹H} NMR (125.705 MHz, 30 °C, C₆D₆): δ = 19.44 (s, CH₃ of Mes), 20.65 (s, CH₃ of Mes), 21.49 (s, CH₃ of Mes), 21.50 (s, CH₃ of Mes), 26.92 (d, *J* = 1.3 Hz, PCy₃), 28.50 (d, *J* = 10 Hz, PCy₃), 30.14 (s, PCy₃), 33.34 (d, *J* = 18 Hz, PCy₃), 51.86 (d, ⁴*J*_{PC} = 2.6 Hz, NCH₂CH₂N), 52.61 (d, ⁴*J*_{PC} = 3.5 Hz, NCH₂CH₂N), 127.30 (s, Mes), 128.17 (s, Mes), 129.26 (s, Mes), 129.51 (s, Mes), 130.11 (s, Mes), 130.52 (s, Mes), 134.68 (d, ⁴*J*_{PC} = 0.7 Hz, *ipso*-C of Mes), 136.85 (s, *ipso*-C of Mes), 138.91 (s, Mes), 138.93 (s, Mes), 139.03 (s, Mes), 139.67 (s, Mes), 217.23 (d, ²*J*_{CP} = 87 Hz, NCN), 218.09 (td, ²*J*_{CP} = 12 Hz, ¹*J*_{CF} = 430 Hz, Ru=CF₂); ¹⁹F NMR (282.192 MHz, 25 °C,

CD₂Cl₂): δ = 133.74 (d, ³*J*_{FP} = 4.5 Hz); ³¹P{¹H} NMR (121.392 MHz, 25 °C, CD₂Cl₂): δ = 32.15 (t, ³*J*_{PF} = 4.4 Hz); IR (KBr pellet): $\tilde{\nu}$ = 1167 and 1172 cm^{−1} (ν_{C-F}).

Crystal structure of 2: The complex was synthesized by the method in ref. [14]. Orange-brown crystals were obtained by slow evaporation of a concentrated hexanes solution. C₄₀H₆₁Cl₂N₂Ru, *M*_w = 772.85, monoclinic, *a* = 12.2168(6), *b* = 18.0193(8), *c* = 18.1162(9) Å, β = 103.651(1)°, *V* = 3875.4(3) Å³, ρ_{calcd} = 1.325 Mg m^{−3}, *T* = 98 K, space group *P*2₁/*n* (No. 14), *Z* = 4, radiation MoKα (λ = 0.71073 Å), μ = 0.613 mm^{−1}, θ_{max} = 28.46°, 56 743 reflections measured, 9201 unique (*R*_{int} = 0.0542), structure solved by direct methods, refinement of *F*² against all reflections using SHELXL-97,^[23] 659 parameters, 0 restraints, final *R*₁ = 0.0414, *wR*₂ = 0.0555 (all data), *GOF* = 1.645, max/min residual density +0.666/−0.476 e Å^{−3}.

Crystal structure of 3: Orange crystals were obtained by slow evaporation of a concentrated hexanes solution. C₄₀H₅₉Cl₂F₂N₂Ru, *M*_w = 808.83, monoclinic, *a* = 12.2295(6), *b* = 18.4137(9), *c* = 18.1269(9) Å, β = 103.709(1)°, *V* = 3965.7(3) Å³, ρ_{calcd} = 1.355 Mg m^{−3}, *T* = 98 K, space group *P*2₁/*n* (No. 14), *Z* = 4, radiation MoKα (λ = 0.71073 Å), μ = 0.610 mm^{−1}, θ_{max} = 28.41°, 58 469 reflections measured, 9430 unique (*R*_{int} = 0.0575), structure solved by direct methods, refinement of *F*² against all reflections using SHELXL-97,^[23] 668 parameters, 0 restraints, final *R*₁ = 0.0528, *wR*₂ = 0.0693 (all data), *GOF* = 1.899, max/min residual density +0.953/−0.636 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-162849 (for **2**) and -162850 (for **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). Structure factors are available on request from xray@caltech.edu.

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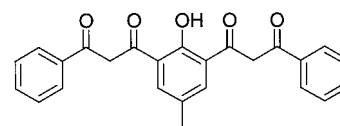
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A Unique Asymmetric [Mn^{II}] Triple-Stranded Helicate from a Symmetric Pentadentate Ligand**

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One of the richest parcels of supramolecular chemistry is that where the various components of a complex assembly recognize and associate with each other “via” the intermediacy of coordination bonds.^[1] In this area, the specific coordination requirements of transition metals are combined with the electronic and structural features of new ligands in the design and construction of unprecedented molecular architectures displaying novel and exciting properties. This approach has been relatively unexplored in the field of molecular magnetism.^[2] We have recently started a program aimed at the design and preparation of novel multidentate ligands as a means of assembling transition metal centers into species displaying new topologies and innovative magnetic properties. In this context, we have built in the same molecule two β-diketone units and a phenol group to produce a symmetric O-pentadentate ligand (H₃L).^[3] The preparation of



some related molecules and their use as dinucleating ligands have been reported in the literature.^[4] We intended to use a basic form of H₃L as a template to bring in proximity a number of transition metals in discrete, molecular arrays. Examples of such systems involving oligo-α-pyridylamine ligands are known.^[2e, 5] We now report the first case with an oxygen donor ligand and introduce an unprecedented asymmetric topology within the context of coordination helicates.

Reaction of H₃L with one equivalent of Mn(AcO)₂ · 4H₂O in the solvent mixture MeOH/py (2/1, v/v) leads to a yellow crude product upon precipitation with hexanes. Recrystallization of this product from CH₂Cl₂/Et₂O/hexanes affords yellow needles of [Mn₃(HL)₃] (**1**) in an overall yield of 15%. The structure of **1**^[6] (Figure 1) consists of a trinuclear array of Mn^{II} ions chelated and bridged by three HL^{2−} ligands wrapped around the molecular axis in an irregular helical

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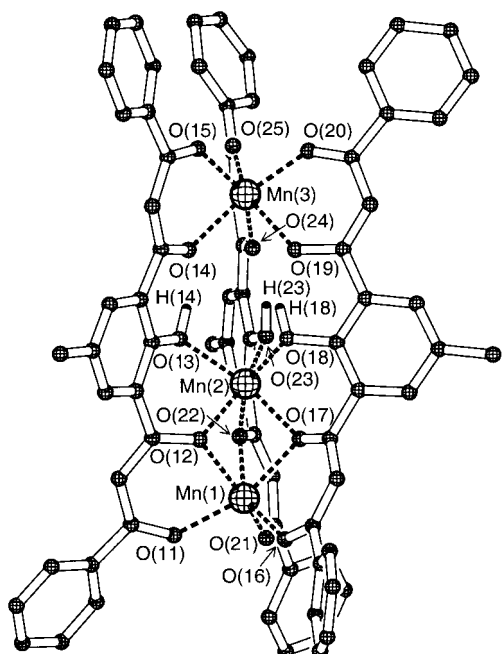


Figure 1. Pluton representation of complex **1**.

manner. The three metal centers are quasi colinear (Figure 2; the Mn(1)–Mn(2)–Mn(3) angle being $179.20(3)^\circ$) and display three distinct Mn...Mn vectors with distances of 3.024(17), 5.044(2), and 8.076(3) Å for Mn(1) ... Mn(2), Mn(2) ... Mn(3), and Mn(1) ... Mn(3), respectively. This topological asymmetry

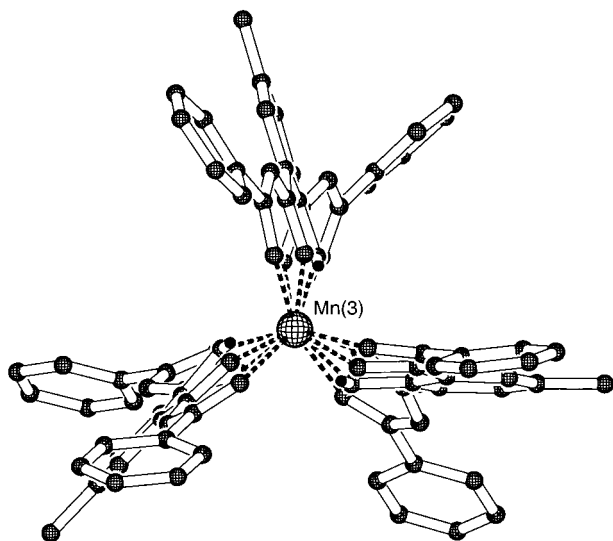


Figure 2. Pluton representation of **1** down the molecular axis emphasizing the amphiverse helical nature of the complex.

of the metals along the molecular axis is what makes this compound unique amongst the family of metallohelicates.^[7] To the best of our knowledge, this is the first asymmetric homometallic helicate obtained from a ligand with idealized C_{2v} symmetry. In addition, it constitutes a new addition to the smaller subset of compounds within this category that are based on oxygen donor ligands.^[4b,c, 7b, 8] The peripheral metals of the complex are chelated by the β -diketonate units of the ligands. Evidence of the high degree of conjugation within

these chelate rings is their quasi planarity. The central metal ion is bridged to Mn(1) by the three inner oxygen donors of the corresponding diketonate groups, with the oxygen atoms from the phenol units completing the hexacoordination around this ion in the form of six-membered chelate rings. In contrast to the case of the external Mn ions, the chelate rings involving Mn(2) display a distorted chair conformation. The coordination around the Mn centers is intermediate between octahedral and trigonal prismatic, with twist angles^[9] and Mn–O distances within the ranges of $16.55–34.87^\circ$ and $2.092(4)–2.261(4)$ Å, respectively. The +2 oxidation state of all the Mn ions is consistent with valence bond sum analysis, the elemental analysis^[10] of the compound, and its magnetic behavior (see below). The cavity left between Mn(2) and Mn(3) is the most likely location of the three protons necessary to insure the electroneutrality of the complex, and indeed residual electron density was found in this area during the crystal structure determination.^[6] The O–H...O contacts between O(13), O(18), O(23) and O(14), O(19), O(24), respectively, $(2.409(6), 2.382(6), \text{ and } 2.385(6) \text{ Å})$ are slightly shorter than in organic molecules containing an equivalent moiety^[11] and fall within the range found in coordination complexes displaying the M–O–H...O–M unit.^[12] These hydrogen-bond interactions are undoubtedly influencing the overall stereochemistry of the molecule (see below). The topological asymmetry of **1** translates into the presence of different helical domains. The irregularity of the helix is further brought about by the change of chirality of the metals along the chain. These show the Δ , Δ , and Δ configurations^[13] for Mn(1), Mn(2), and Mn(3), respectively (Figure 3); the $\Delta\Delta\Delta$ form exists in the crystal in an equal amount by virtue of the inversion center present within the $Pccn$ space group. This alternation of configuration leads to the change of helicity through the molecular axis that renders this helicate amphiverse (Figure 2).

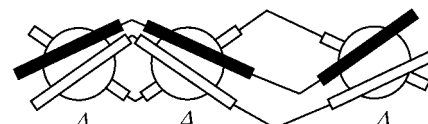


Figure 3. Stereochemistry along the axis of helicate **1**.

Consistent with the relatively slow electronic relaxation of the Mn^{II} ion,^[14] the ^1H NMR spectrum of **1** in $CDCl_3$ shows only very broad, paramagnetically shifted signals. This precludes the study of the enantiomerization dynamics of this new type of helicate using that technique. Diamagnetic versions of **1** with a built in stereochemical probe in the form of diastereotopic protons could be used for that purpose, and this is currently being explored.

Variable-temperature magnetic susceptibility measurements were performed on powdered **1** in the 5–300 K range under a constant magnetic field of 20 kG. A plot of the effective magnetic moment (μ_{eff}/μ_B) versus temperature (T/K) is shown in Figure 4. The value of μ_{eff} at room temperature (10.1) is very close to the spin-only value ($10.25 \mu_B$) expected for three uncoupled Mn^{II} ions per molecule with $g = 2.00$, and the value decreases monotonically to reach $5.87 \mu_B$ at 5 K. This behavior indicates the presence of antiferromagnetic

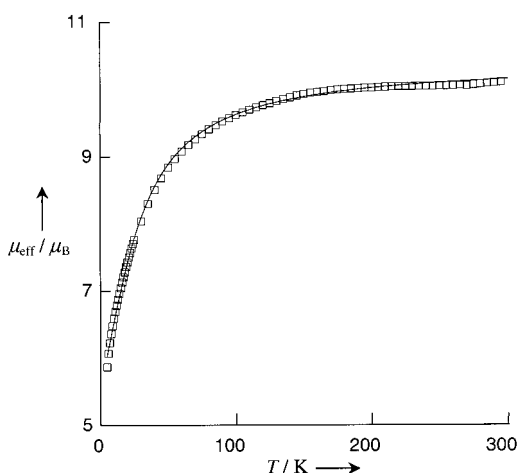


Figure 4. Plot of the effective magnetic moment (μ_{eff}) per molecule versus T for **1** in a 20.0 kG field. The solid line is a fit of the data to the appropriate theoretical equation; see the text for the fitting parameters.

interactions within this system. The magnetic data have been fit to a theoretical expression obtained by use of the van Vleck equation,^[15] the Kambe vector coupling method,^[16] and the spin Hamiltonian given in Equation (1).

$$\mathcal{H} = \mu_B g_3 \hat{S}_3 \vec{B} + \mu_B g_{12} \hat{S}_{12} \vec{B} - 2J \hat{S}_1 \hat{S}_2 \quad (1)$$

This Hamiltonian results from consideration of a simple model consisting of the presence of an exchange-coupled $\text{Mn}^{\text{II}} \cdots \text{Mn}^{\text{II}}$ pair next to a third, uncoupled Mn^{II} center. In Equation (1), S_i is the spin of Mn_i , with $S_1 = S_2 = S_3 = 5/2$, J is the exchange constant of the $\text{Mn}_1 \cdots \text{Mn}_2$ coupling, g_{12} and g_3 are the Landé factors of the Mn_{12} pair and the Mn_3 ion, respectively, μ_B is the Bohr magneton, and B is the external magnetic field. The best fit (Figure 4, solid line) was found for $J = -2.38(2) \text{ cm}^{-1}$, $g_3 = 2.08(2)$, and $g_{12} = 1.90(2)$. A temperature-independent paramagnetism parameter (TIP) was held constant at $600 \times 10^{-6} \text{ cm}^3 \text{ K mol}^{-1}$. A detailed analysis of the magnetic susceptibility data by use of a full-matrix diagonalization method will be necessary in order to evaluate the importance of the three possible $\text{Mn2-O-H} \cdots \text{O-Mn3}$ additional pathways for the magnetic super-exchange.

The unique triple-stranded helicate **1** is the first crystallographically characterized complex with a deprotonated form of H_3L . The study of the coordination properties of this ligand is currently being extended to other metals.

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- [6] Crystal Data for **1**: $\text{C}_{75}\text{H}_{54}\text{Mn}_3\text{O}_{15}$ + solvent (see below). Several crystallization batches yielded only weakly diffracting crystals. Final data were collected on a yellow block-shaped crystal ($0.2 \times 0.2 \times 0.3 \text{ mm}$), orthorhombic, space group *Pccn* (no. 56), $a = 21.998(3)$, $b = 32.757(10)$, $c = 20.784(3) \text{ Å}$, $V = 14977(5) \text{ Å}^3$, $Z = 8$, 106973 reflections measured, 15572 independent, $R_{\text{int}} = 0.112$, $R_{\sigma} = 0.103$, $1.6^\circ < \theta < 27.0^\circ$, $T = 150 \text{ K}$, $\text{MoK}\alpha$ radiation, $\lambda = 0.71073 \text{ Å}$ on a Nonius KappaCCD diffractometer on a rotating anode with no absorption correction. The structure was solved by automated direct methods (SHELXS 86), and refined on F^2 (SHELXL-97). The electron density in disordered solvent areas (two voids of 1783 Å^3 each, filled with a mixture of CH_2Cl_2 , Et_2O , and hexane) was taken into account in the refinement by PLATON/SQUEEZE. A total number of 949 electrons per unit cell was found. One of the phenyl rings (C(211)–C(216)) is disordered. Since no suitable disorder model could be obtained with the present data set, an ideal phenyl ring with isotropic displacement parameters was fitted in this electron density. Hydrogen atoms were included on calculated positions riding on their carrier atoms. Between atom pairs O(13)–O(14), O(18)–O(19), and O(23)–O(24) residual density peaks were observed, which correspond to three symmetric O–H $\cdots$ O hydrogen bonds. As a result of the low crystal quality, these hydrogen atoms could not be refined. Instead, the hydrogen atoms were introduced at ideal hydroxyl positions riding O(13), O(18), and O(23), respectively. Refinement of 793 parameters converged at a final $wR2$ value of 0.2216, $R1 = 0.0956$ (for 7734 reflections with $I > 2\sigma(I)$), $S = 1.042$, $-0.70 < \Delta\rho < 1.20 \text{ e Å}^{-3}$. 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-163316. 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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A Free-Energy Relationship between the Rate of Acidic Hydrolysis of Glycosides and the pK_a of Isofagomines**

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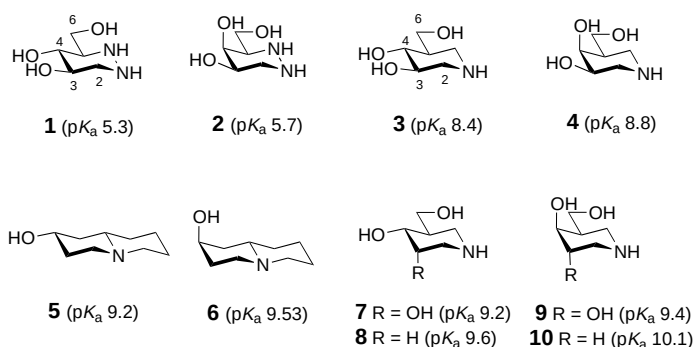
Carbohydrates, the most abundant biomolecules, consist mainly of monomers that are linked together by glycosidic bonds. Since each carbohydrate molecule owes many of its properties to the glycosidic linkages it contains, and because formation and cleavage of these bonds are involved in any carbohydrate synthesis or breakdown, the chemistry of the glycosidic bond has long been under intense scrutiny. As early as 1908 it was noted that the rates of acidic hydrolysis of glycosides depend on the configuration of the hydroxyl groups of the sugar, and that the more axial hydroxyl groups are present in the glycosyl unit, the more rapid is the acidic hydrolysis.^[1] Edward proposed that the rate differences arise because the axial groups facilitate a conformational change necessary for the molecule to reach a half-chair transition-state structure.^[1e] While recent work has suggested that differences in reaction rates may be caused by differences in substituent inductive effects in series with conserved stereochemistry,^[2] Edward's proposal regarding the differences in reaction rates between glycosides with different stereochemistry has not been challenged.

Here we report on a surprising finding that has bearing on this issue. We recently observed^[3] that the basicity of *galacto*-azafagomine (**2**) is slightly higher than that of the *gluco* isomer **1**^[4] (Scheme 1). While this difference in basicity might appear insignificant, the only structural difference between the two hydrazines is whether the 4-hydroxyl group is equatorial or axial, and no difference in base strength was anticipated. We therefore also measured the pK_a of the protonated forms of isofagomine (**3**)^[5] and its *galacto* analogue **4**.^[6] Again the same difference in basicity was observed between the two stereoisomers (Scheme 1).

A literature search revealed that a similar effect was observed in less highly functionalized compounds.^[7, 8] For example, in the conformationally restricted amines **5** and **6**, the isomer with an axial hydroxyl group was found to be the stronger base (Scheme 1).^[7] We also measured the pK_a values of available^[9, 10] isofagomine analogues **7–10** and found a considerable variation (Scheme 1). Furthermore, the pK_a of 1-deoxynojirimycin and its *manno* and *galacto* analogues were reported^[11] to be 6.3, 7.2, and 7.1, respectively. These data show that changing a hydroxyl group from an equatorial to an axial position increases the pK_a of the amine by about 0.8 units when the OH group is β to the amino group, and by about 0.4 units when it is γ to the amino group.

The basicities of amines are known to be affected by a) substituent effects, b) resonance, c) steric hindrance to protonation, d) solvation, and e) internal hydrogen bonding.^[12] In this case, b) and c) can immediately be ruled out of playing any role in explaining the differences in basicity between **1** and **2**. Solvation is mainly significant when the space around the hydrogen atoms attached to the basic center varies, but this is not the case between compounds **3** and **4** and **7–10**.^[13] Internal hydrogen bonding cannot explain the difference in basicity between **3** and **4**, but could possibly affect the basicity of compounds with an axial 3-hydroxyl group, though this is unlikely in water. So the differences in basicity can be attributed mainly to substituent effects. This suggests a difference in electron-withdrawing power between the axial and equatorial 4-hydroxyl groups of **4** and **3**, respectively, whereby the former is apparently de facto less electron withdrawing. This can be explained by differences in charge–dipole interactions in the two molecules. An equatorial hydroxyl group in the β - or γ -position (**A**) will pull electrons away from the charged nitrogen atom to a greater extent than a corresponding axial substituent (**B**), since its vector component in the plane of the charged nitrogen atom is larger (see Scheme 3).^[14] Note that NMR spectroscopic data of **1** and **2** at neutral and acidic pH clearly revealed that the ⁴C₁ conformation was predominant, regardless of pH.

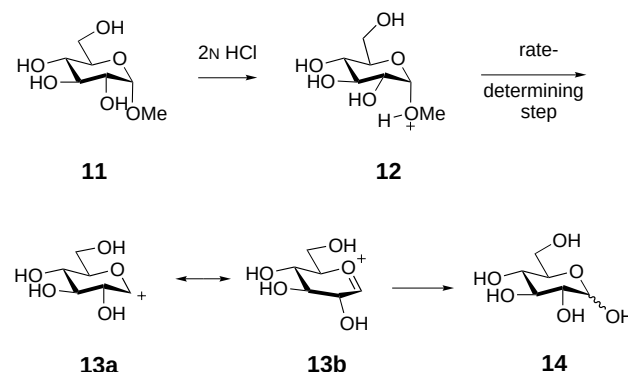
This difference in substituent effects of an axial and equatorial hydroxyl group must be expected to play a role in other compounds of similar structure, particularly in the carbocation **13** (Scheme 2) or in a transition state resembling **13**. The kinetics of acid-catalysed hydrolysis of simple glycosides were studied in detail by Overend et al.^[1f] They found



Scheme 1. Amines **1–10** and the pK_a values of their protonated forms at 25 °C.

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Scheme 2. The mechanism of acid-catalyzed hydrolysis of glycosides in 2N hydrochloric acid (taken from ref. [1f]).

that the reaction follows an A-1 mechanism with a rapid preequilibrium protonation step (**11** → **12**) followed by rate-determining fission of the glycosyl–oxygen bond. Since the transition state was expected to be late in this reaction, it resembles the cation **13a** quite closely, or possibly its oxocarbenium ion resonance form **13b**.

We therefore compared the free-energy differences $\Delta\Delta G^{\ddagger}$, calculated from the differences in pK_a value between the amines **3**, **4**, and **7–10**, with the differences in free energy of activation $\Delta\Delta G^{\ddagger}$ for the hydrolysis of the corresponding glycosides (Table 1).^[17] The values show the effect on acidity and rate of hydrolysis of changes in the 3- and 4-hydroxyl groups and are plotted in Figure 1. There is a remarkable

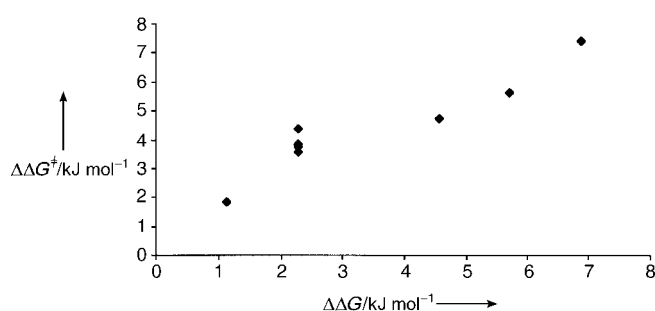
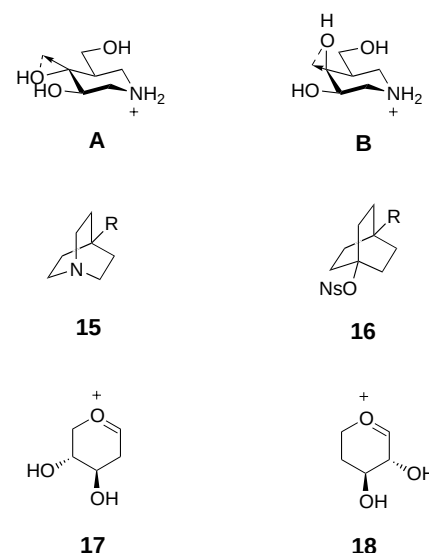


Figure 1. A plot of the differences in activation energy for acidic hydrolysis of glycosides $\Delta\Delta G^{\ddagger}$ versus the differences in free energy of protonation between isofagomines $\Delta\Delta G$.

correlation between $\Delta\Delta G$ and $\Delta\Delta G^{\ddagger}$ as the result of inverting or removing a hydroxyl group, and the best fit was obtained for the equation $\Delta\Delta G^{\ddagger} = \rho\Delta\Delta G$ with $\rho = 0.86$ ($r^2 = 0.94$).^[18] This shows that substituent effects can explain the difference in rate of hydrolysis of stereoisomeric glycosides. It also suggests that positive charge development on the anomeric center is more important in the hydrolysis reaction than previously conceived.

A similar comparison between amine basicity and transition state energy was made by Grob et al., who compared a series of substituted amines **15** with the rate of S_N1 solvolysis of a series of nosylates **16** (Scheme 3).^[19] They found a similar relationship to the above with a ρ value close to unity ($\rho = 1.02$).



Scheme 3. Structures of **A** and **B**, which show the difference in charge–dipole interaction for an axial and an equatorial 4-hydroxyl group, the amines **15** and nosylates **16** (R represents a range of different substituents), and the oxocarbenium ions **17** and **18**, obtained by hydrolysis of methyl 2-deoxy- and 4-deoxyxylopyranoside, respectively.

As mentioned above, the differences in rate between glycosides of different sugars have been explained as being due to the substrate's changing conformation to a half-chair transition state, and the conformational change supposedly becomes easier with an increasing number of axial substituents.^[1e,f] However, it is clear from the present results that the differing effects of substituents on charge development can adequately explain a large fraction of the differences in reactivity observed. This is in agreement with a recent study, which found that field effects largely dictate the rate of hydrolysis of dinitrophenyl glycosides with conservation of stereochemistry.^[2b] It does not directly agree with the postulate that the faster hydrolysis of galactosides is due to a through-space electrostatic interaction between the axial 4-OH group and an oxocarbenium ion,^[2a] but it is possible that both this effect and those reported here could contribute to the difference in rate of hydrolysis between glucosides and galactosides.

The present work also suggests that the transition state has considerable positive charge on the anomeric carbon atom,

Table 1. A comparison of the differences in free energy of protonation between isofagomines ($\Delta\Delta G$) with differences in activation energy for acidic hydrolysis of glycosides ($\Delta\Delta G^{\ddagger}$), both at 25 °C.

Compared amines	$\Delta\Delta G$ [kJ mol ⁻¹]	Compared glycosides	Relative hydrolysis rates ^[a]	$\Delta\Delta G^{\ddagger}$ [kJ mol ⁻¹]
3 and 4	2.28	methyl α -pyranoside, <i>galacto</i> -/ <i>gluco</i> -phenyl α -pyranoside, <i>galacto</i> -/ <i>gluco</i> -methyl β -pyranoside, <i>galacto</i> -/ <i>gluco</i> -phenyl β -pyranoside, <i>galacto</i> -/ <i>gluco</i> -methyl α -pyranoside, <i>altro</i> -/ <i>manno</i> -methyl α -pyranoside, 3-deoxygluco-/ <i>gluco</i> -methyl α -pyranoside, <i>ido</i> -/ <i>manno</i> -methyl α -pyranoside, <i>ido</i> -/ <i>altro</i> -	4.7	3.84
3 and 4	2.28		4.6	3.76
3 and 4	2.28		5.8	4.37
3 and 4	2.28		4.2	3.57
3 and 7	4.56		6.8	4.74
3 and 8	6.84		19.8 ^[b]	7.41
3 and 9	5.70		9.7 ^[b]	5.62
7 and 9	1.14		2.09 ^[b]	1.83

[a] The relative hydrolysis rates are $k_1(1)/k_1(2)$ at 25 °C. The rate constants for acidic glycoside hydrolysis at 60 °C with 2 N HCl were obtained from ref. [1f] and recalculated at 25 °C by using the Arrhenius equation and the activation energies and preexponential factors given in that paper. [b] The rate constant for 3-deoxyglucoside was only available at 58.4 °C, and that for the idoside only at 58.1 °C. The ratios were therefore calculated for these temperatures as it was assumed that the activation energy was identical for the glycosides compared. This is a reasonable assumption.^[14]

although it is reasonable to assume that charge is also present on the ring oxygen atom. The fact that the greatest deviation in the free-energy relationship was seen for the *galacto/gluco* hydrolysis rate seems to suggest this. The finding that a significant amount of the positive charge is located on the anomeric carbon directly contradicts the recent postulate that 97% of the positive charge in the transition state lies on the ring oxygen atom.^[2b] However, if through-space field effects control the rate of hydrolysis, it is not possible that so much charge could be located on the ring oxygen atom, because then 2- and 4-substituents should affect the rate of hydrolysis to a similar extent. Thus the transition states for hydrolysis of methyl 2- and 4-deoxyxylopyranoside **17** and **18** (Scheme 3), respectively, which have a high degree of symmetry, would be affected by substituent field effects of similar size. However since the rate of hydrolysis of 2-deoxyglycosides is about 50 times greater than that of 4-deoxyglycosides, this cannot be the case.^[20] Therefore, if through-space field effects control the rate of hydrolysis, a substantial amount of positive charge must also be present on the anomeric carbon atom.

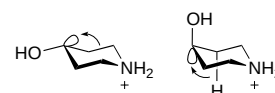
The following conclusions can be made: The greater rate of hydrolysis of galactosides can be explained on the basis of a smaller electron-withdrawing effect of an axial 4-OH group. Similarly, the inductive effects can explain the differences in rate of hydrolysis of other glycosides. Furthermore, it must be expected that other reactions at the anomeric center involving unimolecular mechanisms and charge development should be affected similarly. Secondly, the study shows that isofagomines are quite good transition-state analogues of nonenzymatic glycoside hydrolysis.

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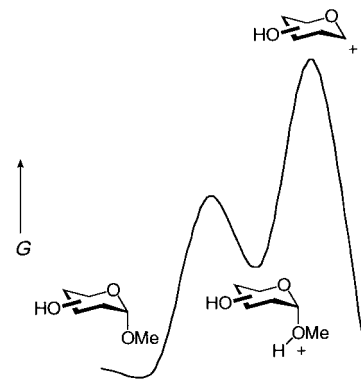
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- [13] It is known that the solvation energies of monosaccharides can differ, and particularly that the solvation energy of glucose and galactose differs (S. A. Galema, M. J. Blandamer, J. B. F. N. Engberts, *J. Am. Chem. Soc.* **1990**, 112, 9665–9666; S. A. Galema, M. J. Blandamer, J. B. F. N. Engberts, *J. Org. Chem.* **1992**, 57, 1995–2001). If such a difference exists between **1** and **2** or **3** and **4**, it would be expected to also exist in the protonated amines and hence not affect pK_a . The difference in solvolysis rates of glycosides does not appear to be an effect of solvation, because galactosides react faster than glucosides in solvents other than water as well.^[2a]
- [14] A referee suggested the following interesting explanation for the difference in electron-withdrawing power of equatorial and axial OH groups: With an equatorial OH group the antibonding orbital of the C–O bond can overlap with the adjacent C–C bond and thus remove electronic charge from the amine (see below left). With an axial OH group the overlap will occur with the adjacent C–H bond, and will not remove charge from the amine (below right).



- [15] Calculated from $\Delta\Delta G = RT \ln [K_a(1)/K_a(2)]$ at 25 °C.
- [16] Calculated from $\Delta\Delta G^\ddagger = RT \ln [k_1(1)/k_1(2)]$ at 25 °C. Since no rate constants were available at this temperature the Arrhenius equation $k_1 = A \exp(-E_a/RT)$ and data from ref. [1c] were used to calculate the rate constants at 25 °C. However, the ratios $k_1(1)/k_1(2)$ did not change much with temperature, as the activation energy E_a only varies by 10% between the different glycosides. Therefore, employing the ratio of rate constants at 60 °C gave a very similar result.
- [17] The observed rate constant k_{obs} of Overend et al. is equal to an equilibrium constant K for exocyclic protonation multiplied by a rate constant k for conversion of the conjugate acid to the transition state (i.e., $k_{\text{obs}} = K \times k$). Our activation energies were derived from k_{obs} , which is the rate constant for going from the unprotonated methyl glycoside to the transition state, and we thereby obtain the activation energy for the entire process, as can be seen from the following energy diagram.



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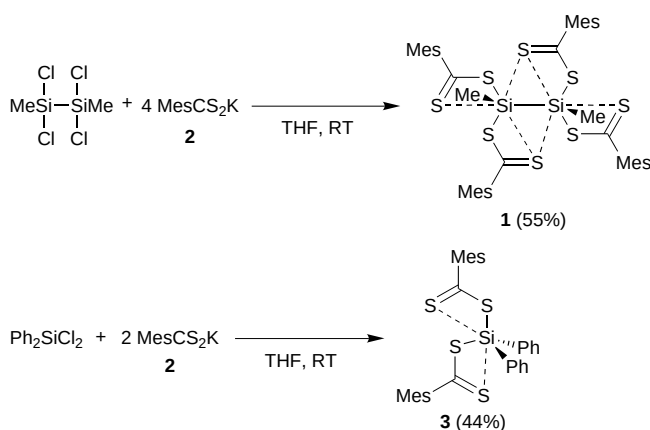
A Disilane Containing Two Heptacoordinate Silicon Atoms and Dithiocarboxylate Ligands**

Naokazu Kano, Norikiyo Nakagawa, and Takayuki Kawashima*

Organosilicon compounds with high coordination numbers have been extensively studied because of their interesting structural features and synthetic utility.^[1] Disilanes that contain exclusively hypervalent silicon atoms are of current interest as components of high-coordination-number polysilanes, which are expected to show unique electronic and optical properties.^[2] In spite of theoretical predictions that Si–Si bonds are not good candidates for hypercoordination, a few examples of pentacoordinate silylsilicates have been reported, as in the cases of disilanes with pentacoordinate or hexacoordinate silicon atom(s) due to intramolecular coordination of oxygen or nitrogen atoms.^[3, 4] However, there have been no reports on disilanes with hepta- or octacoordinate silicon atoms.^[5] Compounds of Group 14 elements containing dithiocarboxylate ligands (RCS_2^-) are known to exhibit intramolecular interaction between the central atom (Ge, Sn, and Pb) and the thiocarbonyl sulfur atom.^[6] Although such an intramolecular coordination is expected for silicon analogues, few examples of RCS_2SiR_3 have been reported, and no X-ray structures are available.^[6a,c, 7] Here we report on a monosilane and a disilane with two dithiocarboxylate ligands on each Si atom; the latter is the first structurally characterized heptacoordinate disilane.

Compound **1** was synthesized in 55% yield by the reaction of 1,1,2,2-tetrachloro-1,2-dimethyldisilane with 4.4 equivalents of potassium 2,4,6-trimethyldithiobenzoate (**2**, Scheme 1), which was prepared from the corresponding arenecarbodithioic acid and potassium hydride.^[8] Compound **3** was similarly synthesized from dichlorodiphenylsilane and 2.1 equivalents of **2** in 44% yield (Scheme 1). Compounds **1** and **3** are thermally quite stable, but highly sensitive to moisture. The alcoholysis of **1** and **3** with absolute ethanol in C_6D_6 at room temperature occurred immediately and quantitatively to give $(\text{EtO})_2\text{MeSiSiMe}(\text{OEt})_2$ and $(\text{EtO})_2\text{SiPh}_2$, respectively, together with 2,4,6-trimethyldithiobenzoic acid.

An X-ray crystallographic analysis was performed on **3** to clarify whether the thiocarbonyl groups interact with the silicon atom.^[9] The unit cell contains two independent molecules of **3** that are very similar to each other. The



Scheme 1. Synthesis of **1** and **3**. Mes = 2,4,6-trimethylphenyl.

ORTEP plot of one molecule is shown in Figure 1. Compound **3** has a slightly distorted tetrahedral structure, as indicated by the bond angles ($99.3(3)$ – $116.5(3)^\circ$) around the silicon atom. Both thiocarbonyl groups of the MesCS_2 ligands are directed towards the central silicon atom, as reported previously for heavier analogues.^[6] Thiocarbonyl sulfur atoms S2 and S4 are

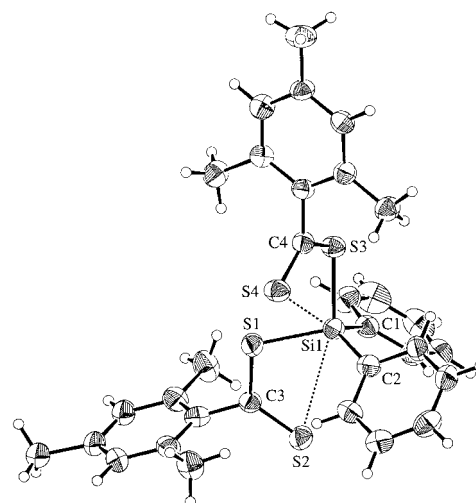


Figure 1. ORTEP plot of **3** (30% probability thermal ellipsoids for all non-hydrogen atoms). Selected bond lengths [Å], interatomic distances [Å], and bond angles [$^\circ$]: Si1–Si2 2.165(3), Si1–S3 2.183(3), Si1–C1 1.842(9), Si1–C2 1.841(10), C3–Si1 1.743(8), C3–S2 1.646(9), C4–S3 1.745(9), C4–S4 1.630(9), Si1...S2 3.315(3), Si1...S4 3.341(3); S1–Si1–S3 102.17(12), S1–Si1–C1 112.0(3), S1–Si1–C2 116.5(3), S3–Si1–C1 99.3(3), S3–Si1–C2 111.2(3), C1–Si1–C2 113.8(4), Si1–Si1–C3 104.6(3), Si1–S3–C4 104.6(3).

located on the opposite side to S3 and C1, respectively, in contrast to the situation of the tin compound $(4\text{-MeC}_6\text{H}_4\text{CS}_2)_2\text{Sn}_2\text{Ph}$.^[6d] The intramolecular distances between the silicon and thiocarbonyl sulfur atoms S2 and S4 are 3.315(3) and 3.341(3) Å, respectively, which are considerably shorter than the sum of the van der Waals radii (3.90 Å), although they are much longer than the sum of the corresponding covalent-bond radii (2.22 Å).^[10] The ratio of $\text{M}\cdots\text{S}$ intramolecular distances and the sum of the corresponding van der Waals radii (14 or 15%) is similar to those of $(4\text{-CH}_3\text{C}_6\text{H}_4\text{CS}_2)\text{MPh}_3$ ($\text{M} = \text{Ge}$, 11%; $\text{M} = \text{Sn}$, 19%; and $\text{M} =$

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Pb, 12 %).^[6d] These structural features of **3** are indicative of intramolecular Si–S interactions and a neutral, hexacoordinate silicon atom. Such an interaction was also observed for silanyl monothioacetate.^[11] The C–S bond length (1.743(8) and 1.745(9) Å) lies between the sums of the covalent radii for a C–S single (1.81 Å) and double bond (1.62 Å), while the C=S bond lengths (1.646(9) and 1.630(9) Å) are close to the value for a double bond. Two Si–S bond lengths (av 2.174 Å) and two Si–C bond lengths (av 1.842 Å) show almost no differences from those previously reported for Me₂Si(SR)₂.^[12] This is the first X-ray analysis of an organosilicon compound bearing dithiocarboxylate ligands.

A similar coordination of thiocarbonyl sulfur atoms to the silicon atoms was also observed in the X-ray structure of **1**·C₆H₆ (Figure 2).^[9] The four Si–S (2.184(3)–2.199(3) Å) and two Si–C bond lengths (1.856(9) and 1.862(9) Å) showed

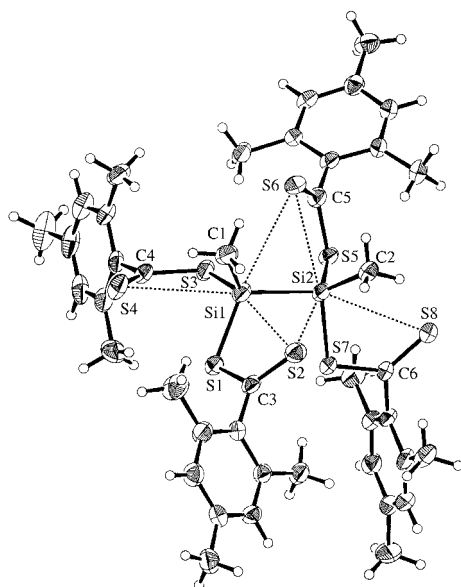


Figure 2. ORTEP plot of **1**·C₆H₆ (30% probability thermal ellipsoids for all non-hydrogen atoms). Benzene molecules are omitted for clarity. Selected bond lengths [Å], interatomic distances [Å], bond angles [°], and torsion angles [°]: Si1–Si2 2.351(3), Si1–C1 1.856(9), Si2–C2 1.862(9), Si1–S1 2.193(3), Si1–S3 2.184(3), Si2–S5 2.199(3), Si2–S7 2.184(3), C3–S1 1.757(8), C3–S2 1.620(8), C4–S3 1.759(8), C4–S4 1.616(9), C5–S5 1.745(8), C5–S6 1.648(9), C6–S7 1.761(8), C6–S8 1.624(9), Si1···S2 3.049(3), Si1···S4 3.461(3), Si1···S6 3.438(4), Si2···S2 3.356(4), Si2···S6 3.076(3), Si2···S8 3.478(3); S1–Si1–S3 99.8(1), S1–Si1–C1 112.3(3), S1–Si1–Si2 111.6(1), S3–Si1–C1 113.6(3), S3–Si1–Si2 99.7(1), C1–Si1–Si2 117.8(3), S5–Si2–S7 97.5(1), S5–Si2–C2 111.1(4), S5–Si2–Si1 112.1(1), S7–Si2–C2 115.8(3), S7–Si2–Si1 100.4(1), C2–Si2–Si1 117.8(3), Si1–Si1–C3 97.9(3), Si1–S3–C4 106.7(3), Si2–S5–C5 99.3(3), Si2–S7–C6 107.3(3), S1–Si1–Si2–S7 14.5(4), S3–Si1–Si2–S5 12.4(4), C1–Si1–Si2–C2 19.9(2).

minor differences from those of **3**. The Si1–Si2 bond length (2.351(3) Å) is almost the same as those of Me(RS)₂–Si–SiMe(SR)₂ (2.3481(11)–2.3642(11) Å).^[12] In the case of Si1, the thiocarbonyl sulfur atoms S4 and S2 are located opposite to Si2 (3.461(3) Å) and S3 (3.049(3) Å), respectively. The sulfur atoms of the other two thiocarbonyl groups have a similar arrangement with respect to the Si2 atom. Thiocarbonyl sulfur atoms S2 and S6 are both located in the vicinity of both silicon atoms, while the other thiocarbonyl sulfur atoms

are close (3.461(3), 3.478(3) Å) to only one silicon atom.^[13] Since the interatomic Si···S distances are within the sum of the van der Waals radii, disilane **1** clearly contains two heptacoordinate silicon atoms with four covalent bonds and three weak Si···S intramolecular interactions. On the other hand, the bond angles (97.5(1)–117.8(3)°) around the silicon atoms indicate retention of the basic tetrahedral structure. Disilane **1** adopts a nearly eclipsed conformation along the Si–Si bond axis according to the corresponding torsion angles (12.4(4)–19.9(2)°). Such a conformation facilitates the coordination of S2 and S6 to the two silicon atoms, which thus avoid steric repulsion of each other. If the silicon atoms adopted a staggered conformation, the thiocarbonyl sulfur atom which is coordinated to two silicon atoms would impinge upon another sulfur atom. This is the first example of a heptacoordinate disilane in which two silicon atoms with tricapped tetrahedral structures are directly connected to each other. The intramolecular Si···S interactions of **1** and **3**, which are weak, as indicated by the interatomic distances, are responsible for the heptacoordinate state of the silicon atoms of **1**, in contrast to previously reported disilanes with high coordination numbers.^[4]

The high coordination number of the silicon atoms in **1** and **3** is firstly attributable to the interaction of the lone pair on the thiocarbonyl sulfur atom with the corresponding σ* orbitals of Si–S, Si–C, or Si–Si bonds on their opposite sides, as observed in other compounds of Group 14 elements with dithiocarboxylate ligands.^[6e] Secondly, their lone pairs may additionally interact with the σ*_{Si–S} orbitals of the same dithiocarboxylate ligand.^[14] The combination of these interactions could be responsible for the widely ranging interatomic Si···S distances in **1**.

In the ¹H and ¹³C NMR spectra of **1** and **3**, the signals for all thiocarbonyl carbon atoms, all mesityl groups, two methyl groups on the silicon atoms of **1**, and two phenyl groups of **3** were equivalent. The chemical shifts of the carbon atoms of thiocarbonyl groups were similar to those of other compounds of Group 14 elements with dithiocarboxylate ligands.^[6e] In the ²⁹Si NMR spectra, signals of **1** (δ_{Si} = –11.9) and **3** (δ_{Si} = –10.1) were observed in a similar region to that of MesCS₂–SiPh₃ (δ_{Si} = –6.4)^[15] and were shifted upfield relative to those of Ph₂Si(S*n*Bu)₂ (δ_{Si} = 10.1) and Me(RS)₂SiSiMe(SR)₂ (δ_{Si} = 9.7 for R = Ph and δ_{Si} = 0.1 for R = *t*Bu).^[16] Some of the intramolecular Si–S interactions appear to be maintained in solution, judging from the upfield shifts. The chemical shifts are in low-field regions for hexacoordinate and heptacoordinate silicon atoms, and this indicates that the coordination of the sulfur atoms to the silicon atom is weak. In the UV/Vis spectra, the n–π* transition of **1** was observed 23 nm to shorter wavelength than that of **3**. This blue shift is most likely to originate in the stabilization of the ground state of **1** by the larger number of orbital interactions between the lone pairs of thiocarbonyl sulfur atoms and σ* orbitals of Si–C, Si–S, or Si–Si bonds, which lie at the relatively low-energy levels of the silicon atoms.

In summary, we have synthesized monosilane **3** and disilane **1**, each of which bears two [2,4,6-trimethyl(thiobenzoyl)]thio ligands on each silicon atom, and determined their structures. Compound **3** has a hexacoordinate structure with weak

intramolecular interactions between the silicon atom and the sulfur atoms of thiocarbonyl groups. X-ray analysis of disilane **1** revealed the direct connection between of two heptacoordinate silicon atoms. Heptacoordination of the silicon atoms in **1** is achieved by an additional intramolecular coordination of one thiocarbonyl sulfur atom to the silicon atom to which its [2,4,6-trimethyl(thiobenzoyl)]thio group is not attached. These results not only showed an interesting structural similarity between compounds of silicon and those of other Group 14 elements, but also suggested the possibility of synthesizing polysilanes with high coordination numbers.

Experimental Section

1: 1,1,2,2-Tetrachloro-1,2-dimethyldisilane (0.038 mL, 0.21 mmol) was added to a solution of **2** (0.216 g, 0.925 mmol) in THF (4 mL) at room temperature. After stirring for 30 min, the solvent was evaporated under reduced pressure. After insoluble materials were removed, the residue was recrystallized from hexane and benzene to give red crystals of **1** (0.101 g, 55 %). M.p. 209–210 °C (decomp); ¹H NMR (500 MHz, C₆D₆): δ = 1.61 (s, 6H), 2.08 (s, 12H), 2.43 (s, 24H), 6.64 (s, 8H); ¹³C NMR (125 MHz, C₆D₆): δ = 3.18 (q), 19.69 (q), 20.90 (q), 128.97 (d), 132.10 (s), 138.00 (s), 146.68 (s), 242.38 (s, C=S); ²⁹Si NMR (99 MHz, C₆D₆): δ = –11.90 (s); UV/Vis (hexane): λ_{max} (lg ε) = 312 (4.5), 496 nm (2.3); IR (KBr): ν̄ = 1255 cm^{–1} (br, C=S); Elemental analysis (%) calcd for C₄₂H₅₀S₈Si₂: C 58.15, H 5.81; found: C 57.38, H 6.15.

3: Dichlorodiphenylsilane (0.095 mL, 0.45 mmol) was added to a solution of **2** (0.225 g, 0.960 mmol) in THF (4 mL) at room temperature. After stirring for 30 min, the solvent was evaporated under reduced pressure. After insoluble materials were removed, the residue was recrystallized from hexane to give red crystals of **3** (0.113 g, 44 %). M.p. 180–184 °C (decomp); ¹H NMR (500 MHz, C₆D₆): δ = 2.05 (s, 6H), 2.29 (s, 12H), 6.61 (s, 4H), 7.10–7.18 (m, 6H), 8.14–8.15 (m, 4H); ¹³C NMR (125 MHz, C₆D₆): δ = 19.27 (q), 20.90 (q), 128.83 (d), 129.46 (s), 131.19 (d), 131.75 (s), 135.18 (d), 136.48 (d), 137.68 (s), 147.29 (s), 238.42 (s, C=S); ²⁹Si NMR (99 MHz, C₆D₆): δ = –10.14 (s); UV/Vis (hexane): λ_{max} (lg ε) = 318 (4.4), 519 nm (2.0); IR (KBr): ν̄ = 1261 (C=S), 1241 cm^{–1} (C=S); Elemental analysis (%) calcd for C₃₂H₃₄S₄Si: C 67.08, H 5.63; found: C 66.84, H 5.85.

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Generation and Stabilization of D_{6h} and C_{2v} Valence Tautomeric Structures of the Rhodizonate Dianion in Hydrogen-Bonded Host Lattices**

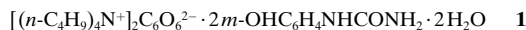
Chi-Keung Lam and Thomas C. W. Mak*

Dedicated to Dr. Tze-Lock Chan
on the occasion of his 60th birthday

The monocyclic oxocarbon dianions, $C_nO_n^{2-}$ ($n=3$, delatate; $n=4$, squarate; $n=5$, croconate; $n=6$, rhodizonate), constitute a series of nonbenzenoid aromatic systems whose historical significance and interesting chemistry are well-documented in several books and reviews.^[1] A wealth of spectroscopic and X-ray crystallographic data are available for numerous squarate^[2] and croconate^[3] metal complexes, including a recent report on the crystal structure of $K_2C_5O_5 \cdot 2H_2O$.^[4] On the other hand, owing to the relative instability of the delatate and rhodizonate dianions, very meager structural information has been gathered for these two species.^[5–7] Until now, the known rhodizonate compounds consist of salts of the alkali metals, alkaline earth metals, transition elements, and ammonium ions. It is noteworthy that the only well-characterized rhodizonate salts are $K_2C_6O_6$ and $Rb_2C_6O_6$, whose X-ray structures were described in a doctoral dissertation published in 1965^[7] and widely quoted in the literature.^[1]

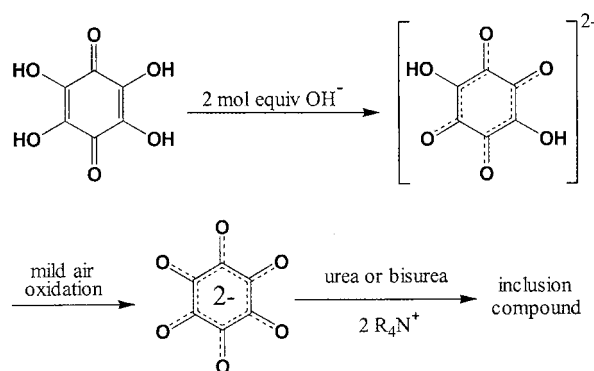
For over four decades, interest in the whole family of oxocarbon dianions has focused on their aromaticity and molecular structure. The latest theoretical studies indicated that aromaticity decreases with increasing ring size, and that $C_6O_6^{2-}$ possesses only a small degree of aromatic character.^[8] Furthermore, while it is firmly established that the squarate and croconate ions have planar D_{nh} structures, infrared spectroscopy^[6] and X-ray analysis^[7] indicates that the rhodizonate ion conforms to idealized D_{6h} geometry in $Rb_2C_6O_6$, but nonplanar D_{3d} and C_2 forms coexist in $K_2C_6O_6$.^[1, 7]

In the course of our systematic investigation on inclusion compounds of urea and thiourea with quaternary ammonium salts,^[9] we found that an elusive anionic species such as allophanate^[10] or dihydrogen borate^[11] can be generated in situ and stabilized in a hydrogen-bonded host lattice. In our follow-up studies, the squarate ion proved to be an effective divergent multisite hydrogen-bond acceptor for supramolecular assembly.^[12] Against this background, we planned the synthesis (Scheme 1, starting with tetrahydroxy-1,4-quinone rather than rhodizonic acid) of two air-stable crystalline inclusion compounds of tetra-*n*-butylammonium rhodizonate with urea derivatives, namely **1** and **2**, and determined their X-ray structures.^[13]



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Scheme 1.

In the host lattice of **1**, the planar rhodizonate dianion resides at an inversion center, being directly linked to a pair of (3-hydroxyphenyl)urea molecules through pairs of $N-H \cdots O$ (**A**, $N_2=R_3^2(9)$)^[14] and phenyl $C-H \cdots O$ (**B**, $N_2=R_1^2(6)$) hydrogen bonds to form a puckered trimer (Figure 1). The trimer is consolidated by a pair of bridging water molecules

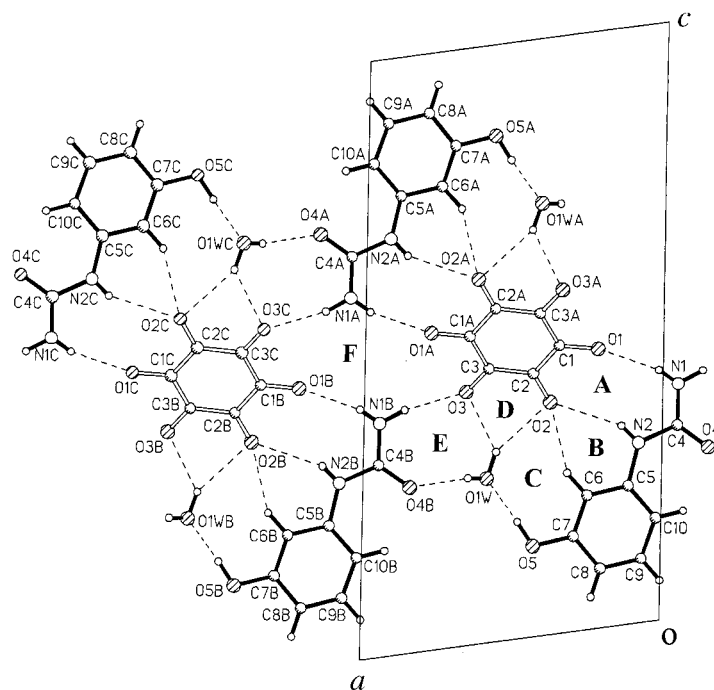


Figure 1. Projection down the *b* axis showing extensive hydrogen bonding interactions around the centrosymmetric rhodizonate dianion in the host lattice of $[(n-C_4H_9)_4N^+]_2C_6O_6^{2-} \cdot 2m-OHC_6H_4NHCONH_2 \cdot 2H_2O$ (**1**). Symmetry transformations: A: $1-x, 1-y, 1-z$; B: $1+x, y, z$; C: $2-x, 1-y, 1-z$.

that form strong $O-H \cdots O$ hydrogen bonds (**C**, $N_3=R_3^2(8)$ and **D**, $N_2=R_1^2(5)$) with the hydroxy and rhodizonate carbonyl groups to generate a pentamer. Adjacent pentamers are further connected together by strong $O-H \cdots O$ and $N-H \cdots O$ hydrogen bonds (**E**, $N_3=R_3^2(8)$ and **F**, $N_4=R_4^4(14)$) to produce a wide, puckered ribbon running parallel to the *a* axis. Well-ordered $(n-C_4H_9)_4N^+$ ions are accommodated between broken sinusoidal layers formed by the alternately stacked puckered ribbons, with an interlayer spacing of about 8.0 Å.

In the host lattice of **2**, two independent 1,1'-ethylenediurea molecules are directly bound to the rhodizonate dianion, which occupies a general position, by four pairs of strong N–H···O hydrogen bonds (**A**, **B**, **C**, and **D**, $N_2 = R_2^2(9)$) to yield a trimer (Figure 2). Adjacent trimers related by simple

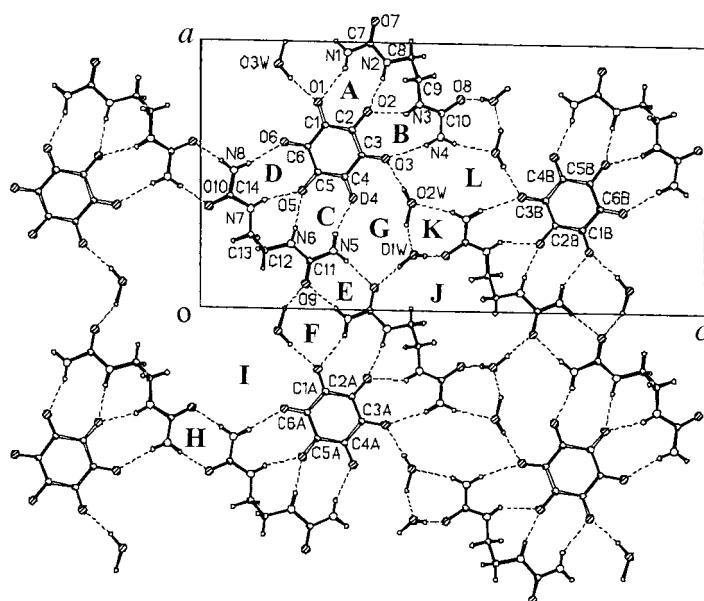


Figure 2. Projection down the *b* axis showing the hydrogen bonding interactions within the puckered rhodizonate-bisurea-water layer of $[(n\text{-C}_4\text{H}_9)_4\text{N}^+]_2\text{C}_6\text{O}_6^{2-} \cdot 2\text{NH}_2\text{CONHCH}_2\text{CH}_2\text{NHCONH}_2 \cdot 3\text{H}_2\text{O}$ (**2**). Symmetry transformations: A: $x - 1, y, z$; B: $-x, 1 - y, 1 - z$.

translation along the *a* axis are connected together by pairs of amido N–H···O hydrogen bonds in the usual shoulder-to-shoulder manner^[9] (**E**, $N_2 = R_2^2(8)$) to produce a zigzag ribbon, which is further consolidated by three independent water molecules through additional strong O–H···O hydrogen bonds to generate two new motifs (**F**, $N_4 = R_4^4(8)$ and **G**, $N_5 = R_5^5(13)$). Two antiparallel ribbons related by $\bar{1}$ are cross-linked by pairs of N–H···O (**H**, $N_2 = R_2^2(8)$) hydrogen bonds to yield a double ribbon with large voids (**I**, $N_8 = R_8^8(36)$). Adjacent double ribbons related by the *c* translation are interwoven with three additional ring motifs (**J**, $N_4 = R_4^4(22)$, **K**, $N_3 = R_3^3(8)$ and **L**, $N_6 = R_6^6(12)$) by strong N–H···O and O–H···O hydrogen bonds to generate a highly corrugated layer. Well-ordered $(n\text{-C}_4\text{H}_9)_4\text{N}^+$ ions are sandwiched between adjacent layers with an interlayer spacing of about 7.8 Å.

A recent theoretical study concluded that while the oxocarbon dianions $\text{C}_n\text{O}_n^{2-}$ ($n = 3\text{--}5$) all favor D_{nh} symmetry, the $\text{C}_6\text{O}_6^{2-}$ ground state has C_2 symmetry, and all isomeric structures are very close in energy, for example, the energy difference is 0.34 kcal mol^{−1} between the D_{6h} and nonplanar C_2 forms.^[8b] Notably the measured dimensions of the relatively unstable $\text{C}_6\text{O}_6^{2-}$ species in **1**

and **2** (the mean deviation from planarity is 0.020(4) and 0.010(3) Å for **1** and **2** respectively) nearly conform to idealized D_{6h} and C_{2v} molecular symmetry, corresponding to distinct valence tautomeric structures that manifest non-benzenoid aromatic and enediolate character, respectively (Figure 3).

The measured C–C bond lengths in **1** (1.440(5)–1.442(5) Å) and **2** (1.404(5)–1.472(5) Å) are markedly shorter than those (1.488 and 1.501 Å) in $\text{Rb}_2\text{C}_6\text{O}_6$ ^[7] and the computed values (1.500 and 1.501 Å) for the C_2 structure,^[8b] implying that π -electron delocalization in the aromatic ring of $\text{C}_6\text{O}_6^{2-}$ is much more extensive than hitherto recognized. The strong negative charge-assisted hydrogen bonding interaction between rhodizonate and 1,1'-ethylenediurea causes the latter to assume a less stable *gauche* conformation, in preference to its favored *anti* conformation. Occurrence of the charge-localized structure of $\text{C}_6\text{O}_6^{2-}$ in **2**, as well as its noticeable deviation from idealized C_{2v} molecular symmetry, can be attributed to unequal hydrogen bonding interaction with its two neighboring bisurea donors and a pair of water molecules (Figure 2). The present finding would seem to provide a challenge for further theoretical work on monocyclic oxocarbon dianions and related species, despite the fact that the most recent publication on this topic appeared only a year ago.^[8b]

Experimental Section

General: Tetra-*n*-butylammonium hydroxide (40 wt% in water) was purchased from Aldrich. (3-Hydroxyphenyl)urea,^[15] 1,1'-ethylenediurea,^[16] and tetrahydroxy-1,4-quinone^[17] were prepared according to literature methods. IR spectra were recorded of KBr pellets on a Nicolet Impact 420 FT-IR spectrometer in the region of 4000–400 cm^{−1}. Melting points (uncorrected) were measured on a IA 9100 Electrothermal Digital Melting Point Apparatus.

Bis(tetra-*n*-butylammonium) rhodizonate-(3-hydroxyphenyl)urea-water (1/2/2) (**1**): To a 50 mL round-bottomed flask containing tetrahydroxy-1,4-quinone (0.035 g, 0.168 mmol), two molar equivalents (0.71 mL) of tetra-*n*-butylammonium hydroxide (40 wt% in water, diluted to 0.486 M) were added. The flask was stoppered and the solution stirred until all solid material had been completely dissolved. (3-Hydroxyphenyl)urea (0.102 g) was then added to the deep orange-brown solution and stirred for about half an hour. The solution was concentrated to dryness under reduced pressure, and the solid residue redissolved in absolute ethanol. The filtrate was allowed to evaporate in a desiccator charged with anhydrous calcium chloride. Well-formed deep brownish red polyhedra were obtained in

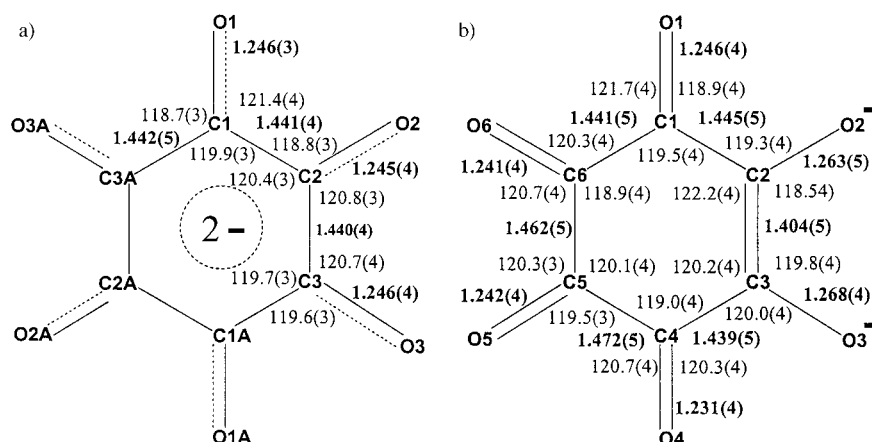


Figure 3. Bond lengths and angles of the a) D_{6h} and b) C_{2v} valence tautomers of the rhodizonate dianion in **1** and **2**, respectively.

nearly quantitative yield after about one week. The crystals were found to be stable in air. M.p. 148–150 °C (decomp); IR(KBr): $\tilde{\nu}$ = 3435 (s, br), 3315 (ms, sh), 3261 (ms, sh), 3091 (w, sh), 2961 (m, sharp), 2874 (w), 1699 (s, sharp), 1678 (m, sh), 1604 (s, sharp), 1547 (vs, sharp), 1522 (vs, sh), 1494 (s, sh), 1353 (m, sh), 1327 (m, br), 1178 (m, sharp), 1158 (m, sharp), 875 (m, sharp), 778 (m, sharp), 699 (w) cm^{-1} ; C, H, N analysis calcd for $[(n\text{-C}_4\text{H}_9)_4\text{N}^+]_2\text{C}_6\text{O}_6^{2-} \cdot 2m\text{-OHC}_6\text{H}_4\text{NHCONH}_2 \cdot 2\text{H}_2\text{O}$ (M_r = 993.32): C 62.88, H 9.33, N 8.46; found: C 62.48, H 9.37, N 8.41.

Bis(tetra-*n*-butylammonium) rhodizonate–1,1'-ethylenediurea–water (1/2/3) (**2**): The above procedures were employed using tetrahydroxy-1,4-quinone (0.051 g), tetra-*n*-butylammonium hydroxide (1.00 mL), and 1,1'-ethylenediurea (0.107 g). Air-stable rose red prisms were obtained in nearly quantitative yield after about ten days. M.p. 187.9–191.5 °C, IR(KBr): $\tilde{\nu}$ = 3426 (vs, br), 3295 (s, sh), 3100 (w, sh), 2958 (m, sharp), 2873 (w), 1666 (s, sharp), 1550 (vs, sharp), 1521 (vs, sh), 1360 (w, sharp), 1158 (w, sharp), 790 (w, sh), 702 (m, sh), 543 (ms, br) cm^{-1} ; C, H, N analysis calcd for $[(n\text{-C}_4\text{H}_9)_4\text{N}^+]_2\text{C}_6\text{O}_6^{2-} \cdot 2\text{NH}_2\text{CONHCH}_2\text{CH}_2\text{NHCONH}_2 \cdot 3\text{H}_2\text{O}$ (M_r = 999.34): C 55.29, H 9.88, N 14.01; found: C 54.18, H 10.04, N 13.81. The results of elemental analysis are in better agreement with the presence of an extra water molecule in the structural formula of **2**, which might be due to absorption of atmospheric moisture by the mailed sample.

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$2\text{NH}_2\text{CONHCH}_2\text{CH}_2\text{NHCONH}_2 \cdot 3\text{H}_2\text{O}$ (**2**), M_r = 999.34, monoclinic, space group $P2_1/c$ (no. 14), a = 13.933(2), b = 15.576(2), c = 26.713(3) Å, β = 91.042(2)°, V = 5797(1) Å³, Z = 4, ρ_{calcd} = 1.145 Mg m^{-3} , $F(000)$ = 2192, $\mu(\text{Mo K}\alpha)$ = 0.080 mm^{-1} , 25 578 reflections measured, 7585 unique (R_{int} = 0.0685), final R = 0.0494, $wR2$ = 0.1165 for 3310 observed reflections [$I > 2\sigma(I)$]. Data collection was performed at 293 K on a Bruker SMART 1000 CCD diffractometer using frames of oscillation range 0.3°, with 3° < θ < 28°. The structures were solved by direct methods, and all non-hydrogen atoms were refined anisotropically by full-matrix least-squares on F^2 using the SHELXTL program.^[18] 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-163556 (**1**) and CCDC-163557 (**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).

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A Practical and Highly Efficient Aminohydroxylation of Unsaturated Carboxylic Acids**

Valery V. Fokin* and K. Barry Sharpless*

Dedicated to Professor Harry H. Wasserman on the occasion of his 80th birthday

Osmium-catalyzed aminohydroxylation is a powerful synthetic transformation that allows conversion of olefins to commonly occurring β -amino alcohols. Products obtained by this route are important intermediates in the synthesis of natural products, pharmaceutically important compounds,

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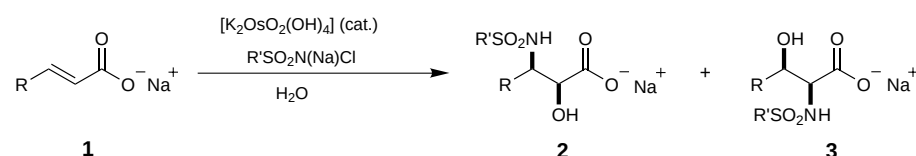
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Supporting information for this article is available on the WWW under <http://www.angewandte.com> or from the author.

new materials, and catalysts. Both racemic^[1] (“A”) and asymmetric^[2] (“AA”) variants of this reaction have been reported, and various classes of olefins have been studied as substrates. In efforts to develop this process further, α,β -unsaturated carboxylic acids were examined as potential substrates. The ready availability of these olefins from natural sources, outstanding synthetic methods for their preparation, and importance of α,β -hydroxyamino acid derivatives obtained make them one of the most attractive classes of olefins for aminohydroxylation.

The present asymmetric versions of both the osmium-catalyzed dihydroxylation and aminohydroxylation reactions depend crucially on the ligand-acceleration effect.^[3] Additionally, the cinchona alkaloid ligands enhance chemoselectivity in the aminohydroxylation by suppressing diol formation. Herein, we report that with α,β -unsaturated carboxylates as substrates the aminohydroxylation proceeds rapidly *in the absence* of cinchona alkaloid ligands (Scheme 1). A consequence of this remarkable ligand-independent reactivity is that the process is not enantioselective, even in the presence of a large excess of the chiral ligand.

However, this new ligand-independent aminohydroxylation process is otherwise superior to the AA with alkaloid ligands in the following ways: 1) it requires much lower loading of the osmium catalyst (0.1–1.0 mol % as opposed to 4–5 mol %); 2) it needs only a stoichiometric amount of the nitrogen source (cf. the >3 equivalents needed in the AA); 3) diol



Scheme 1. Aminohydroxylation of α,β -unsaturated carboxylates.

formation, an inevitable side reaction under standard A and AA conditions, and which, in worst cases, accounts for as much as 40% of the product, has not been observed; 4) a range of solvents can be employed for the reaction (e.g. water/*tert*-butyl alcohol and water/acetonitrile), but very importantly, *it often proceeds just as well in water without any organic cosolvent*; 5) scale-up is greatly facilitated by the ability to run the reaction at high concentrations (0.3–0.8 M in olefin being typical), whereas the AA requires concentrations <0.1 M for optimal results; and finally, 6) since the only coproduct is sodium chloride, product isolation is very easy for, upon acidification, most of the α,β -hydroxyamino acid derivatives precipitate and are collected by simple filtration as pure materials. It should be noted that carboxylic acids must be converted to the corresponding salts before addition of the chloramine. Given that aminohydroxylation is markedly impeded in a highly alkaline medium (pH > ca. 10), sodium bicarbonate appears to be among the best neutralizing agents. It can be used in slight excess without raising the pH significantly, thus further simplifying the experimental procedure.

A wide range of unsaturated acids was examined in this study. Simple alkenoic acids, such as acrylic, methacrylic, and

fumaric, participate readily in the reaction providing easy access to α,β -hydroxyamino acids. When unsymmetrically substituted alkenes are used, only one product results, always bearing nitrogen on the less substituted carbon. Selected results are presented in Table 1. For example, *N*-(*p*-toluene-

Table 1. Aminohydroxylation of simple alkenoic acids.

Entry	Substrate	Os [mol %]	Time [h]	Yield [%]
1		0.5	8	94
2		0.5	6	98
3		0.2	6	98
4		0.5	6	98
5		1.0	12	88

sulfonyl)-protected isoserine and hydroxyaspartic acids were easily prepared in one step in excellent yields. When necessary, the tosyl protecting group can be reductively removed, liberating the free amino acid.^[4]

When variously substituted aryl-acrylic acids (Table 2) were subjected to aminohydroxylation, mixtures of both regioisomers were obtained, favoring nitrogen in the benzylic position (**2**) in all cases. Most of the time, separation of these regioisomers did not present a problem as their solubility in water and/or water/alcohol mixtures differs significantly.^[5]

Although at present this process yields racemic mixtures of products, their resolution with chiral bases is a well-established methodology that can be easily carried out on a large scale.^[6]

The scope of the reaction regarding viable sulfonamide precursors of the electrophilic nitrogen was also examined and found to be excellent, including, for example, 4-nitrobenzene- and methanesulfonamide.^[7] The former one, devised by Fukuyama et al. to allow removal under very mild conditions,^[8] enables liberation of parent hydroxyamino acids in high yields.

In conclusion, we have developed a new method for converting α,β -unsaturated carboxylic acids to α,β -hydroxyamino acid derivatives in one step. The reaction exhibits wide scope and excellent chemoselectivity. It is the latest, and most impressive, example to date in the ligand-independent family of aminohydroxylations. Hence, unsaturated acids now join α,β -unsaturated amides^[9] and Baylis–Hillman olefins^[10] as privileged substrates for this transformation. These ligand-

Table 2. Aminohydroxylation of arylacrylic acids.^[a]

Entry	Substrate	Time [h]	Ratio 2:3	Yield [%]
1		10	1.6:1	96
2		10	1.6:1	91
3		10	1.8:1	88
4		5	3.0:1	92
5		8	1.6:1	90
6		12	3.0:1	88

[a] All reactions were performed with 1.0 mol % of Os catalyst.

independent catalytic processes are several orders of magnitude more efficient than the other versions of the osmium-catalyzed aminohydroxylations which have been reported since its discovery in 1976. The mechanistic foundation for this exceptional catalytic behavior, as well as additional practical applications it has enabled, will be reported elsewhere.^[11]

Experimental Section

General experimental procedure as described for fumaric acid: Fumaric acid (116 g, 1.0 mol) and sodium bicarbonate (186 g, 2.2 mol) were dissolved in tap water (1.5 L) in a 3 L Erlenmeyer flask. After gas evolution ceased, Chloramine-T trihydrate (281 g, 1.0 mol) was added, followed by potassium osmate (0.74 g, 0.2 mol %). The dark brown color of the reaction mixture is characteristic at this point. The reaction mixture was stirred for 8 h, after which time it cleared and turned green. No oxidant could be detected at this point (starch/iodide paper). HCl (5 M, 500 mL) was then added, and the reaction mixture was left in the refrigerator overnight. Crystals formed were filtered, washed with a small amount of cold water, and dried to yield 280 g (92 %) of pure product. M.p. 201–203 °C (decomp, H₂O); ¹H NMR (500 MHz, [D₆]DMSO): δ = 7.65 (d, *J* = 7.8 Hz, 2H), 7.29 (d, *J* = 7.8 Hz, 2H), 4.05 (d, *J* = 3.4 Hz, 1H), 3.88 (d, *J* = 3.4 Hz, 1H), 2.33 (s, 3H); ¹³C NMR (125 MHz, [D₆]DMSO): δ = 173.93, 171.29, 142.26, 138.33, 129.27, 126.78, 71.63, 58.84, 21.03.

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Palladium-Catalyzed Synthesis of Aryl Ketones from Boronic Acids and Carboxylic Acids or Anhydrides **

Lukas J. Gooßen* and K. Ghosh

The acylation of carbon nucleophiles with carboxylic acid derivatives is an important C–C bond-forming reaction that is extensively used in the synthesis of natural products and pharmaceutical compounds. Several carboxylic acid derivatives, for example, nitriles, amides, anhydrides, or acid chlorides, can be alkylated or arylated with a variety of organometallic species to produce ketones in moderate to excellent yields.^[1]

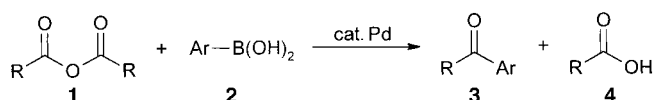
Carboxylic acids themselves can only be transformed into the corresponding ketones by treatment with lithium, magnesium, or aluminum reagents under rather elaborate conditions that are intolerant of most functional groups.^[2] Various procedures have been developed to stop the reaction at the stage of the ketone and avoid the formation of the tertiary alcohols.^[3] Milder reagents, for example organotin, -zinc, and -copper compounds or boronic acids, are acylated only by more reactive acid derivatives (usually acid chlorides), which have to be generated in an additional reaction step.^[4] Recently, Liebeskind et al. disclosed a versatile, base-free synthesis of aryl ketones by the palladium-catalyzed coupling of thioesters with boronic acids.^[5] However, this transformation requires the preformation of the thioester and calls for a stoichiometric amount of copper thiophene carboxylate.

Our target was to develop a convenient preparation of ketones directly from carboxylic acids under mild conditions and with minimal waste production by using a strategy similar to the one that Yamamoto and co-workers utilized for the reduction of carboxylic acids.^[6] Boronic acids appeared to be the carbon nucleophiles of choice, as they are readily available,^[7] non-toxic, air- and moisture-stable compounds that tolerate the presence of many sensitive functionalities.

Since most carboxylic acids can easily (and potentially in situ) be converted into their anhydrides,^[8] our first goal was the development of a catalytic system capable of coupling anhydrides with boronic acids. Although the palladium-catalyzed coupling of the more reactive acid chlorides with boronic acids is known,^[9] no reports have been made on whether anhydrides are also suitable for this reaction.^[10] In principle, a similar catalytic process for anhydrides that consists of the oxidative addition of the anhydride to an acyl palladium complex, exchange of the carboxylate for the aryl group by transmetalation of the boronic acid, and reductive elimination of the ketone should also be possible.

We chose the reaction of hexanoic anhydride (**1a**, R = *n*-C₅H₁₁) with phenylboronic acid (**2a**, Ar = Ph) as our model

system and screened several palladium catalysts under various conditions (Scheme 1). Selected results are shown in Table 1. We initially employed anhydrous conditions to prevent hydrolysis of the anhydrides. However, with various catalytic systems, only sluggish turnovers and moderate yields were observed (Table 1, entry 1). Upon the addition of water, the reaction surprisingly became faster while the competing hydrolysis of the anhydride remained slow, so that high yields of the desired ketone **3a** were obtained.^[11] The addition of two equivalents of water with respect to the boronic acid was found to be optimal (Table 1, entries 1–3).



Scheme 1. Coupling of anhydrides with boronic acids (R, Ar: see below).

Table 1. Coupling of hexanoic anhydride with phenylboronic acid.

Entry	Phosphane	Solvent	Water [mmol]	Yield [%]
1	PPh ₃	THF	0	29
2	PPh ₃	THF	2.5	97
3	PPh ₃	THF	10	38
4	PPh ₃	DME	2.5	53
5	PPh ₃	DMF	2.5	92
6	PPh ₃	toluene	2.5	77
7	PPh ₃	CH ₃ CN	2.5	54
8 ^[a]	PPh ₃	THF	2.5	92
9	PCy ₃	THF	2.5	91
10	P(<i>o</i> -Tol) ₃	THF	2.5	31
11	BINAP	THF	2.5	< 5
12	P(<i>p</i> -MeOPh) ₃	THF	2.5	97
13	DPPF	THF	2.5	< 5
14	P(Fur) ₃	THF	2.5	46
15	P(<i>t</i> Bu) ₃	THF	2.5	28

Reagents and conditions: hexanoic anhydride (1 mmol), phenylboronic acid (1.2 mmol), Pd(OAc)₂ (0.03 mmol), ligand (0.07 mmol; 0.035 mmol for chelating phosphanes), 16 h. The yields were determined by using GC. [a] The reaction was performed at 20 °C. DME = 1,2-dimethoxyethane.

DMF (*N,N*-dimethylformamide) and THF proved to be the most effective solvents (Table 1, entries 2, 4–7). Owing to its lower toxicity, we considered THF to be more convenient. The choice of phosphane also has a significant effect on the reaction outcome (Table 1, entries 9–15). The optimum reaction temperature was 60 °C, but reasonable yields were also obtained at lower temperatures (Table 1, entry 8).

To study the scope of this transformation, we varied both the boronic acids and the anhydrides. A few representative results are shown in Table 2. We found that for best results, different phosphanes need to be chosen with regard to the steric and electronic properties of the anhydrides. Tri-*p*-methoxyphenylphosphane is generally suitable for all carboxylic acid anhydrides and the superior choice for most alkyl derivatives. For more electron-poor aryl derivatives, however, triphenylphosphane or diphenylferrocenylphosphane are more effective.

Most substrates gave excellent yields, and even sensitive compounds such as furanboronic acid were smoothly converted. Alkyl, vinyl, and aryl anhydrides are equally suitable

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Table 2. Synthesis of aryl ketones according to Scheme 1.

Entry	R	Ar	Yield [%]
1	<i>n</i> -C ₅ H ₁₁	<i>o</i> -tolyl	98
2	<i>n</i> -C ₅ H ₁₁	<i>p</i> -MeO-phenyl	91
3 ^[a]	<i>n</i> -C ₅ H ₁₁	<i>p</i> -CH ₃ CO-phenyl	96
4	<i>n</i> -C ₅ H ₁₁	<i>m</i> -Cl-phenyl	97
5	<i>n</i> -C ₅ H ₁₁	2-furyl	84
6	<i>n</i> -C ₅ H ₁₁	3-thienyl	88
7	CH ₃	phenyl	98
8	<i>sec</i> -butyl	phenyl	98
9	-C(CH ₃)=CH ₂	phenyl	71
10 ^[b]	<i>p</i> -MeO-Ph	phenyl	90
11 ^[b]	phenyl	phenyl	96
12	<i>tert</i> -butyl	phenyl	0

Reagents and conditions: anhydride (1 mmol), boronic acid (1.2 mmol), Pd(OAc)₂ (0.03 mmol), P(*p*-MeOPh)₃ (0.07 mmol), 16 h, 60 °C; all yields refer to isolated products. [a] PCy₃ (0.07 mmol) was used as ligand. [b] PPh₃ (0.07 mmol) was used as ligand.

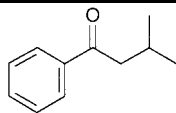
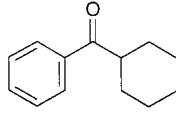
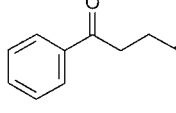
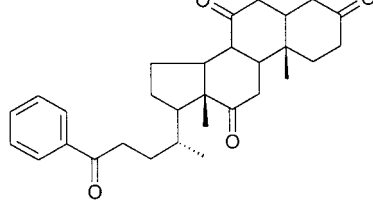
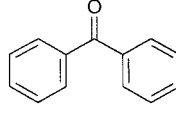
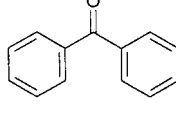
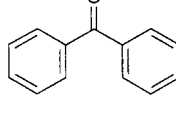
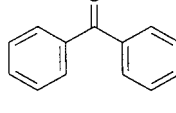
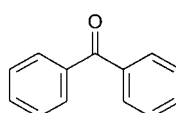
for this transformation (Table 2, entries 1, 7–11). However, no reaction was observed for the sterically demanding pivalic anhydride (Table 2, entry 12).

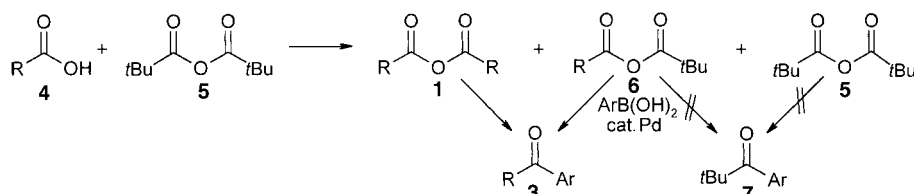
The low reactivity of pivalic anhydride (**5**) opened up an opportunity for achieving an in situ activation of sterically less hindered carboxylic acids **4** by the generation of the corresponding mixed anhydrides **6** (Scheme 2).

We tested our hypothesis on the reaction of 3-phenylpropionic acid (**4b**) with phenylboronic acid (**2a**) (Scheme 3, R = 3-phenylpropyl, Ar = Ph) and indeed observed the desired conversion exclusively into 3-phenylpropyl phenyl ketone **3b**.

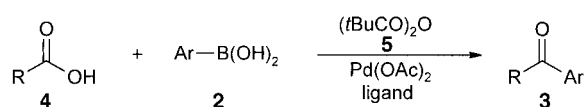
The optimum reaction conditions for the one-pot reaction were found to be similar to the best conditions for the homoanhydrides. The influence of the water content is profound: in the absence of water, less than 1% of the ketone was obtained. However, with two equivalents of water more than 90% of the ketone was formed, but with ten equivalents less than 80% was produced. P(*p*-MeOPh)₃ (65%), PPh₃ (68%), DPPF (91%; DPPF = 1,1'-bis(diphenylphosphino)ferrocene), and PCy₃ (55% yield) were found to be effective ligands on palladium. The scope of the one-pot reaction was investigated by using different combinations of carboxylic acids and boronic acids. Selected results are displayed in Table 3. P(*p*-MeOPh)₃ is most effective for alkyl carboxylic acids (Table 3, entries 1–5), whereas PPh₃ or DPPF are usually superior for

Table 3. Synthesis of aryl ketones from carboxylic acids and aryl boronic acids.

Entry	Product ^[a]	Ligand ^[b]	Yield [%]
1		A	90
2 ^[c]		A	60
3		A	65
4		A	81
5		C	68
6		B	80
7		C	75
8		D	54
9		B	85



Scheme 2. In situ activation of carboxylic acids.

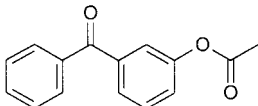
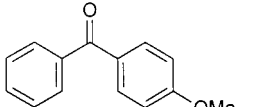
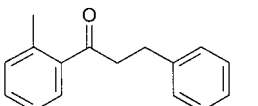
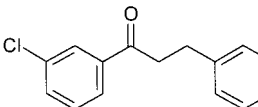
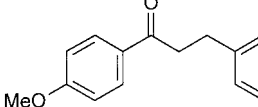
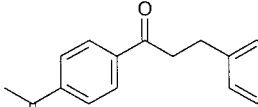
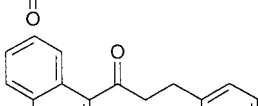
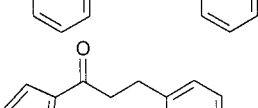
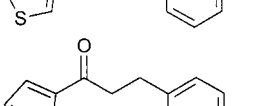


Scheme 3. One-pot synthesis of aryl ketones.

aryl derivatives (Table 3, entries 1–10). For aryl alkyl derivatives such as 3-phenylpropionic acid, the properties of the boronic acids also have to be taken into account when choosing the ligand (Table 3, entries 12–18).

Many functional groups are tolerated: carboxylic acids and boronic acids that contain halo, keto, cyano, ester, nitro, or protected amino groups were successfully converted. Some heterocyclic ketones were synthesized as well. However, alkylboronic acids were not converted under any of the given conditions.

Table 3. (Continued).

Entry	Product ^[a]	Ligand ^[b]	Yield [%]
10		D	48
11		D	55
12		C	65
13		C	55
14		A	78
15		D	55
16		C	75
17		A	72
18		B	47

Reagents and conditions: carboxylic acid (1 mmol), boronic acid (1.2 mmol), pivalic anhydride (1.5 mmol), Pd(OAc)₂ (0.03 mmol), ligand (0.07 mmol; 0.035 mmol for DPPF), solvent (4 mL), H₂O (2.5 mmol), 60 °C, 16 h; all yields refer to isolated products. [a] Aryl groups that originate from the boronic acids are drawn on the left side of the keto groups. [b] Ligands: A = P(*p*-MeOPh)₃; B = PPh₃; C = DPPF; D = PCy₃. [c] DME was used as solvent.

In summary, the palladium-catalyzed cross-coupling reaction disclosed herein represents a one-step, high-yielding synthesis of aryl ketones directly from the plethora of available carboxylic acids and boronic acids. The reaction is easily performed with many functionalized derivatives, requires only commercially available nontoxic chemicals, and produces a minimum amount of waste. It is thus a valuable alternative to the standard procedures, especially for applications in drug discovery or combinatorial chemistry.

Experimental Section

3b: A 100-mL round-bottomed flask equipped with a pressure equalizer and a magnetic stirring bar was charged with palladium acetate (67.3 mg, 0.30 mmol), diphenylferrocenylphosphane (194 mg, 0.35 mmol), 3-phenyl-

propionic acid (1.50 g, 10 mmol), and phenylboronic acid (1.46 g, 12 mmol). Subsequently, THF (40 mL), water (45 mg, 2.5 mmol), and pivalic anhydride (2.79 g, 15 mmol) were added through a syringe. The reaction vessel was purged with argon and the brown reaction mixture was heated at 60 °C overnight. After removal of the volatiles under vacuum, the residue was taken up in a minimum amount of hexane and filtered through aluminum oxide (10 cm) by using a hexane/MTBE (MTBE = *tert*-butyl methyl ether) gradient as eluent. The first fraction contained mainly biphenyl formed during the reduction of the palladium(II) precatalyst. The second fraction, which eluted with 10% MTBE in hexane, contained the almost pure product. After the removal of the volatiles and crystallization of the residue from hexane, 3-phenylpropyl phenyl ketone (1.73 g, 83%) was obtained as colorless crystals. ¹H NMR (200 MHz, CDCl₃, 25 °C, TMS): δ = 7.95 (m, 2H), 7.61–7.19 (m, 8H), 3.32 (t, ³J(H,H) = 6 Hz, 2H), 3.13 (t, ³J(H,H) = 6 Hz, 2H); ¹³C NMR (50 MHz, CDCl₃, 25 °C, TMS): δ = 198.8, 141.0, 136.5, 132.7, 128.3, 128.2, 128.1, 127.7, 125.8, 40.1, 29.8; MS (70 eV): *m/z* (%): 210 (53) [*M*⁺], 105 (100), 77 (46), 51 (17); HR-MS: calcd for C₁₅H₁₄O [*M*⁺]: 210.104465; found: 210.10447; Elemental analysis calcd for C₁₅H₁₄O (210.3): C 85.68%, H 6.71%, N 0.00%; found: C 85.37, H 6.67, N 0.00%.

The reactions in Tables 1–3 were performed on a 1-mmol scale, with tetradecane (0.05 mL) as an internal GC standard. The products were isolated by column chromatography (basic Al₂O₃ or SiO₂, ethyl acetate/hexane 1:30–1:10) and characterized by means of ¹H and ¹³C NMR spectroscopic analysis as well as by GC-MS and HR-MS.

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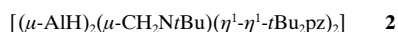
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A Polyhedral Aluminum Compound with an $\text{Al}_4\text{C}_4\text{N}_4$ Framework**

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Dedicated to Professor Philipp Gülich

Recently the oligomeric and polyhedral compounds of aluminum containing Al–N and Al–C cores have attracted considerable interest following the preparation of a few three-coordinate iminoalanes^[1] and the determination of the structures of carbaalanes.^[2] Heteroatom-containing organo-aluminum cage compounds^[3] are potential precursors for various applications such as catalysts, reagents, and for chemical vapor deposition,^[3a, 4, 5] as well as being of theoretical importance.^[6] Although the formation of iminoalanes and carbaalanes is now almost understood,^[1–3] compounds containing an $\text{Al}_n\text{C}_n\text{N}_n$ framework ($n > 2$) are still unknown.^[7] We figured that such species should exhibit some interesting features. Herein we report the structure of the polyhedron **1** and the synthesis of the bridging compound with bulky pyrazolato ligands **2** ($t\text{Bu}_2\text{pz} = 3,5\text{-di-}t\text{-tert-butylpyrazolato}$).



Treatment of $\text{H}_3\text{Al} \cdot \text{NMe}_3$ ^[8] with one equivalent of $\text{CN}t\text{Bu}$ in refluxing toluene smoothly gives **1** in 75% yield. Compound **1** is well soluble in toluene and readily crystallizes.

The X-ray structure analysis of **1** reveals a remarkable $\text{Al}_4\text{C}_4\text{N}_4$ cage configuration in which the C atoms adopt *exo* positions and thus may be formally viewed as inserting into four Al–N bonds of an Al_4N_4 cube (Figure 1A).^[9] It is surprising that a significant difference in the arrangement of the atoms within the cage skeleton of **1** is found in comparison with those of other known aluminum compounds with a 12-membered core ($(\text{AlE})_6$, E = N,^[3d–g] O,^[10a] S,^[10b] P,^[10c] As^[10d]) which exclusively adopt the form of a hexagonal-prismatic array. The core of **1** consists of six faces formed by two boat-shaped six-membered $\text{Al}_2\text{C}_2\text{N}_2$ rings and four puckered five-membered Al_2CN_2 rings. The two six-membered heterocycles are positioned crosswise to each other, and the four five-membered rings are arranged between the two. Each atom of the core participates in two different types of ring systems, and the geometries of both Al and N atoms are slightly distorted

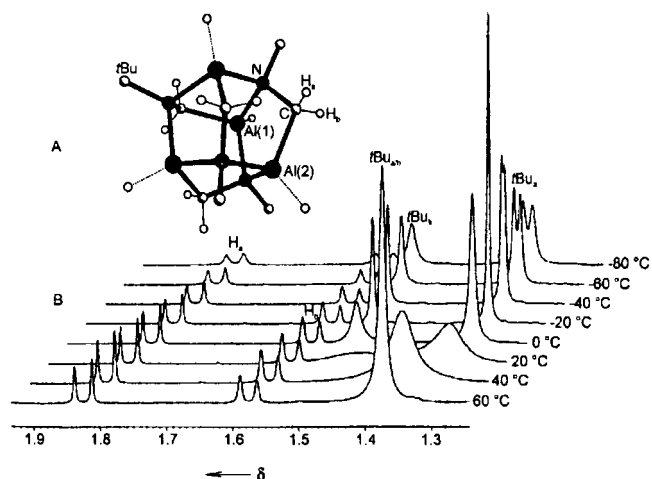


Figure 1. A) Molecular structure of **1** (*t*Bu groups are represented by C atoms). Selected bond lengths [Å] and angles [°]: Al(1)–N(1) 1.968(2), Al(1)–N(2) 1.9693(19), Al(1)–C(4) 1.992(2), N(1)–C(1) 1.529(3); N(1)–Al(1)–N(2) 104.44(8), N(1)–Al(1)–C(4) 107.09(9), N(2)–Al(1)–C(4) 98.60(9), C(1)–N(1)–Al(1) 105.42(13), C(1)–N(1)–Al(2) 106.33(13), Al(1)–N(1)–Al(2) 102.49(8), N(1)–C(1)–Al(3) 109.95(14), N(2)–C(2)–Al(2) 110.07(14). B) Variable-temperature ¹H NMR spectra of **1** in [D₈]toluene.

tetrahedral. Four Al atoms have a common center to which the distance of each Al atom is 1.95(1) Å. The Al–C bond lengths (1.979(2) to 1.992(2) Å) are all within the expected range in comparison with the carbaalanes (Al–C 1.948(3)–2.212(2) Å),^[2] whereas the Al–N bond lengths (1.966(2) to 1.978(2) Å) are slightly longer than those in iminoalanes with the 12-membered Al_6N_6 core structure (e.g. Al–N 1.898(3)–1.955(3) Å in $(\text{HAlNiPr})_6$).^[3e] The molecular symmetry is quite close to S_4 . No critical intermolecular contacts were observed since the closest Al⋯Al distance (e.g. Al(1)⋯Al(2)) is more than 3.073 Å.

The mass spectrometry data and elemental analysis data are in complete agreement with the formula of **1**. The ²⁷Al NMR spectrum shows only one resonance ($\delta = 148.5$), which indicates four-coordinate Al atoms.^[11] However, the ¹H NMR (C_6D_6 , RT) spectrum displays a quite broad resonance ($\delta = 1.4$, $W_{1/2} = 60$ Hz, 36 H) and two sets of doublets ($\delta = 1.61$ and 1.85, $^2J = 12.65$ Hz, 8 H; ratio 1:1). The resonances of the ¹³C NMR spectrum are too broad to be distinguished. Broad resonances in the ¹H and ¹³C NMR spectra suggest that **1** is involved in dynamic processes in solution at room temperature.^[12] Preliminary information regarding these processes was elucidated from the variable-temperature ¹H NMR spectrum in [D₈]toluene (Figure 1B). At 60 °C the spectrum clearly shows one sharp resonance at $\delta = 1.37$ (36 H) indicating fast rotation of the *t*Bu groups. This signal (*t*Bu_{a/b}) coalesces at about 10 °C, and below this temperature the exchange is slow as demonstrated by the two new shoulders ($\delta = 1.33$ (*t*Bu_a) and 1.50 (*t*Bu_b), ratio 2:1 (–20 °C)). Surprisingly, the *t*Bu_a signal further splits into two sharp singlets below –40 °C (the ratio is close to 1:1). In any case the integration of the resonances ($\text{H}_a\text{:H}_b\text{:}t\text{Bu}_a\text{:}t\text{Bu}_b$) are strictly in the ratio of 1:1:3:6. Such dynamic behavior is unprecedented and it is currently not clear whether three sharp resonances (3:3:3) of the *t*Bu_{a/b} group below –40 °C really correspond to the three frozen non-

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equivalent methyl groups or indicate a dissociation of the molecules in solution. However, the two sets of coupled resonances may be tentatively assigned to the two unequal hydrogen atoms (H_aH_b) of the core of **1** due to steric effects. The IR spectrum shows two strong bands in the range of Al–H stretching frequencies (1831 and 1858 cm^{-1}), which implies terminal Al–H bonds in the solid state.^[13]

Further insight into the molecular structure of **1** is gained by *ab initio* calculations^[14] on the model compound $[(\mu_3\text{-AlH})(\mu_3\text{-CH}_2\text{NMe})]_4$ (**1a**)^[15] with molecular symmetry S_4 .

A suggestion of the bonding situation in **1** (**1a**) is offered by the representation of the Laplacian of the electronic density.^[16] The negative zones (dark areas in Figure 2) display the tendencies for charge accumulation, for example in the terminal hydrides and the σ skeleton of the embedded CH_2NMe fragments. The positive Laplacian shows the depletion of the electron density around the positively charged aluminum atoms.

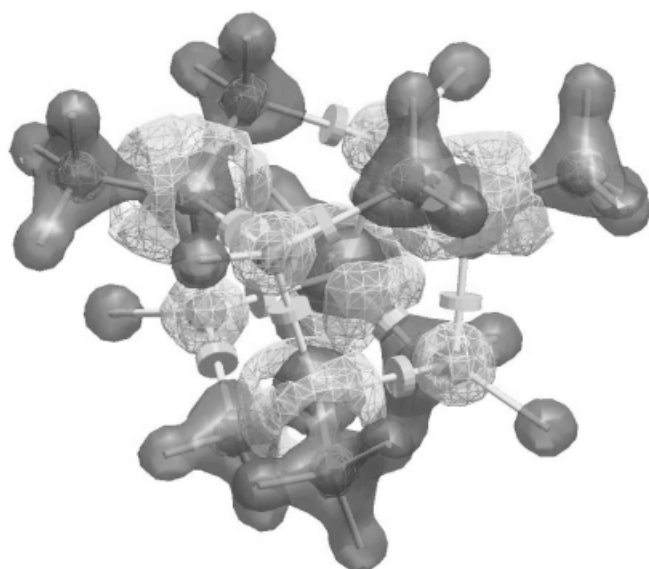


Figure 2. The isosurfaces (at the $0.2\text{ e}\text{\AA}^{-3}$ value) for the Laplacian of the electron density in $[(\mu_3\text{-AlH})(\mu_3\text{-CH}_2\text{NMe})]_4$ (**1a**). The dark solid surface corresponds to density accumulation (negative Laplacian) and illustrates the lone pairs at C and N. The zones with depletion of electron density (e.g. metal centers) are depicted with the light-colored mesh. The positions of the $(3, -1)$ bond critical points are highlighted with circular sectors.

The Al–N and Al–C contacts reveal accumulated charge on N and C with the lone pairs of nonmetals directed toward the metal atom. The Laplacian analysis shows that the CH_2NMe fragments can be regarded as μ_3 -coordinating ligands. The $(3, -1)$ bond critical points are placed closer to the electropositive partner, as is clearly seen for the Al–C and Al–N bonds. Moreover the analysis reveals the $(3, +1)$ critical points (cycle) on all the faces of the cluster and a $(3, +3)$ critical point in the center of gravity. This is specific for a closed cage. The small ellipticities of the Hessian eigenvalues of the Al–C, Al–N, and C–N $(3, -1)$ critical points show the σ nature of the bonds.

The coordination-type of the bonding is evident from the results of natural bond orbital (NBO) analysis,^[17] which shows that in both types of Al–N bonds the content of nitrogen AOs is $\sim 92\%$, which corresponds to a ligand-field regime. The Al–C bonds contain 85% nonmetal character. The composition of the NBOs of the Al–N bonds shows sp^3 -like hybrids in the bonding with s character ranging from 22 to 25% (the content of d orbitals is low $\sim 1.5\%$). The Al–C bonds reveal 32.8% s and 67.2% p participation for the carbon AOs. However, the apparent sp^2 character of carbon does not correspond to the π bonding. The lower p content in the natural hybrids of the C atom is only the counterpart of higher p character in the C–N bond (79.3%).

The dynamic NMR experiment detected changes in the resonance for the *t*Bu groups with temperature. This phenomenon can be assigned to the promotion of rotational disorder around the C–N bond of the terminal fragments. The differentiation of protons probably takes place by through-space dipolar interactions of the corresponding *t*Bu and CH_2 groups.

Reaction of the dimeric aluminum dihydride $[(\eta^1\text{-}\eta^1\text{-}t\text{Bu}_2\text{pz})(\mu\text{-Al})\text{H}_2]_2$ ^[18a] and $\text{CN}t\text{Bu}$ at room temperature leads to the formation of **2** in 91% yield. However, a further reaction of **2** using an excess of $\text{CN}t\text{Bu}$ was not observed at room temperature.

The ^1H NMR spectrum of **2** displays three different sharp resonances for the *t*Bu groups ($\delta = 1.39, 1.46$, and 1.52 , ratio $2:1:2$) indicating the asymmetric nature of the molecule. However, the resonance in the ^{27}Al NMR spectrum appears too broad to be observed. The X-ray structure analysis of **2** reveals a structure with two Al_2CN_3 rings and one Al_2N_4 heterocycle (Figure 3).^[9] To our surprise the Al(1)–N(5) bond

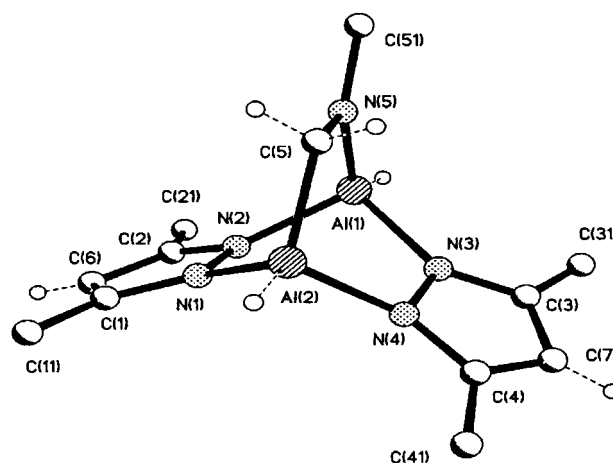


Figure 3. Molecular structure of **2** (C(11, 21, 31, 41, 51) represent *t*Bu groups). Selected bond lengths [$\text{\AA}$] and angles [$^\circ$]: Al(1)–N(5) $1.778(2)$, Al(1)–N(2) $1.9698(18)$, Al(2)–N(4) $1.9335(18)$; N(2)–Al(1)–N(3) $99.38(8)$, N(1)–Al(2)–N(4) $99.87(8)$, Al(2)–C(5)–N(5) $118.43(15)$, Al(1)–N(5)–C(5) $110.59(14)$, N(1)–Al(2)–C(5) $102.54(9)$, N(2)–Al(1)–N(5) $106.73(9)$.

length ($1.778(2)\text{ \AA}$) is significantly shorter than those in comparable iminoalanes with four-coordinate aluminum atoms ($1.898(3)–1.955(3)\text{ \AA}$)^[3e] and even shorter than those in compounds with three-coordinate aluminum atoms (Al–N $1.804(\text{av})–1.824\text{ \AA}$)^[1] whereas the other Al–N bond lengths

of **2** fall in the expected range (1.9265(19)–1.9698(18) Å). The Al(2)–C(5) distance (1.966(2) Å) is in accordance with those in $[(\eta^1-\eta^1-t\text{Bu}_2\text{pz})(\mu\text{-Al})\text{Me}_2]_2$ (Al–C 1.962(2) Å).^[18b]

In summary, we have reported the preparation of the polyhedron aluminum compound **1** and the dinuclear aluminum species **2** by hydroalumination of CNtBu. The $\text{Al}_4\text{C}_4\text{N}_4$ core of **1** is unique and thus can be considered as a fused carbaaminoalane. The exact mechanism for the formation of **1** and **2** is unknown, but probably an initial donor–acceptor intermediate is involved, in which the isocyanide carbon coordinates to the aluminum center followed by hydrogen migration from aluminum to carbon to yield the CH_2 moiety.^[7]

Experimental Section

1: CNtBu (1.4 mL, 12.4 mmol) was added at room temperature by syringe to a solution of $\text{H}_3\text{Al}\cdot\text{NMe}_3$ ^[8] (15.0 mL, 0.8 M solution in toluene, 12.0 mmol) in toluene (30 mL). The solution was refluxed for 0.5 h and then the solvent from the resulting red solution was removed under reduced pressure. The resulting residue was dried for 3 h in vacuo and then dissolved in warm toluene (10 mL) to afford colorless crystals within 2 h at -26°C (1.02 g, 75%). M.p. 261°C ; ^1H NMR (C_6D_6 , 500 MHz): $\delta = 1.4$ (br ($W_{1/2} = 60$ Hz), 36 H; tBu), 1.61 (d, $^2J(\text{H}_a, \text{H}_b) = 12.65$ Hz, 4 H; Al–CH₃), 1.85 (d, $^2J(\text{H}_b, \text{H}_a) = 12.65$ Hz, 4 H; Al–CH₃), 4.3 (v br ($W_{1/2} = 550$ Hz), 4 H; Al–H); ^{13}C NMR ($[\text{D}_8]\text{toluene}$, 125 MHz, 90°C): $\delta = 27.6$ (s, CH₃), 33.0 (br Al–C), 57.5 (s, N–C(CH₃)₃); ^{27}Al NMR (C_6D_6 , 65 MHz): $\delta = 148.5$ (s); IR (Nujol mull): $\bar{\nu}_{\text{Al-H}} = 1831, 1858\text{ cm}^{-1}$; MS(EI): m/z (%): 451 ($[\text{M}^+ - 1]$, 1), 395 ($[\text{M}^+ - t\text{Bu}]$, 100); elemental analysis (%) calcd for $\text{C}_{20}\text{H}_{48}\text{Al}_4\text{N}_4$: C 53.09, H 10.62, N 12.39; found: C 52.86, H 10.54, N 11.92. Single crystals suitable for X-ray diffraction analysis were obtained from toluene at room temperature. The thermogravimetric analysis of **1** shows a complete conversion into AlN in the temperature range of 350 to 400°C .

2: CNtBu (0.24 mL, 2.1 mmol) was added at room temperature by syringe to a suspension of $[(\eta^1-\eta^1-t\text{Bu}_2\text{pz})(\mu\text{-Al})\text{H}_2]_2$ ^[18a] (0.83 g, 2.0 mmol) in hexane (50 mL). After the mixture had been stirred for 3 h, the solvent of the clear solution was removed under reduced pressure to afford **2** (0.91 g, 91%) as a pure white solid. M.p. 176°C ; ^1H NMR (C_6D_6 , 200 MHz): $\delta = 1.39$ (s, 18 H; C(11,41)CH₃), 1.46 (s, 9 H; C(51)CH₃), 1.52 (s, 18 H; C(21,31)CH₃), 6.08 (s, 2 H; C(6,7)H), 2.30 (s, 2 H; NC–H); ^{13}C NMR (C_6D_6 , 125 MHz): $\delta = 28.5$ (br Al–C(5)), 30.5 (s, C(51)C), 31.1 (s, C(2,3)C–CH₃), 31.0 (s, C(1,4)C–CH₃), 32.6 (s, C(2,3)C), 32.3 (s, C(1,4)C), 53.2 (C(51)), 102.0 (s, C(6,7)), 163.8 (s, C(1,4)), 165.2 (s, C(2,3)); IR (Nujol mull): $\bar{\nu}_{\text{Al-H}} = 1887, 1918\text{ cm}^{-1}$; MS(EI): m/z (%): 498 ($[\text{M}^+ - 1]$, 10), 442 ($[\text{M}^+ - t\text{Bu}]$, 100); elemental analysis (%) calcd for $\text{C}_{27}\text{H}_{51}\text{Al}_2\text{N}_5$: C 51.20, H 7.73, N 7.47; found: C 50.51, H 7.58, N 7.23. Single crystals suitable for X-ray diffraction analysis were obtained from Et₂O at -26°C .

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Anion-Templated Syntheses of Rhombohedral Silver–Alkynyl Cage Compounds

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The interest in metal alkynyl complexes stems not only from their range of organometallic transformations, but also from their flexible structural features.^[1] The linear and rigid arrangement of atoms in these compounds makes them attractive building blocks for a wide range of complex architectures.^[2, 3] The alkynyl moiety has a flexible coordination environment, being able to form metal–alkynyl σ and π bonds.^[4] Alkynyl complexes of the coinage metals are of particular interest since these metals can display additional noncovalent (metallophilic) $M \cdots M$ interactions,^[5] which provide an extra degree of complexity. These metallophilic interactions are well-established for gold(II) complexes,^[6] but have not been as widely studied for silver(I)^[7] and copper(I).^[8]

Alkynyl complexes of the coinage metals tend to form polymeric materials.^[9] A potential route to oligomeric species (e.g., macrocyclic and cage-type structures) rather than polymeric products could involve the use of templating agents.^[10] Most templating agents are cationic or neutral, but recently increasing interest has focused on templating anions.^[11, 12]

The synthesis and novel structure of the gold(II) catenane $[[[\text{Au}(\text{C}\equiv\text{CtBu})]_6]_2]$ ^[13] stimulated us to carry out similar

studies with silver(I). Here we report the remarkable anion-templated syntheses of silver–alkynyl cage-type compounds with general formula $[\text{Ag}_{14}(\text{C}\equiv\text{CtBu})_{12}\text{X}]\text{Y}$ (X = Cl, Br; Y = OH, BF_4).

When a solution of AgBF_4 in THF is treated with a mixture of $t\text{BuC}\equiv\text{CH}$ and NEt_3 in THF, a white precipitate is obtained. On the basis of IR spectroscopy, FAB^+ mass spectrometry, and elemental analyses, this solid was formulated as polymeric $[\text{Ag}(\text{C}\equiv\text{CtBu})]_n$ (**1**).^[14] To establish the structure of **1**, attempts were made to recrystallize it. Although a wide variety of common solvents (THF, acetone, chloroform, dichloromethane, ethanol, methanol, toluene, hexane) were tried, **1** was found to dissolve only in chloroform. Addition of diethyl ether to this solution gave a white precipitate **2**, and the fact that this could then be redissolved, not only in chloroform, but also in other solvents such as acetone, ethanol, and THF (in which **1** is insoluble), suggested that a chemical modification had occurred. Crystals of this compound were obtained by slow evaporation of an ethanolic solution.

A single-crystal X-ray analysis^[15, 16] showed **2** to be the novel silver cage compound $[\text{Ag}_{14}(\text{C}\equiv\text{CtBu})_{12}\text{Cl}]\text{OH}$ (Figure 1). The cage comprises fourteen silver atoms and twelve

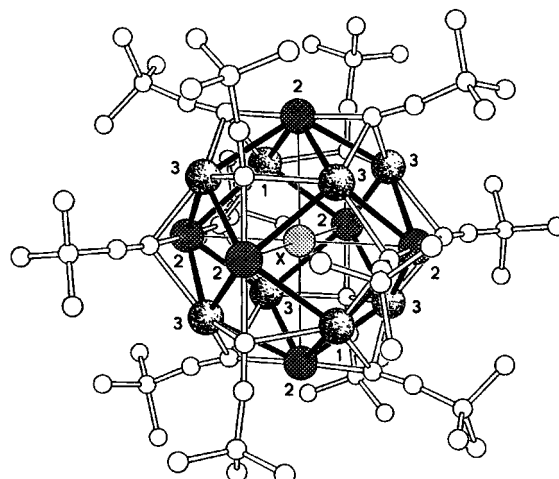


Figure 1. The structure of the silver cage complexes **2** (X = Cl) and **4** (X = Br), showing the rhombic dodecahedron of silver atoms (solid bonds) and the encapsulation of a halide anion at the center of an octahedron of type 2 silver atoms. Silver atoms of types 1 and 3 cap the faces of this octahedron and define a cubic array.

tert-butylethynyl ligands, and has a chloride anion at its center. The silver atoms are arranged so as to form a near-regular rhombic dodecahedron having crystallographic S_6 symmetry and molecular O_h symmetry, with $\text{Ag} \cdots \text{Ag}$ distances for the “edges” in the range of 2.953(2)–2.986(2) Å. These distances are comparable to those observed, for example, in the silver double salts reported by Mak et al. and attributed to argentophilic $\text{Ag} \cdots \text{Ag}$ interactions.^[17] The cage contains three crystallographically unique silver centers (labeled 1, 2, and 3 in Figure 1); six of these (of type 2) can be envisaged as forming an octahedron (with the anion position at its center) which interpenetrates a cubic array made up of silver atoms of types 1 and 3 such that each silver atom of the “cube” caps a trigonal face of the octahedron. The silver atoms of the

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octahedron each bear two linearly disposed, σ -bonded *tert*-butylethynyl groups ($\text{Ag}(2)-\text{C}$ ca. 2.07 Å, $\text{C}-\text{Ag}(2)-\text{C}$ 175.9(6)°, the $\text{C}=\text{C}$ groups of which also bridge pairs of silver atoms that form the edges of the cube ($\text{C}-\text{Ag}$ ca. 2.42 Å; Figure 2). Within the cage, the $\text{Cl}\cdots\text{Ag}$ distances, which range between 3.116(2) and 3.297(1) Å, are significantly longer than those of conventional silver–chlorine bonds (ca. 2.6 Å). Thus, there is clearly a small amount of residual free volume within the cage, and hence the potential for the encapsulation of larger anions.

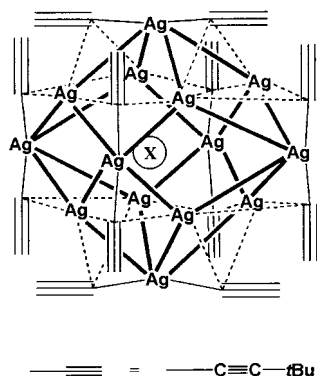


Figure 2. Schematic representation of the coordination sphere of the silver cages 2–4.

The formation of the cation in **2** was confirmed by IR, FAB^+ mass spectrometry, and elemental analyses. These provided clear evidence for the complete transformation of the presumably polymeric species **1** into the cage-type structure **2** when the former was treated with CHCl_3 . The IR spectrum of **2** shows a $\nu(\text{C}=\text{C})$ band at 2042 cm^{-1} (cf. 2053 and 2034 cm^{-1} for **1**). The FAB^+ mass spectrum of **2** is also consistent with this formulation: the intense molecular-ion peak at m/z 2519 was assigned to $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{Cl}]^+$. Elemental analyses were consistent with the formulation $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{Cl}]\text{OH}$.

A more rational route to **2** directly from AgBF_4 and $t\text{BuC}\equiv\text{CH}$ in the presence of NEt_3 and stoichiometric amounts of NMe_4Cl as a chloride source in THF has now been developed. The initial precipitation but rapid redissolution of a white solid suggested that **1** was not being produced or was being rapidly converted into a soluble material. After two hours the solvent was removed under reduced pressure, and the remaining white solid washed several times with water. This solid was characterized as $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{Cl}]\text{BF}_4$ (**3**). Its IR spectrum shows a $\nu(\text{C}=\text{C})$ band at 2042 cm^{-1} and an additional strong band at 1069 cm^{-1} , characteristic of BF_4^- . In the FAB^+ mass spectrum, the molecular-ion peak appears at m/z 2519, which corresponds to $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{Cl}]^+$. A single-crystal X-ray analysis of this compound, however, showed only the presence of **2**, and it can be concluded that the OH^- salt crystallizes in preference to the BF_4^- salt (vide infra).

The reaction between AgBF_4 and $t\text{BuC}\equiv\text{CH}$ in THF (with NEt_3 to deprotonate the alkyne) was repeated in the presence of NBu_4Br . In this reaction no solid precipitated; this suggests that **1** was not being formed. The reaction mixture was treated

as described for the synthesis of **3** to give $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{Br}]\text{BF}_4$ (**4**). The IR spectrum of this compound showed the characteristic $\nu(\text{C}=\text{C})$ band at 2038 cm^{-1} together with an intense band for BF_4^- at 1068 cm^{-1} . The FAB^+ mass spectrum showed the expected molecular-ion peak for $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{Br}]^+$ at m/z 2564. A single-crystal X-ray study^[18] revealed **4** to be isomorphous with **2** and **3**, but with a bromide ion encapsulated at its center. Again the compound crystallized more readily as an OH^- salt than as a BF_4^- salt. The dimensions of the cage differ little from those in **2** and **3**, with $\text{Ag}\cdots\text{Ag}$ edge distances in the rhombic dodecahedron between 2.972(2) and 3.006(2) Å. The $\text{Ag}\cdots\text{Br}$ distances are very similar to the $\text{Ag}\cdots\text{Cl}$ distances in **2**, and range between 3.148(2) and 3.313(1) Å, that is, they are longer than those of conventional $\text{Ag}-\text{Br}$ bonds.

The rhombohedral structure of these silver cages resembles the geometry of the nickel cages $[\text{Ni}_2\{\text{Ni}(\text{atu})_2\}_4\text{X}]\text{X}_3$ ($\text{atu} = \text{H}_2\text{NC}(\text{NH})\text{NHC}(\text{NH})\text{S}^-$; $\text{X} = \text{Cl}, \text{Br}$).^[11] The nickel atoms in these assemblies can be envisaged as forming, around the halide ion, an octahedron which interpenetrates a cubic array of NH groups. In spite of the chemical differences between the two types of cages, the $\text{M}\cdots\text{X}$ ($\text{M} = \text{Ag}, \text{Ni}$; $\text{X} = \text{Cl}, \text{Br}$) distances are strikingly similar and range between 3.116(2) and 3.313(1) Å for $\text{Ag}\cdots\text{X}$ and between 3.123(1) and 3.140(1) for $\text{Ni}\cdots\text{X}$.^[11]

An anomalous feature of the silver cage structures is the apparent absence of a second counteranion in the crystals. Clearly there is insufficient space for a second anion to be located within the cage itself. The exterior of the structure is populated by the bulky, hydrophobic *tert*-butyl groups, and inspection of the packing of the molecules shows that the clefts in the region of the type 2 silver atoms in one molecule are occupied by the *tert*-butyl groups of their symmetry-related counterparts. However, the *tert*-butyl groups all exhibit rotational disorder, and it is thus possible that small cavities are formed within which the required counteranions could reside with partial occupancy (because of symmetry restraints). This scenario would be consistent with the presence of a small anion such as OH^- , which could be formed from adventitious water, distributed over several sites; a similar situation was previously reported for a gold cluster.^[19] In the bromide-containing cage structure of **4**, for example, the residual electron density in the final ΔF map of only 0.50 $\text{e} \text{Å}^{-3}$ indicates that the “missing” counteranion must be small, of low molecular weight, and highly disordered.^[20]

The assignment of OH^- as the counteranion in **2** (i.e., the cage formed by treating **1** with chloroform) is consistent with all the spectroscopic, structural, and analytical data. However, the assignment of the counteranion for the chloride-containing cage **3** and the bromide-containing cage **4**, both of which are formed directly from AgBF_4 , the alkyne, and stoichiometric amounts of templating halide, is more difficult. The structural analyses of both these cages again indicate that only small counteranions can be accommodated within the crystal lattice. However, spectroscopic (^{19}F , ^{11}B NMR and IR spectroscopy) and analytical data show that BF_4^- is indeed present in the bulk crystalline sample. This observation is consistent with **3** and **4** being mixtures of $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{X}]\text{BF}_4$ and $[\text{Ag}_{14}(\text{C}\equiv\text{C}t\text{Bu})_{12}\text{X}]\text{OH}$ ($\text{X} = \text{Cl}, \text{Br}$), from which only

the OH[−]-containing compounds give crystals suitable for X-ray analyses. Since the analytical data of the bulk material suggest that BF₄[−] is the main counteranion present in both **3** and **4**, it is possible that the required small amounts of the OH[−] anion are generated in the crystallization process, which was carried out by using standard solvents that were not predried.^[21]

The results presented here strongly suggest that the formation of the silver–alkynyl cage compounds **2–4** is templated by spherical, mono-anionic species such as chloride and bromide. When these halides are not present, the formation of the insoluble, presumably polymeric material **1**, which in turn can be converted to the cages, is favored. The ability of larger anions to act as potential templating agents is currently being explored.

Experimental Section

Synthesis of 2 from 1: [Ag(C≡CtBu)]_n (0.6 g, 3.2 mmol of Ag(C≡CtBu)) was treated with warm CHCl₃ to give a colorless solution. After filtration while hot, the reaction mixture was concentrated under reduced pressure, and addition of diethyl ether yielded **2** as a white powder. Yield: 45% (0.26 g). Elemental analysis (%) calcd for Ag₁₄C₇₂ClH₁₀₉O: C 34.10, H 4.32; found: C 34.36, H 4.28; IR (KBr): $\tilde{\nu}$ = 2042 (vs, C≡C); ¹H NMR (270 MHz, CDCl₃): δ = 1.36 (s, CCH₃); FAB⁺-MS: *m/z*: 2519 [Ag₁₄(C≡CtBu)₁₂Cl]⁺, 1241 [Ag₇(C≡CtBu)₆]⁺, 1053 [Ag₆(C≡CtBu)₅]⁺, 863 [Ag₅(C≡CtBu)₄]⁺.

Synthesis of 3: Solid AgBF₄ (0.5 g, 2.6 mmol) was added to a mixture of *tert*-butylacetylene (0.3 mL, 2.6 mmol), triethylamine (0.4 mL, 2.6 mmol), and tetramethylammonium chloride (0.2 g, 0.2 mmol) in THF (10 mL). A white precipitate formed but gradually redissolved, and after 10 min a clear solution was obtained. After stirring the mixture for 4 h, the solvent was evaporated under reduced pressure, and the resulting white solid was treated with water and diethyl ether to give **3** as a fine white powder. Yield: 85% (0.4 g). Elemental analysis (%) calcd for Ag₁₄BCl₇F₄H₁₀₈: C 33.19, H 4.15; found: C 33.09, H 4.02; IR (KBr): $\tilde{\nu}$ = 2042 (vs, C≡C), 1069 (vs, BF₄[−]); ¹H NMR (270 MHz, CDCl₃): δ = 1.36 (s, CCH₃); ¹¹B NMR (CDCl₃): δ = −1.83 (s, BF₄); FAB⁺-MS: *m/z*: 2519 [Ag₁₄(C≡CtBu)₁₂Cl]⁺, 1241 [Ag₇(C≡CtBu)₆]⁺, 1053 [Ag₆(C≡CtBu)₅]⁺, 863 [Ag₅(C≡CtBu)₄]⁺.

Synthesis of 4: Solid AgBF₄ (1.5 g, 7.7 mmol) was added to a mixture of *tert*-butylacetylene (0.9 mL, 7.7 mmol), triethylamine (1.1 mL, 7.7 mmol), and tetrabutylammonium bromide (0.55 mmol) in THF (10 mL). A colorless solution formed immediately. After stirring the mixture for 2 h, the solvent was evaporated under reduced pressure. The resulting white solid was treated with water and subsequently recrystallized from THF/diethyl ether to give **4** as a white fine powder. Yield: 92% (0.9 g). Elemental analysis (%) calcd for Ag₁₄BBrc₇₂F₄H₁₀₈: C 32.63, H 4.08; found: C 32.62, H 4.00; IR (KBr): $\tilde{\nu}$ = 2038 (vs, C≡C), 1068 (vs, BF₄[−]); ¹H NMR (270 MHz, [D₆]acetone): δ = 1.44 (s, CCH₃); ¹¹B NMR ([D₆]acetone): δ = −1.86 (s, BF₄); ¹⁹F NMR ([D₆]acetone): δ = −150.74 (s, ¹¹BF₄), −150.69 (s, ¹⁰BF₄); FAB⁺-MS: *m/z*: 2564 [Ag₁₄(C≡CtBu)₁₂Br]⁺, 1287 [Ag₁₂(C≡CtBu)₁₀Br]⁺, 1241 [Ag₇(C≡CtBu)₆]⁺, 1053 [Ag₆(C≡CtBu)₅]⁺, 863 [Ag₅(C≡CtBu)₄]⁺.

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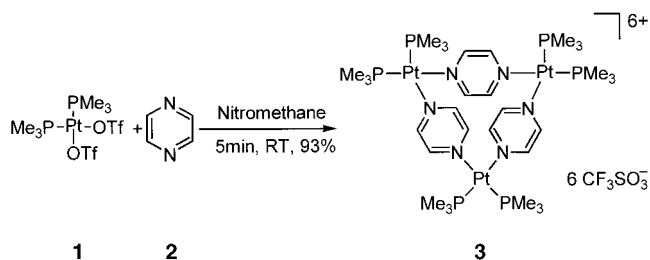
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- [21] The crystalline samples of **3** and **4** used for the structure determinations were uniform by simple visual inspection. However, it was found consistently that within the same sample some of the material was indeed composed of single crystals (from which the X-ray structures were obtained), whereas the remaining crystalline material was not suitable for X-ray analysis since no resolvable diffraction pattern could be obtained. This observation is consistent with the suggestion that **3** and **4** are mixtures of [Ag₁₄(C≡CtBu)₁₂X]BF₄ and [Ag₁₄(C≡CtBu)₁₂X]OH (X = Cl, Br), from which only the OH⁻-containing compounds give crystals suitable for X-ray analyses.

The Self-Assembly of an Unexpected, Unique Supramolecular Triangle Composed of Rigid Subunits**

Manuela Schweiger, S. Russell Seidel, Atta M. Arif, and Peter J. Stang*

In recent years, coordination-driven self-assembly has been employed in the synthesis of a large assortment of supramolecular entities.^[1] Among the many members of this family are a wide array of two-dimensional systems, such as rhomboids, squares, pentagons, and hexagons, and three-dimensional systems, such as truncated tetrahedra, adamantoids, cuboctahedra, dodecahedra, and a variety of other cages.^[2] In contrast, only a relatively small number of self-assembled triangles exist in the literature.^[3–6] Typically, they have been either the product of rational design, where the outcome of the reaction is dictated by the relative ratios of preprogrammed building blocks, or have been in equilibrium mixtures with larger entities, such as squares. The latter usually results when flexible and/or larger subunits are employed, allowing the formation of multiple species.

Herein, we report a novel, self-assembled, unexpected supramolecular triangle **3** formed from the stoichiometric reaction of ditopic platinum acceptor unit **1**^[7] and ditopic donor unit pyrazine (**2**) in nitromethane (Scheme 1).



Scheme 1. Formation of self-assembled triangle **3** from rigid subunits **1** and **2**. ⁻OTf = triflate = CF₃SO₃⁻.

Pyrazine (**2**) is the smallest, and hence most rigid, linear aromatic linker available for self-assembly processes, while platinum corner **1**, with its two bonding sites oriented approximately 90° to one another, is also quite compact. Based solely on these considerations, the combination of **1** and **2** would be predicted to yield a square as the final aggregate, but triangle **3** instead results. This distinctive occurrence, where inflexible linkers lead to unexpected products, is rare^[8] in the field of discrete molecular assembly, and we attribute it to both entropy considerations and the unique electronics incurred by having two platinum centers attached to each pyrazine ring.

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The assembly was monitored by multinuclear NMR spectroscopy, which revealed a highly symmetrical product as the predominant species in solution. Single crystals suitable for X-ray structure analysis (Figure 1)^[9] were grown by diffusing diethyl ether into **3** in nitromethane.

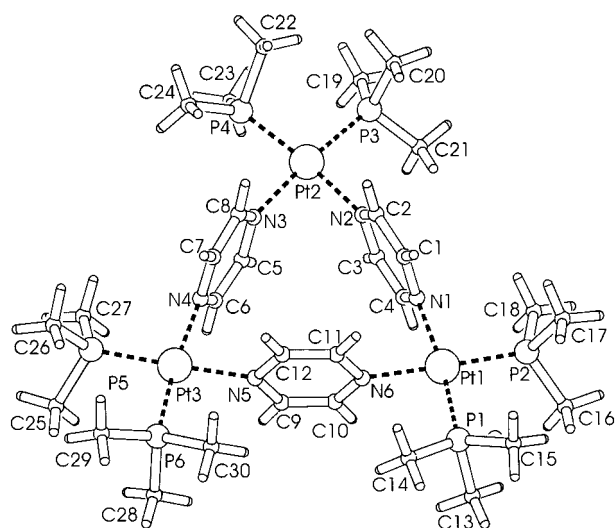


Figure 1. PLUTON plot of supramolecular triangle **3**. Solvent and counterions omitted for clarity. Selected bond lengths [Å] and angles [°]: Pt1–N1 2.13(2), Pt1–N6 2.11(2), Pt2–N2 2.12(2), Pt2–N3 2.14(2), Pt3–N5 2.12(2), Pt3–N4 2.13(2); N1–Pt1–N6 81.9(9), N2–Pt2–N3 83.2(8), N4–Pt3–N5 82.1(9).

The cross-ring distances from each platinum center to the centroid of the opposing pyrazine unit are within the range of 6.4–6.5 Å. Surprisingly, despite the strain present in **3**, the coordination geometry about the platinum atoms is not distorted enough from their ideal 90° angle to account for the triangular assembly (N–Pt–N angles range from 81.9(9)° to 83.2(8)°). Instead, a considerable portion of the tension generated from creating the smaller species is concentrated in the Pt–N connections themselves. While the pyrazine moieties are not noticeably bent (N–centroid(pyrazine)–N angles are all ~179°), the bonds are significantly distorted (centroid(pyrazine)–N–Pt angles range from 167–171°) from their ideal value of 180°.

The triangles stack in staggered columns, with an inter-ring, Pt–Pt distance of 15.6 Å (Figure 2). Interestingly, each ensemble has two triangular arrays of triflate anions aligned above and below the plane of the assembly, with their oxygen atoms pointing toward the positively charged platinum centers. As a consequence, the trifluoromethyl groups of the counterions are positioned diametrically, indicating the formation of a hydrophobic buffer between negatively charged sulfonate groups.

Adjacent columns are offset with respect to one another, leading to sheets in which each assembled system is surrounded by six trigonal anion clusters of alternating, opposite orientation (Figure 3). Disordered nitromethane molecules are interspersed throughout the lattice at various locations, yet, due to the small size of the cavity, never reside within a ring.

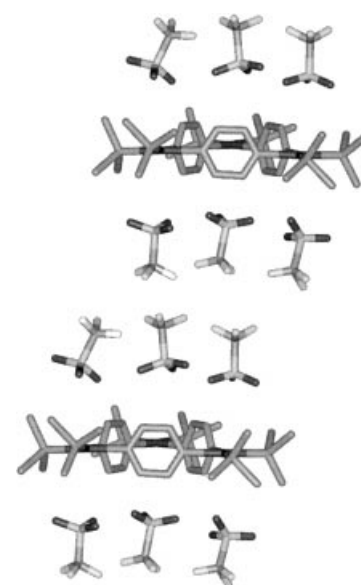


Figure 2. Stacking of **3** into staggered columns (side view; stick representation; dark portions of triflate anions represent oxygen atoms, light portions represent fluorine atoms). Protons omitted for clarity.

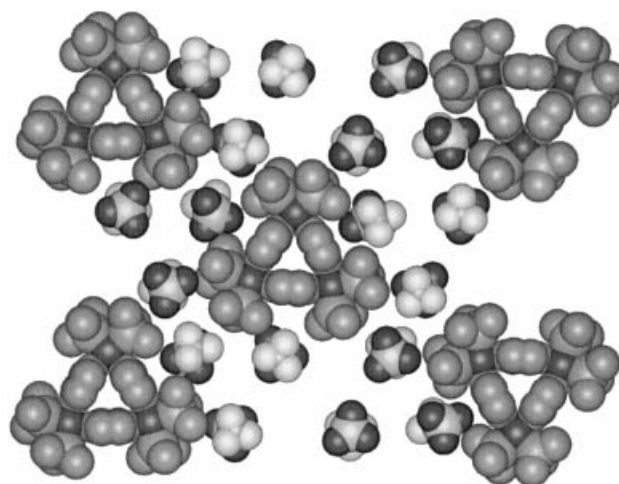


Figure 3. Top view, Van der Waals representation of a single sheet (dark portions of triflate anions represent oxygen atoms, light portions represent fluorine atoms). Protons and solvent omitted for clarity.

In conclusion, we herein report the formation and characterization of a unique, self-assembled, supramolecular aggregate formed from platinum subunit **1** and pyrazine (**2**). By all accounts, with **2** being the most rigid, linear aromatic linker conceivable and **1** being quite compact itself, the most logical outcome of this reaction should be a square. It is not; it is a triangle. This result, which we believe to be due to a combination of entropy factors and the electronic effects of having two platinum centers per pyrazine unit, suggests that there are likely limitations and exceptions to the coordination-directed synthetic strategy of “rational design”.^[2e]

Experimental Section

A solution of **2** (0.62 mg, 0.0077 mmol) in CD₃NO₂ (0.3 mL) was added to **1** (5.00 mg, 0.0077 mmol) dissolved in CD₃NO₂ (0.3 mL). Multinuclear NMR shows an instantaneous and quantitative reaction. At very high concen-

trations a second by-product (<5%) could be observed, which is most likely attributable to a square. The solvent was removed under a stream of N₂, and the resulting white precipitate was dried in vacuo (yield 93%). ¹H NMR (CD₃NO₂, 300 MHz): δ = 9.41 (s, 2H; H_{pyr}), 1.79 (d, J_{PH} = 11.4 Hz, 9H; P-CH₃); ³¹P{¹H} NMR (CD₃NO₂, 121 MHz): δ = -25.6 (s, ¹⁹⁵Pt satellites, J_{PP} = 3269 Hz); ¹⁹F NMR (CD₃NO₂, 282 MHz): δ = -78.1; ¹³C{¹H} NMR (CD₃NO₂, 75 MHz): δ = 151.8 (s, C_{pyr}), 122.2 (q, J_{C,F} = 319 Hz, OTf), 14.7 (m, P-CH₃).

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- [10] Further details on the crystal structure investigation 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-163666.

Microencapsulated Palladium Catalysts: Allylic Substitution and Suzuki Coupling Using a Recoverable and Reusable Polymer-Supported Palladium Catalyst**

Ryo Akiyama and Shū Kobayashi*

While palladium catalysts find wide-spread utility in a variety of transformations in organic synthesis,^[1] they are expensive, air-sensitive, and cannot be recovered in many cases. Immobilized palladium catalysts have been expected to solve these problems, and several polymer-supported palladium catalysts have been developed for allylic substitution,^[2, 9a,b,e] oligomerization,^[2c, 3, 4] decarboxylation,^[2d] hydrogenation,^[4, 9g] isomerization,^[5] telomerization,^[6] Suzuki coupling,^[7, 9c,d,h] and the Mizoroki–Heck reaction,^[4c, 8, 9f,h] etc. In most of these cases, however, recovery and reuse of the polymer catalysts have not been satisfactory.^[9] Recently, we developed novel polymer-supported catalysts, microencapsulated scandium trifluoromethanesulfonate (MCSc(OTf)₃)^[10] and osmium tetroxide (MCOsO₄).^[11] Our work has demonstrated a new method for immobilizing catalysts onto polymers based on physical envelopment by the polymers and on electronic interaction between the π electrons of the benzene rings of the polystyrene-based polymers and vacant orbitals of the catalysts. We now apply this new technology to immobilizing palladium catalysts. Herein, we describe the use of microencapsulated triphenylphosphane palladium for allylic substitution and Suzuki coupling. In both cases, the catalysts were recovered quantitatively and reused. Moreover, valuable information on the structure of microencapsulated catalysts was obtained.

Preparation of microencapsulated triphenylphosphane palladium was as follows:^[12] polystyrene (1.0 g, *M_w* ca. 280 000) was dissolved in cyclohexane (20 mL) at 40 °C, and to this solution was added tetrakis(triphenylphosphane)palladium(0) ([Pd(PPh₃)₄] 0.20 g) as a core ([Pd(PPh₃)₄] was dissolved). The mixture was stirred for 1 h at this temperature (and changed from brown to black), then slowly cooled to 0 °C. Coacervates (phase separation) were found to envelop the core dispersed in the medium, and hexane (30 mL) was added to harden the capsule walls. The mixture was left to stand at room temperature for 12 h, and the catalyst capsules were then washed with acetonitrile several times and dried at room temperature for 24 h. Three equivalents of triphenylphosphane (PPh₃) were recovered from the washings and one equivalent of PPh₃ remained in the catalyst capsules. We measured ³¹P swollen-resin magic angle spinning (SR-MAS)

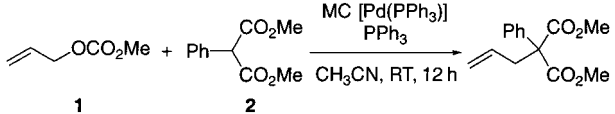
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NMR spectra^[13] of the catalyst capsules, and only one resonance signal arising from PPh_3 coordinating to the palladium was observed.^[14] From these results, we assumed that the catalyst was encapsulated as $[\text{Pd}(\text{PPh}_3)]$.

The microencapsulated $[\text{Pd}(\text{PPh}_3)]$ ($\text{MC}[\text{Pd}(\text{PPh}_3)]$) thus prepared was used in the allylation reaction of allyl methyl carbonate (**1**) with dimethyl phenylmalonate (**2**). When **1** was combined with **2** in the presence of 20 mol % $\text{MC}[\text{Pd}(\text{PPh}_3)]$, the reaction did not proceed at all. However, it was found that the reaction proceeded smoothly after adding PPh_3 (external ligand). We examined the effect of different amounts of PPh_3 (Table 1). The best results were obtained when 20 mol % of PPh_3 was used.^[15] Of note is that the palladium catalyst was recovered quantitatively and reused, and that the high activity of the catalyst was maintained even after the fifth use.

Table 1. Effect of the amount of triphenylphosphane.



MC[Pd(PPh ₃)] [mol %]	PPh ₃ [mol %]	Yield [%] (Recovery [%])		
		1st	2nd	3rd
5	0	0	—	—
5	5	97 (quant)	75 (quant)	57 (quant)
20	0	0	—	—
20	10	94 (quant)	61 (99)	30 (99)
20	20	83 (quant)	90 (quant)	84 (quant) ^[a]
20	40	92 (quant)	81 (99)	77 (quant)
20	60	69 (95)	58 (98)	65 (99)

[a] 4th run: 94 (quant), 5th run: 83 (quant).

Several examples of the $\text{MC}[\text{Pd}(\text{PPh}_3)]$ -catalyzed allylation reactions of C-nucleophiles with allylic carbonates are summarized in Table 2. Malonates and β -ketoesters smoothly

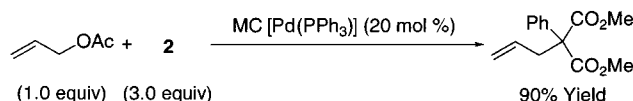
Table 2. Allylic substitution using $\text{MC}[\text{Pd}(\text{PPh}_3)]$.^[a]

Entry	Allylic carbonate	Nucleophile	Product	Yield [%]
1	1	2		83
2	1			86
3	1			60
4		2		69
5		2		92 ^[b]
6	4			79 ^[c]
7		2		64 ^[b]

[a] All reactions were carried out using $\text{MC}[\text{Pd}(\text{PPh}_3)]$ (20 mol %) and PPh_3 (20 mol %) in CH_3CN at room temperature for 12 h. [b] $E/Z = >99/ <1$. [c] $E/Z = 64/36$.

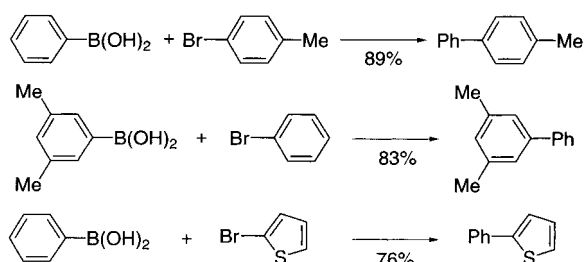
reacted under these conditions to afford the corresponding allylation products in high yields. While the reaction of ethyl acetoacetate with (*E*)-cinnamyl methyl carbonate (**4**) gave a mixture of *E/Z* stereoisomers ($E/Z = 64/36$), only *E* isomers were obtained in the reactions of **2** with **4** or *Z* carbonate **5**. The recovery was quantitative in all cases and the recovered catalyst could be reused.

$\text{MC}[\text{Pd}(\text{PPh}_3)]$ was successfully used in other reactions. Allyl acetate reacted with dimethyl phenylmalonate in the presence of $\text{MC}[\text{Pd}(\text{PPh}_3)]$, PPh_3 , *N,O*-bis(trimethylsilyl)-acetamide (BSA), and a catalytic amount of potassium acetate (KOAc), to afford the corresponding product in 90% yield (Scheme 1). In addition, Suzuki coupling reac-

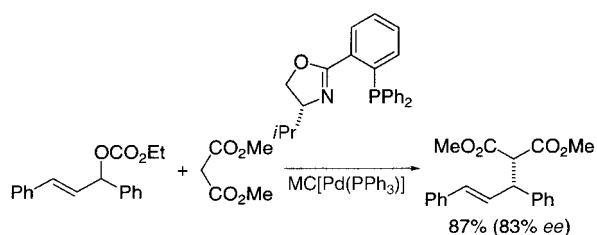


Scheme 1. Allylic substitution of allyl acetate with **2**. Reaction carried out using $\text{MC}[\text{Pd}(\text{PPh}_3)]$ (20 mol %), PPh_3 (20 mol %), BSA (3.0 equiv), and KOAc (0.10 equiv) in CH_3CN under reflux for 12 h.

tions^[16] of boronic acids with aryl bromides were found to proceed smoothly in the presence of $\text{MC}[\text{Pd}(\text{PPh}_3)]$ to afford the corresponding products in high yields. 2-Bromothiophene also worked well. In these reactions, the best results were obtained by using tri-*o*-tolylphosphane ($\text{P}(\text{o-Tol})_3$) as an external ligand (Scheme 2). Finally, a catalytic asymmetric allylation reaction was carried out using $\text{MC}[\text{Pd}(\text{PPh}_3)]$ and a chiral ligand (Scheme 3). The reaction of 1,3-diphenyl-2-



Scheme 2. Suzuki coupling. All reactions were carried out using $\text{MC}[\text{Pd}(\text{PPh}_3)]$ (20 mol %) and $\text{P}(\text{o-Tol})_3$ (20 mol %) in CH_3CN under reflux for 6 h.



Scheme 3. Asymmetric allylic substitution. Reaction carried out using $\text{MC}[\text{Pd}(\text{PPh}_3)]$ (20 mol %), chiral ligand (20 mol %), BSA (3.0 equiv), and KOAc (0.10 equiv) in CH_3CN under reflux for 12 h.

propen-1-yl ethyl carbonate (1.0 equiv) with dimethyl malonate (3.0 equiv) was performed in the presence of $\text{MC}[\text{Pd}(\text{PPh}_3)]$ (20 mol %), 2-(*o*-diphenylphosphanophenyl)-(*4R*)-isopropylloxazoline (20 mol %),^[17] BSA (3.0 equiv), and

KOAc (0.10 equiv) under reflux conditions in acetonitrile. The allylation adduct was obtained in 87 % yield with 83 % *ee*.

For the structure of the microencapsulated species, we assume an 18-electron Pd⁰ center that is coordinated by PPh₃ and the benzene ring(s) of polystyrene. After adding an external ligand, 14- or 16-electron Pd⁰ would be formed and the catalytic reaction proceeds.^[18] The coordination of the external ligand to Pd⁰ was confirmed by ³¹P SR-MAS NMR analysis which revealed that the recovered catalyst in Suzuki coupling reactions contained P(*o*-Tol)₃.^[19]

In summary, we have immobilized triphenylphosphane palladium onto a polymer using a microencapsulating technique. The polymer-supported catalyst (MC[Pd(PPh₃)]) has been successfully used in several palladium-catalyzed reactions. In all cases, the reactions proceeded in high yields, and the catalyst was recovered simply by filtration and reused. It should be noted that the air-sensitivity of the palladium complex is substantially suppressed by this immobilization. Moreover, NMR spectroscopic analyses have provided valuable information on the structure of the MC catalysts.

Experimental Section

A typical experimental procedure for the allylation reaction of **1** with **2**: **1** (0.55 mmol), **2** (0.5 mmol), PPh₃ (0.1 mmol), and MC[Pd(PPh₃)] (0.1 mmol, 20 mol %) were combined in acetonitrile (5 mL). The mixture was stirred for 12 h at room temperature. After ethanol was added to quench the reaction, the catalyst was collected by filtration and washed with ethanol and acetonitrile, and then dried. The solvents of the filtrate were removed under reduced pressure, and the crude product was purified by preparative thin-layer chromatography (TLC) to afford the desired adduct (83 %). The recovered MC[Pd(PPh₃)] was reused several times without loss of activity.

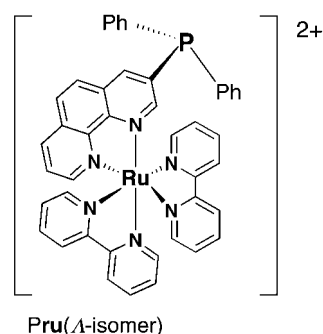
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A Light-Harvesting *tert*-Phosphane Ligand Bearing a Ruthenium(II) Polypyridyl Complex as Substituent

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Polypyridine ruthenium(II) complexes have been used extensively as visible-light active photosensitizers because they display metal-to-ligand charge transfer (MLCT) excited states with outstanding photochemical and photophysical properties.^[1] Numerous works are devoted to activating suitable substrates by pathways that involve intermolecular energy transfer and/or electron transfer. While an alternative approach that involves intramolecular sensitization is also described in the literature,^[2] to date this method is mainly directed to photophysical observation and photochemical net reactions are less explored. Since chromophore and substrate are fixed in close proximity, the photochemical reaction by



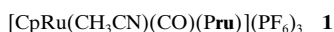
intramolecular sensitization has the expected advantage of minimized diffusion effect. As a new organometallic approach to such sensitization, we prepared the first *tert*-phosphane ligand (**Pru**) that has a covalently bound Ru^{II} polypyridyl complex ("sensitizer fragment") as a substituent. With such a "colored phosphane"

ligand coordinated to a transition metal center, we should be able to construct a system that can perform reactions promoted by visible photo-energy absorbed at the sensitizer fragment in the phosphane ligand and transmitted to the metal center through the metal–P bond. To our knowledge, however, there is no precedent for triplet electronic energy efficiently migrating to a transition metal through a coordinating *tert*-P atom and, therefore, this is the most challenging point of the present study. If this approach works, then photo-active phosphanes such as those presented here can open the way to a wide variety of complexes and applications because *tert*-phosphane is one of the most frequently used ligands classes in organometallic chemistry and homogeneous catalysis. Herein we report one such complex, a CpRu–**Pru** (Cp = cyclopentadiene) system that actually demonstrates efficient visible-light activity at the CpRu center.

Lithiation of [(bipy)₂Ru(3-bromo-1,10-phenanthroline)](PF₆)₂^[3] (bipy = 2,2'-bipyridine) with *n*BuLi at –78 °C in CH₂Cl₂ took place smoothly and was followed by addition of an equimolar amount of PClPh₂. After purification by column

chromatography under Ar, eluting with acetonitrile, the desired [(bipy)₂Ru(1,10-phenanthroline-3-yl)PPh₂](PF₆)₂ (**Pru**(PF₆)₂) was obtained as orange red powder in 83 % yield. In the ³¹P NMR spectrum (in CD₂Cl₂) of **Pru**(PF₆)₂ the signal at $\delta = -7.07$ is close to that of free PPh₃ ($\delta = -5.65$). Free **Pru** is easily converted into its oxide in CH₂Cl₂ solution under air when illuminated with visible light, in sharp contrast to related triarylphosphanes such as PPh₃. But in the dark, a solution of **Pru** is fairly stable and does not react with oxygen.

The reaction of **Pru**(PF₆)₂ with one equivalent of [CpRu(CH₃CN)₂(CO)]PF₆^[4] in CH₂Cl₂ at room temperature gave **1** almost quantitatively. FT-infrared (IR) spectrum of **1** in CH₃CN shows ν (CO) at 1996 cm^{–1}, which is comparable to the PPh₃ analogue **2** (1989 cm^{–1}), which indicate that the electron-donating ability of the ground-state **Pru** in **1** is a little less than that of PPh₃.



The ¹H NMR spectrum of **1** shows two singlets at $\delta = 5.18$ and 5.14 (ca. 1:1) attributable to Cp protons, and two doublets (J (P,H) = 1.20 Hz) at $\delta = 2.00$ and 1.97 (ca. 1:1) assigned to the coordinated acetonitrile, thus suggesting that two isomers are present. Apparently, the combination of two asymmetric centers, Δ and Λ on **Pru** and the CpRu-centered (*R*)- and (*S*)-epimers, results in two diastereomers.^[5] Photophysical properties of **Pru**(PF₆)₂ and **1** are compared in Figure 1, the reduced emission intensity of coordinated **Pru** in **1** compared to that of free **Pru** is clear. In **1** an intramolecular triplet energy transfer

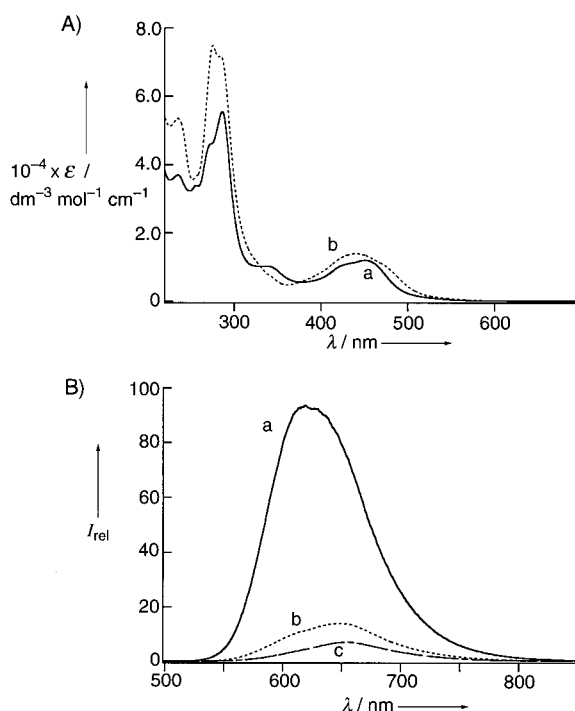


Figure 1. A) Absorption spectra of a) **Pru**(PF₆)₂ and b) **1** in CH₃CN. B) Emission spectra excited at 440 nm in de-aerated CH₃CN at 293 K: a) **Pru**; $\lambda_{\text{max}} = 619$ nm, b) **1**; $\lambda_{\text{max}} = 653$ nm, and c) **1** in aerated CH₃CN; $\lambda_{\text{max}} = 655$ nm.

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process takes place between the “sensitizer unit” in **Pru** and the $[\text{CpRu}]^+$ fragment, which leads to quenching of the luminescence from the excited state of the Ru^{II} –polypyridyl unit by about 85 %. The remaining 15 % emission from Ru^{II} –polypyridyl fragment in **1** was further quenched when the spectrum was recorded in aerated solution (curve c in Figure 1B). Quenching of the emission from $[\text{Ru}(\text{bipy})_3]^{2+}$ by oxygen is known.^[6]

The CH_3CN ligand in **1** and **2** is strongly coordinated to the metal center and replacement by CD_3CN or $[\text{D}_5]\text{pyridine}$ was not observed at room temperature in the dark after 48 h. In contrast, the facile substitution of CH_3CN in **1** by the solvent took place on irradiation with visible light (400 W high-pressure mercury lamp, $\lambda > 450 \text{ nm}$). Thus, after 40 min irradiation, $[\text{CpRu}(\text{CD}_3\text{CN})(\text{CO})(\text{Pru})](\text{PF}_6)_3$ (**3**), or $[\text{CpRu}(\text{C}_5\text{D}_5\text{N})(\text{CO})(\text{Pru})](\text{PF}_6)_3$ (**4**) were obtained in quantitative yields. The reaction was monitored by ^1H NMR spectroscopy. The time-conversion curve (Figure 2) was

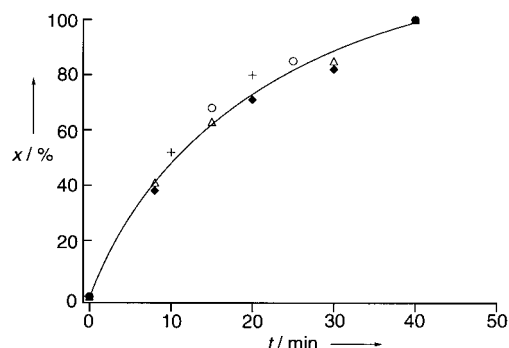
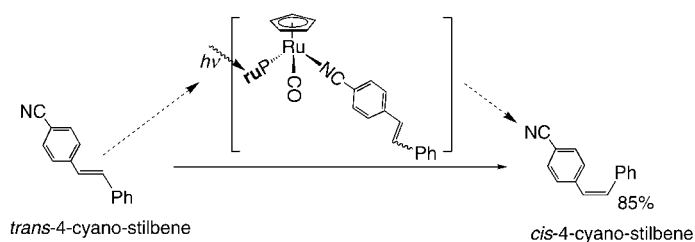


Figure 2. Rate for the CH_3CN –solvent exchange reaction of **1** on irradiation ($\lambda > 450 \text{ nm}$) in: (+) CD_3CN ; (○) $\text{CD}_2\text{Cl}_2/\text{CD}_3\text{CN}$ (9/1); (Δ) $[\text{D}_5]\text{pyridine}$; (◆) aerated CD_3CN . t = irradiation time, x = extent of CH_3CN substitution in **1**.

independent of solvent (CD_3CN or $\text{C}_5\text{D}_5\text{N}$) and of CD_3CN concentration (neat or 9/1 mixture of $\text{CD}_2\text{Cl}_2/\text{CD}_3\text{CN}$). Also, the presence of air exerts no effect though oxygen is known to quench the emission from polypyridine Ru^{II} species. All these observations are consistent with unimolecular dissociation of the coordinated CH_3CN in **1**, brought about by efficient intramolecular photosensitization from the Ru^{II} –polypyridyl group in **Pru**. As expected, similar irradiation of **2** did not cause exchange of the coordinated CH_3CN . Evidence that the dissociation of CH_3CN is not caused by mutual intermolecular sensitization between two molecules of **1** was obtained when a CD_3CN solution of **2** was irradiated in the presence of an equimolar amount of $[\text{Ru}(\text{bipy})_3]^{2+}$: there was no reaction.

When *trans*-4-cyano-stilbene,^[7] was employed instead of acetonitrile this visible-light induced substitution of the nitrile ligand resulted in concomitant isomerization of the C–C double bond to the *cis* form (Scheme 1). In the presence of a catalytic amount of **1** (8.0 mg, 0.006 mmol), pure *trans*-4-cyano-stilbene (55 mg, 0.27 mmol) in CH_2Cl_2 (5 mL) was irradiated by visible light (400 W lamp, $\lambda > 450 \text{ nm}$) at 25°C . After 2 h, a *cis* rich product mixture (*cis/trans* = 5.7/1) was formed as determined by gas chromatography (GC) and ^1H NMR spectroscopy. The reaction was carried out under



Scheme 1. The *trans* → *cis* isomerization on photo-induced nitrile ligand substitution.

aerated conditions to minimize direct (intermolecular) sensitization of 4-cyano-stilbene by the Ru^{II} –polypyridyl unit. Addition of CH_3CN (1.2 mL), thereby blocking the coordination of 4-cyano-stilbene to the CpRu center, leads to a dramatic retardation of the *trans* → *cis* isomerization to less than 10 % of the original rate (Figure 3). The curve b in

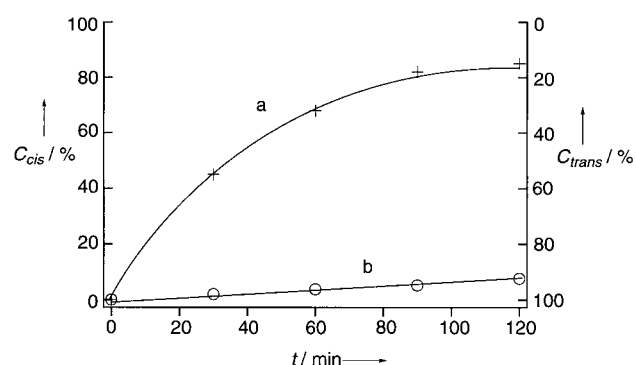


Figure 3. Rate curves for the *trans* → *cis* photo-isomerization of 4-cyano-stilbene on irradiation in the presence of **1** (2 mol %): a) in CH_2Cl_2 (5 mL); b) in the mixture solvent of CH_2Cl_2 (3.8 mL) and CH_3CN (1.2 mL). t = irradiation time.

Figure 3 is independent of further addition of CH_3CN (2.0 mL), which suggests that the slow isomerization observed under these conditions may be attributed to direct intermolecular sensitization. The *trans* → *cis* isomerization of stilbene and styrylpyridines by intermolecular triplet–triplet energy transfer from $[\text{Ru}(\text{bipy})_3]^{2+}$ or $[\text{Ru}(\text{phen})_3]^{2+}$ has been observed in acetonitrile solution.^[8] Under the concentration used here, intramolecular sensitization is much more effective than intermolecular (Figure 3).

In conclusion, herein we have shown that coordination of the new phosphane ligand **Pru** is a useful and convenient way of changing a transition metal complex into visible-light harvesting reaction center. Synthesis of other metal complexes containing **Pru** and the development of their photochemical reactions are now in progress.

Experimental Section

All reactions were carried out under an atmosphere of Ar, unless otherwise indicated.

Pru(PF_6)₂: *n*BuLi (170 mL, 1.54 M in hexane) was added by microsyringe to a solution of $[(\text{bipy})_2\text{Ru}(3\text{-bromo-1,10-phenanthroline})](\text{PF}_6)_3$ (250 mg, 0.26 mmol) in CH_2Cl_2 (20 mL) at -78°C . A brown precipitate formed immediately. After 10 min, neat PPh_2Cl (80 mL, 0.52 mmol) was added by microsyringe. After stirring for 30 min at -78°C the reaction mixture was

allowed to warm to room temperature. The crude compound was purified by flash chromatography on alumina eluting with CH₃CN to give analytically pure **Pru**(PF₆)₂ (200 mg, 83 %). ³¹P NMR (162 MHz, CD₂Cl₂): δ = −7.07 (s); MS (ES): *m/z* 922.9 [*M* − PF₆]⁺, 389.0 [*M* − 2PF₆]²⁺; UV/Vis (CH₃CN): λ_{max} [nm] (ε [dm³mol^{−1}cm^{−1}]): 450 (12000), 339 (10000), 285 (56000), 273sh (48000), 255 (35000), 235 (38000); elemental analysis (%) calcd for C₄₄H₃₃F₁₂N₆P₃Ru₁: C 49.49, H 3.11, N, 7.87; found C 49.77, H 3.21, N, 7.69.

1: A solution of **Pru**(PF₆)₂ (100 mg, 0.094 mmol) in CH₂Cl₂ (10 mL) was added to a stirred solution of [CpRu(CH₃CN)₂(CO)]PF₆^[4] (39.4 mg, 0.094 mmol) in CH₂Cl₂ (10 mL). After 24 h the solvent was evaporated under vacuum. The crude product was purified by column chromatography on alumina, eluting first with CH₃CN/Toluene (1/1) and then with CH₃CN to afford the pure product **1** (94.3 mg, 88 %). FT-IR (CH₃CN): ν̄ = 1996 cm^{−1} (C=O); ¹H NMR (400 MHz, CD₃CN, TMS): δ = 5.18 (s, 5H, C₅H₅), 5.14 (s, 5H, C₅H₅), 2.00 (d, *J*(P,H) = 1.20 Hz, 3H, CH₃CN), 1.97 (d, *J*(P,H) = 1.20 Hz, 3H, CH₃CN); ³¹P NMR (162 MHz, CD₃CN): δ = 48.77 (s), 48.67 (s); UV/Vis (CH₃CN): λ_{max} [nm] (ε [dm³mol^{−1}cm^{−1}]): 441 (14000), 284 (72000), 275 (75000), 235 (53500); elemental analysis (%) calcd for C₅₂H₄₁F₁₈N₇O₁P₄Ru₂: C 43.13, H 2.85, N 6.77; found C 43.52, H 2.96, N 6.54.

Photosubstitution of CH₃CN in **1**: The photochemical reaction was carried out with a 400 W high-pressure mercury lamp through a Toshiba Y-47 glass filter (λ > 450 nm). The exchange reaction was monitored by the intensity of the ¹H NMR resonance signals of the CH₃CN and Cp units.

3: 40 min irradiation of **1** in CD₃CN; yield 100%; spectroscopic data are identical to **1**.

4: 40 min irradiation of **1** in [D₅]pyridine; yield 92%; FT-IR (CH₃CN): ν̄ = 1979 cm^{−1} (C=O); ¹H NMR (400 MHz, CD₃CN, TMS): δ = 5.30 (s, 5H, C₅H₅), 5.24 (s, 5H, C₅H₅); ³¹P NMR (162 MHz, CD₃CN): δ = 51.26 (s), 51.16 (s).

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The Hexaphosphapentaprismane P₆C₄tBu₄: A “Jaws-Like” Cage Molecule That Bites!*

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Organophosphorus cage compounds are of considerable current interest.^[1] Two major synthetic routes to these compounds are the cyclooligomerization of phosphalkynes (often metal-mediated)^[2] and the coupling of polyphospholyl anions.^[3] Examples include 1) the tetraphosphacubane P₄C₄tBu₄ from the thermal oligomerization of PCtBu or treatment of [ZrCp₂(P₂C₂tBu₂)] with C₂Cl₆,^[4] and 2) the FeCl₃-mediated oxidative coupling of a mixture of the anions [1,2,4-P₃C₂tBu₂][−] (**1**) and [1,3-P₂C₃tBu₃][−] (**2**) to afford the pentaphospha cage compound P₅C₅tBu₅.^[5] More recently, we reported the structurally characterized hexaphospha cage compound P₆C₆tBu₆, which to date is the largest phosphalkyne oligomer known.^[6]

We were interested in expanding this area to include cages containing an additional heteroatom, and recently we showed that treatment of **1** with EI₄ (E = Si, Ge) leads to the two structurally different cage compounds P₆C₄tBu₄GeI₂ (**3**) and P₆C₄tBu₄SiI₂ (**4**).^[7] These cages are probably formed by two successive [2+2] cycloadditions of adjacent triphospholyl rings of the bis-η¹ intermediate (P₃C₂tBu₂)₂EI₂. We now report the synthesis of the related phosphorus–chalcogen cages P₆C₄tBu₄E (E = S, Se, Te), with full structural characterization (E = Se, Te) or NMR spectroscopic characterization (E = S). These compounds were obtained by an unprecedented reaction involving the facile specific insertion of the chalcogen atom into a P–P bond of the hexaphosphapentaprismane P₆C₄tBu₄ (**5**). Compound **5** was first synthesized by Breit, Mack, and Regitz by an indirect method, but more recently improved syntheses involving coupling of two (P₃C₂tBu₂)[−] anions were independently developed by Nixon et al. and Zenneck et al.^[1b,8]

In view of the ease and specificity of the insertion reactions of several carbene-like species into **5**, we have likened this behavior to that of the well-known shark “Jaws”. This novel feature of its reactivity is further exemplified by the ready reaction of **5** with the stable germylene GeR₂, stannylene SnR₂ (R = N(SiMe₃)₂), and the plumbylene PbR₂ (R' = C₆H₃(NMe₂)₂) to afford the structurally characterized

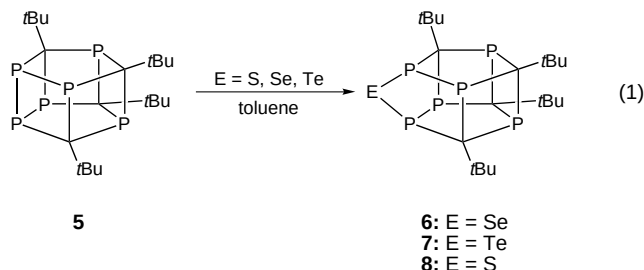
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insertion products $P_6C_4tBu_4GeR_2$, $P_6C_4tBu_4SnR_2$, and $P_6C_4tBu_4PbR_2$.^[9, 10]

Treatment of **5** with elemental S, Se, or Te in toluene at room temperature (S, Se) or 60 °C (Te) readily affords the new cage compounds $P_6C_4tBu_4E$ (**6–8**) by specific insertion of the heteroatom into the unique P–P bond which joins the two component $P_3C_2tBu_2$ ring systems [Eq. (1)]. All the com-



pounds are stable towards air and moisture and are thermally robust, melting without decomposition above 240 °C. Each exhibits a molecular-ion peak in its EI mass spectrum, and the multinuclear solution spectra of **6**, **7**, and **8** are fully consistent with their proposed cage structures, which were subsequently confirmed by single-crystal X-ray diffraction studies on **6** and **7** (vide infra).

The ^1H NMR spectrum of **6**, which is typical, displays two singlets for the two inequivalent *t*Bu groups. The $^{31}P\{^1H\}$ NMR spectrum consists of three complex multiplets arising from an AA'MM'XX' spin system, but despite their complexity the large $^1J_{PP}$ coupling constant of 235 Hz can be clearly distinguished. A $^1J_{PSe}$ coupling of 202 Hz was observed both in the ^{77}Se NMR spectrum and for the satellites in the $^{31}P\{^1H\}$ spectrum.

The molecular structure of **6** (Figure 1 a) is very similar to that of **3**^[7] and can be formally viewed as arising from simple substitution of the GeI_2 moiety with a selenium atom. The P–Se–P angle of 91.69(9)° is marginally smaller than usual, but the P–Se bond lengths of 2.2615(19) Å in **6** lie in the expected range for single bonds. Compound **7** (Figure 1 b), which as expected has a molecular structure similar to that of **6**, is only the third structurally characterized compound containing a P–Te–P structural motif, along with $[(CO)_4Cr(\mu-PtBu_2)_2Te]^{[11]}$ (**11**) and $[(MeCH)_2N_2P]_2Te^{[12]}$ (**12**). The P–Te–P angle in **7** (85.94(3)°) lies between those of **11** (81.4°) and **12** (94.5°), while the P–Te bond lengths of 2.4728(9) and 2.4790(5) Å are comparable with those of 2.481(1) and 2.491(1) Å in **11** and 2.56 Å in **12**.

Solution NMR data for **7** are also consistent with the above structure and are similar to those of **6**; the $^1J_{PP}$ coupling constant is 240 Hz. The ^{125}Te satellites in the $^{31}P\{^1H\}$ NMR spectrum yielded a $^1J_{PTe}$ value of 358 Hz, which was confirmed by the ^{125}Te NMR spectrum, which in addition gave a $^2J_{PTe}$ coupling constant of 57 Hz. As expected, coupling constants involving tellurium are generally around two to three times greater than the corresponding couplings involving selenium.^[13]

To better understand why only the unique central P–P bond is specifically involved in these insertion reactions, quantum

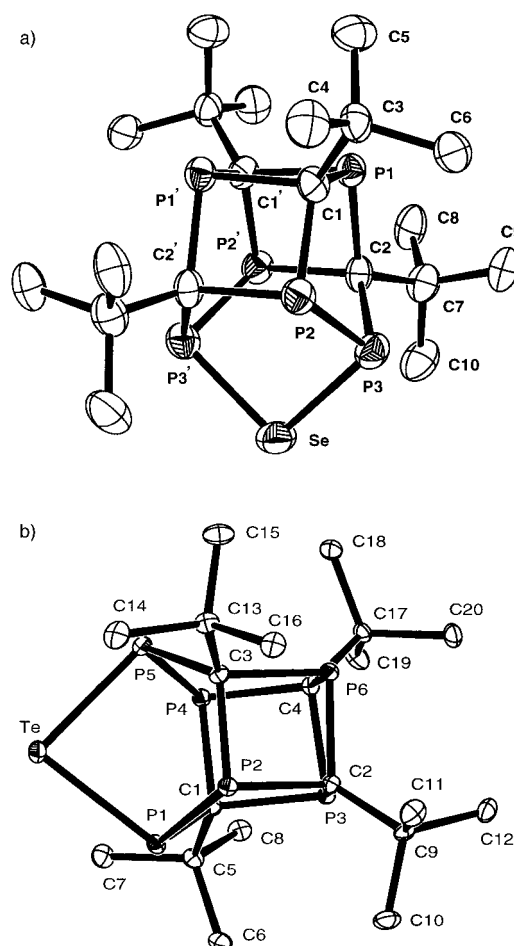
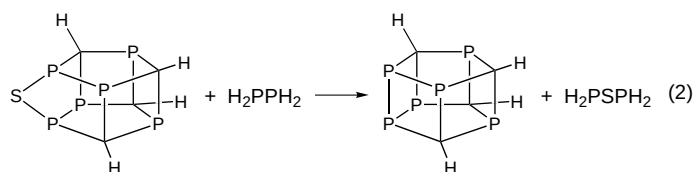


Figure 1. View of the molecule of **6** (a) and **7** (b) in the crystal.

chemical calculations were carried out.^[14] Optimization of the parent hexaphosphapentaprismane $P_6C_4H_4$ (**5H**) at the B3LYP/6-31G* level of theory resulted in a structure similar to that obtained from the X-ray diffraction study on **5**.^[8a] The calculated length of the central P–P bond in **5H** is 2.331 Å (2.2809(6) Å in **5**), while the other P–P bond length is 2.297 Å (2.2200(6) Å in **5**); this indicates slight weakening of the central P–P bond. In the hypothetical tetraphosphane $H_2PPHPPH_2$, the central P–P bond is calculated to be slightly shorter than the terminal bonds (2.248 and 2.274 Å, respectively), while in $Me_2PPMePMePMe_2$ all three P–P bonds are nearly identical in length. The Wiberg bond index in **5H** is slightly larger for the central P–P bond (0.964) than for the two other P–P bonds (0.957). It seems therefore that the two types of P–P bonds in **5H** have similar strengths. Likewise, the three P–P bonds in tetraphosphane do not differ much in energy. For the isodesmic reaction shown in Equation (2), a net stabilization of only 1.4 kcal mol^{−1} was



obtained, and this indicates that the two P–P bonds in tetraphosphane have similar strengths.

The electronic structure of **5H**, as evidenced by the shape of the HF/6-31G**/B3LYP/6-31G* frontier orbitals shown in Figure 2, indicates that the central P–P unit is involved not

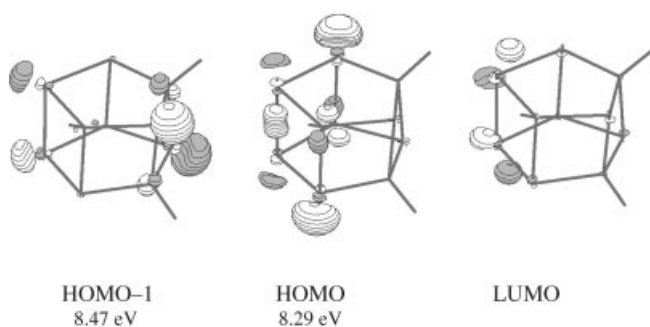


Figure 2. Frontier orbitals of **5H** at the HF/6-31G**/B3LYP/6-31G* level of theory.

only in both the HOMO and LUMO but also in HOMO – 1.^[15] This is strong evidence that the central P–P bond is likely to be involved in a bond-cleavage reaction of **5H**. Interestingly, the lowest B3LYP/6-31G* harmonic frequency (224 cm⁻¹) involves a vibration in which the unique central P–P bond length is changing while the other atoms of the cage show hardly any movement.

The triplet state of **5H** is only 18.6 kcal mol⁻¹ higher in energy (UB3LYP/6-31G*) than **5H** itself, and the cage opens at the central P–P bond. A search for a singlet biradical (UB3LYP/6-31G*) resulted in **5H'**, which has a similar geometry to and almost the same energy as the triplet (Figure 3); however, the formal *S*² value of the UB3LYP

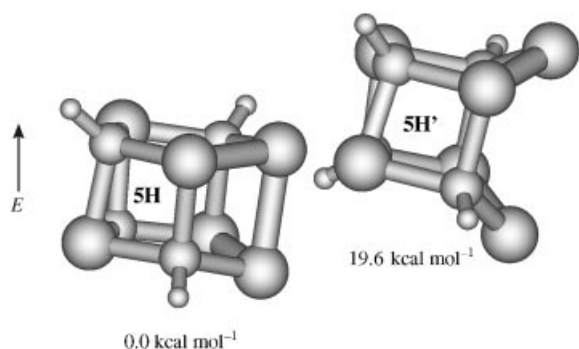


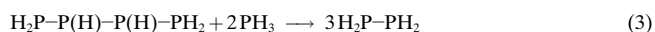
Figure 3. Structure and energy (UB3LYP/6-31G*) of **5H** and **5H'**.

wavefunction is 1.03. Optimization at the UMP2/6-31G* level resulted in a similar structure, again with an *S*² value of 1.03 instead of 0.0, which is the expected value for the singlet state. The energy difference (MP2CAS(2,2)/6-31G**/B3LYP/6-31G*) of 19.6 kcal mol⁻¹ between **5H** and **5H'** provides support for the above energy difference between the closed and opened structures.

As evidenced by the structural data and Wiberg bond indices discussed above, there is no significant difference in the electronic nature of P–P single bonds in general and that of the unique P–P bond in the hexaphosphapentaprismane **5**

in particular. However, opening the latter bond might cause the rest of the molecule to relax, so that the product is stabilized relative to **5**. Since the P–P bond energy is about 50 kcal mol⁻¹,^[16] and **5H'** is less stable than **5H** by 19.6 kcal mol⁻¹, the destabilization of the central P–P bond in **5** can be estimated to be about 30 kcal mol⁻¹. The destabilization is also in accord with the rules set up by Böcker and Häser^[17] for four-membered phosphorus ring systems.

Thus, the product stability plays a decisive role in reactions which involve opening of the central P–P bond in **5**. Calculating the relative energies of the pentaprismane-based C₄H₄P₆S isomers, we found the lowest energy for **8H**, in which the sulfur atom inserted into the central P–P bond. The structure in which the sulfur atom inserted into the other P–P bond of **5H** is higher in energy by 13.88 kcal mol⁻¹, and possible isomers in which the sulfur atom is doubly bonded to the other phosphorus atoms of the P₄ unit in **5H** are all at least 20 kcal mol⁻¹ less stable than **8H**. The isodesmic reaction shown in Equation (3), which is endothermic by



30.8 kcal mol⁻¹, also clearly shows that the stability of the product relative to the reactant is the driving factor that opens the unique central P–P bond of **5**. The destabilization energy deduced from the isodesmic reaction also favorably matches that deduced above for the central P–P bond of **5** and thus offers a ready explanation for its unexpected reactivity. The overall reaction sequence involving the reactant cage compound, the biradical intermediate, and the product cage is shown pictorially in Figure 4.

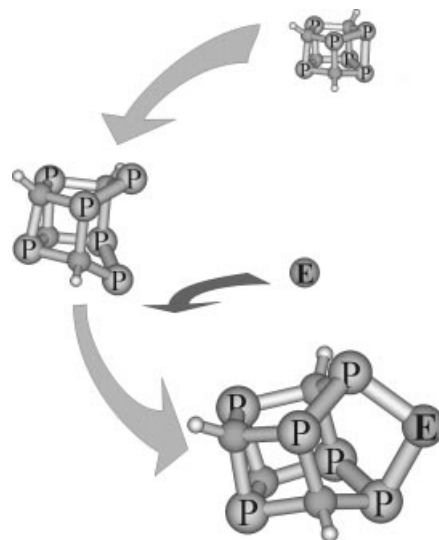


Figure 4.

Experimental Section

Spectroscopic data: **6**: ¹H NMR (300 MHz, [D₆]benzene, 25 °C): δ = 1.13 (s, 18 H, *t*Bu), 1.33 (s, 18 H, *t*Bu); ³¹P{¹H} NMR (121.68 MHz, [D₆]benzene, 25 °C, 85 % H₃PO₄): δ = 138.5 (m, ¹J(P,P) = 235 Hz, P3), 141.0 (m, ¹J(P,P) = 235 Hz, P2), 145.5 (m, P1); ⁷⁷Se NMR (95.39 MHz, [D₆]benzene, 25 °C,

Me₂Se): δ = 290 (tt, ¹J(P,Se) = 202, ²J(P,Se) = 22 Hz); MS (70 eV): *m/z* (%): 542 (6) [*M*]⁺, 485 (10) [*M* – *t*Bu]⁺, 231 (23) [*P*₃C₃*t*Bu₃]⁺; m.p. 245 °C; elemental analysis (%) found (calcd): C 44.56 (44.38), H 6.59 (6.70). **7**: ¹H NMR (300 MHz, [D₆]benzene, 25 °C): δ = 1.16 (s, 18H, *t*Bu), 1.41 (s, 18H, *t*Bu); ³¹P{¹H} NMR (121.68 MHz, [D₆]benzene, 25 °C, 85 % H₃PO₄): δ = 91.5 (m, ¹J(P,P) = 240 Hz, P1, P5), 134.0 (m, P3, P6), 146.8 (m, ¹J(P,P) = 240 Hz, P2, P4); ¹²⁵Te NMR (157.86 MHz, [D₆]benzene, 25 °C, Me₂Te): δ = 424.8 (tt, ¹J(P,Te) = 358, ²J(P,Te) = 57 Hz); MS (70 eV): *m/z* (%): 592 (43) [*M*]⁺, 535 (6) [*M* – *t*Bu]⁺, 464 (77) [*P*₆C₄*t*Bu₄]⁺, 231 (100) [*P*₃C₃*t*Bu₃]⁺; m.p. 264 °C; elemental analysis (%) found (calcd): C 41.40 (40.72), H 6.11 (6.15). **8**: ¹H NMR (300 MHz, [D₆]benzene, 25 °C): δ = 1.12 (s, 18H, *t*Bu), 1.28 (s, 18H, *t*Bu); ³¹P{¹H} NMR (121.68 MHz, [D₆]benzene, 25 °C, 85 % H₃PO₄): δ = 138.7 (m, ¹J(P,P) = 239.6 Hz, P3), 156.0 (m, ¹J(P,P) = 235 Hz, P2), 154.9 (m, P1); MS (70 eV): *m/z* (%): 494 (100) [*M*]⁺, 437 (15) [*M* – *t*Bu]⁺.

Crystal data: **6**: C₂₀H₃₆P₆Se, *M*_r = 541.27; crystal dimensions 0.2 × 0.1 × 0.05 mm, monoclinic, space group *C2/c* (No. 15); *a* = 13.780(2), *b* = 14.862(3), *c* = 12.1833(13) Å, β = 94.981(1)°, *V* = 2485.8(7) Å³, ρ_{calcd} = 1.45 g cm^{−3}; θ = 4.64–22.98°, MoK α radiation (λ = 0.71073 Å), *T* = 173(2) K, 5013 reflections, 1708 independent reflections (*R*_{int} = 0.095); *R*₁ = 0.057, *wR*₂ = 0.128 (*I* > 2σ*I*); *R*₁ = 0.086, *wR*₂ = 0.141 (all data); Multiscan absorption correction, μ = 1.90 mm^{−1}, *T*_{max} = 0.842, *T*_{min} = 0.789. **7**: C₂₀H₃₆P₆Te, *M*_r = 690.91; crystal dimensions 0.3 × 0.2 × 0.02 mm; triclinic, space group *P1* (No. 2); *a* = 10.4038(3), *b* = 14.8533(3), *c* = 16.5591(15) Å, α = 91.435(1), β = 94.981(1), γ = 99.001(2)°, *V* = 2526.4(1) Å³, ρ_{calcd} = 1.55 g cm^{−3}; θ = 3.76–27.91°, MoK α radiation (λ = 0.71073 Å), *T* = 173(2) K, 43231 reflections, 12034 independent reflections (*R*_{int} = 0.058); *R*₁ = 0.039, *wR*₂ = 0.081 (*I* > 2σ*I*); *R*₁ = 0.062, *wR*₂ = 0.089 (all data); Multiscan absorption correction, μ = 1.56 mm^{−1}, *T*_{max} = 0.829, *T*_{min} = 0.756.

Diffraction data for both structures were recorded on a Kappa CCD instrument. Both structures were solved and refined by using the SHELX-97 suite of programs for crystal structure analysis (G. M. Sheldrick, University of Göttingen, Germany, 1997). Structures were refined by full-matrix least-squares methods on $|F^2|$. Hydrogen atoms were placed in calculated 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 no. CCDC-161083 and -161084. 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). Figures 2 and 3 were drawn with the program MOLDEN.^[18]

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