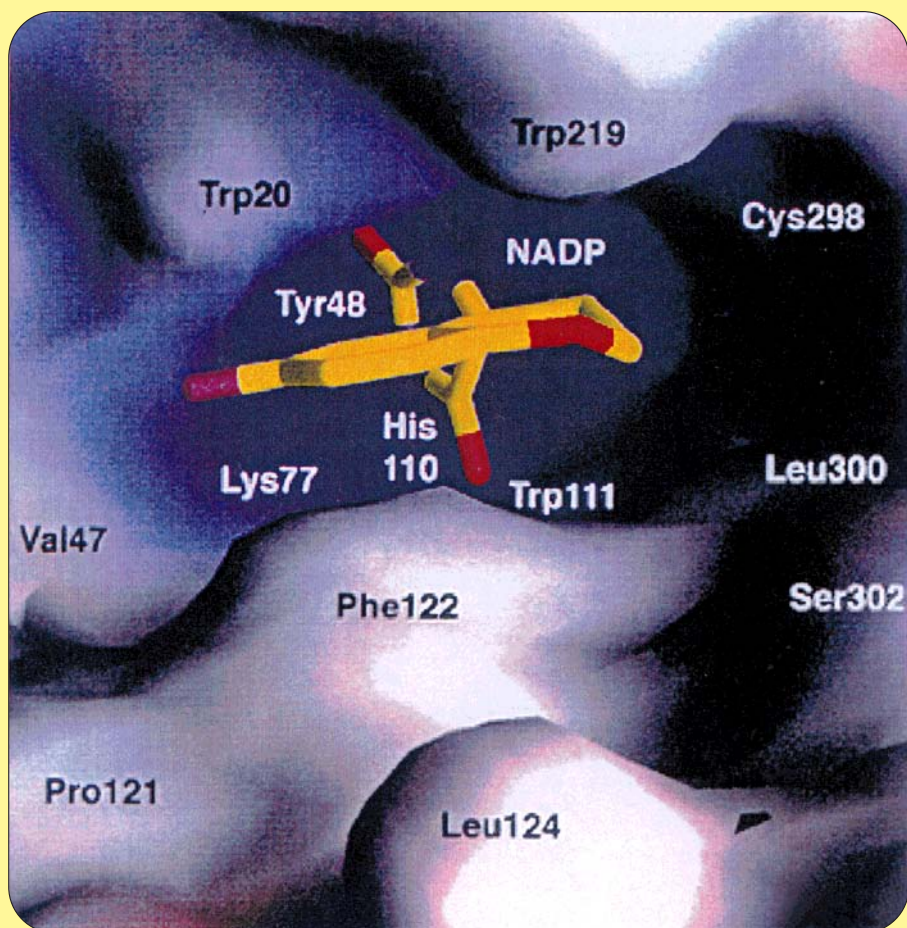
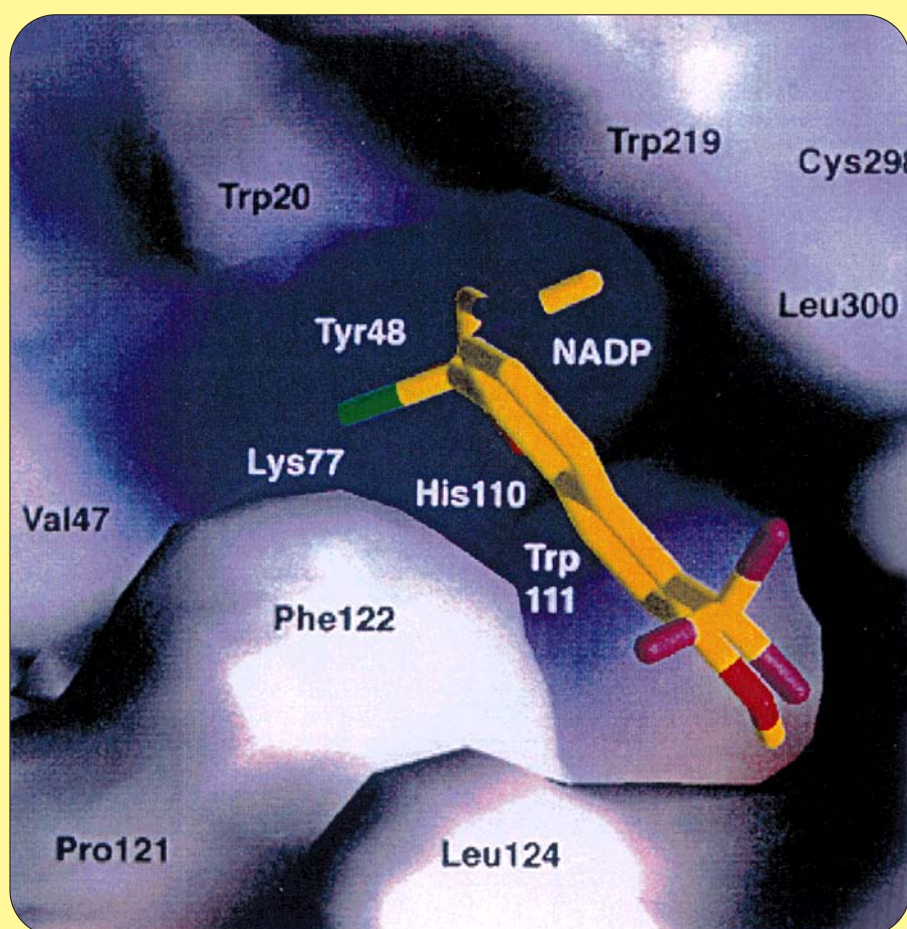




Crystallization of aldose reductase with the inhibitors sorbinil (top) and tolrestat (bottom) reveals that their polar head groups both bind near the coenzyme, but that their hydrophobic moieties are at 90° to each other. Sorbinil binds with few changes in the enzyme structure, whereas the binding of tolrestat induces a displacement of Leu300 and Phe122. This figure was produced with permission from Podjarny et al (*Structure* 1997, 5, 601).



Hydrogen Bonding, Hydrophobic Interactions, and Failure of the Rigid Receptor Hypothesis

Andrew M. Davis and Simon J. Teague*

Why does experimental determination of the structure of drug–receptor complexes so often result in surprises? The literature is scattered with examples of drugs which bind to their receptors in orientations quite different from that expected from simple overlay or indeed from molecular modeling. Charge-reinforced hydrogen bonds are very strong, but neutral–neutral hydrogen bonds are much weaker and their contribution to the binding constant can range from 15-fold to zero. Hydrophobic interactions are less easily visualized, but appear to play a crucial role in the binding of many drugs to their receptors. In a number of cases, where the structure of a series of complexes was determined during structure–activity studies, tighter binding has been observed in analogues where hydrophobic interactions have been optimized, even at the

expense of possible hydrogen bonds. The optimization of hydrophobic interactions also plays a major role in “induced fit” of receptors to ligands. One consequence of induced fit is that several molecules, with differing shapes, can bind and fit well to the same part of a certain receptor. Overemphasis of the importance of hydrogen bonds has often resulted in the incorrect prediction of binding orientation within a series of related ligands. The balance between hydrogen bonds and hydrophobic interactions is addressed to differing extents by existing molecular modeling packages. At present most assume the receptor is almost rigid. None are able to predict the large or small changes often observed in experimentally determined drug–receptor complexes. In validations some programs were more successful in docking polar ligands than hydropho-

bic ones. However, a balance between polar and hydrophobic properties is required for druglike molecules. Tight binding of drugs to receptors is achieved through polar interactions and most importantly through the optimization of *specific* hydrophobic interactions. The bulk properties of drugs, which control solubility, absorption, metabolism, and pharmacokinetic properties, is achieved mainly with polar groups, together with sufficient lipophilicity to achieve partition into lipid bilayers. The present status of rational design tools still necessitates the employment of a range of approaches in the discovery of new drugs.

Keywords: drug research • hydrogen bonds • hydrophobic effect • molecular modeling • protein structures

1. Introduction

The successful practice of medicinal chemistry is crucially dependent upon an understanding of the principles of molecular recognition. Surely the greatest success in the field of molecular recognition was one of the first, the discovery of the principles underlying the structure of DNA by Watson and Crick in the early 1950s.^[1] The mutual recognition of adenine (A) and thymine (T) as well as guanine (G) and cytosine (C) bases for each other is determined by their hydrogen-bonding

and shape complementarity. More recently a great deal of information about protein structure has become available from X-ray crystallography and NMR spectroscopy. This, together with the use of energy calculations and docking procedures from molecular modeling, has resulted in predictions of the bound geometry of new ligands and made the concept of rational design of drugs a reality. Although it is well accepted that the binding of a drug to its receptor is mediated by ion–ion interactions, hydrogen bonding, dipole–dipole interactions, lipophilicity, and shape complementarity, the relative contributions of each of these interactions is still surprisingly poorly understood. This is because, although many studies have tried to quantify these interactions through mathematical deconvolution of observed binding energies^[2, 3] or experiment,^[4, 5] few have attempted to experimentally dissect the relative contribution of these interactions under conditions that are relevant to the biological situation, that is, in water.

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The contribution of hydrogen bonding to drug–receptor interactions varies greatly. It is often overlooked that formation of a hydrogen bond in a drug–receptor complex is an exchange process, and that in order to form this bond similar bonds have to be broken between the drug and water and between the receptor and water. Likewise new hydrogen bonds are formed between the drug and receptor and between the previously hydrogen bonded water molecules and bulk water. The favorability of the drug–receptor hydrogen bond depends upon the overall energy change involved in breaking and forming all these hydrogen bonds. The work of Fersht et al.^[6] and Williams et al.^[7] represents two important and complementary approaches to quantifying the contribution of hydrogen bonding to drug–receptor binding interactions; structural alterations to the receptor and to the drug were investigated. Fersht and co-workers studied the coupling of tyrosine to ATP to yield tyrosyl-AMP, the first step in the transfer of tyrosine to t-RNA catalyzed by the enzyme tyrosyl t-RNA synthase.^[6] Site-directed mutagenesis was used to probe the energetics of this coupling through the effects of mutations upon the kinetics of the reaction. Because the crystal structure of the complex was known, mutations could be interpreted directly as losses in specific hydrogen bonds, salt bridges, and hydrophobic contacts. Through a series of point mutations, they were able to determine that a neutral–neutral hydrogen bond contributes only about 0.5–1.5 kcal mol^{−1} in binding (equivalent to a 2- to 15-fold increase in affinity), but the presence of a charged hydrogen bond contributes up to 4.7 kcal mol^{−1} (equivalent to a 3000-fold increase in affinity).

Williams et al. studied the binding of model peptides of the D-ala-D-ala terminus of the growing peptidoglycan bacterial cell wall to the antibiotic vancomycin by NMR spectroscopy.^[7] Structural modification of the peptide caused changes in the overall binding affinity to vancomycin. The NMR investigations provided information on both the macroscopic binding constants and individual interactions between the peptide and vancomycin. Williams et al. estimated the contributions of neutral–neutral hydrogen bonds to be only 0.5–1.5 kcal mol^{−1}, as observed by Fersht et al. Further confirmation of these

estimates of the contribution of hydrogen bonds to binding can be found in a study of the affinities of fluorodeoxy sugar analogues to glycogen phosphorylase, where hydroxyl groups were serially replaced with hydrogen bond accepting fluorine atoms.^[8] This study also found that a neutral–neutral hydrogen bond is worth up to about 1.5 kcal mol^{−1}, which is equivalent to a maximum 15-fold increase in binding. The degree of agreement between these studies is remarkable, and are further supported by many observations from drug design investigations.^[9–11] Even though a hydrogen bond between a charged and a neutral component can contribute up to 3000-fold to the binding of a drug, a neutral–neutral hydrogen bond is worth less than 15-fold in binding. Very recently Williams and Westwall have revised even these very modest contributions to binding from neutral–neutral hydrogen bonds by suggesting they are a gross overestimate, and that their true local contribution to binding is near zero!^[12] Even more orthodox views concerning the importance of hydrogen bonds, for instance in the cross-linking of collagen 4-hydroxyproline residues, has recently been called into question.^[13]

The contribution of hydrophobicity to drug–receptor interactions is well described. Removal of the hydrophobic surface area from water, by binding into a hydrophobic region of a receptor, is generally worth a minimum of 28 cal Å^{−2} mol^{−1}, which is the equivalent of 0.68 kcal mol^{−1} or a 3.2-fold increase in binding constant per methyl group.^[14] This is similar to the energy gain in partitioning of a drug from water to a hydrophobic solvent such as *n*-octanol.^[15] In some circumstances, where the complementarity between the drug's hydrophobic surface and the receptor is particularly high, the contribution can be significantly greater than this.^[16] Large numbers of hydrophobic atoms are present in drug molecules, and it is apparent that hydrophobicity is a major source of binding in drug–receptor interactions. This conclusion is supported by a survey of the properties of marketed oral drugs as listed in the Physicians Desk Reference.^[17] This shows that on average drugs contain only one to two donors and three to four acceptors, whereas the average number of hydrophobic atoms in a drug molecule is 16 (Figure 1).

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This view of the energetics of drug–receptor interactions is not new, though it does not appear to be universally appreciated. It is often assumed that, although hydrophobicity

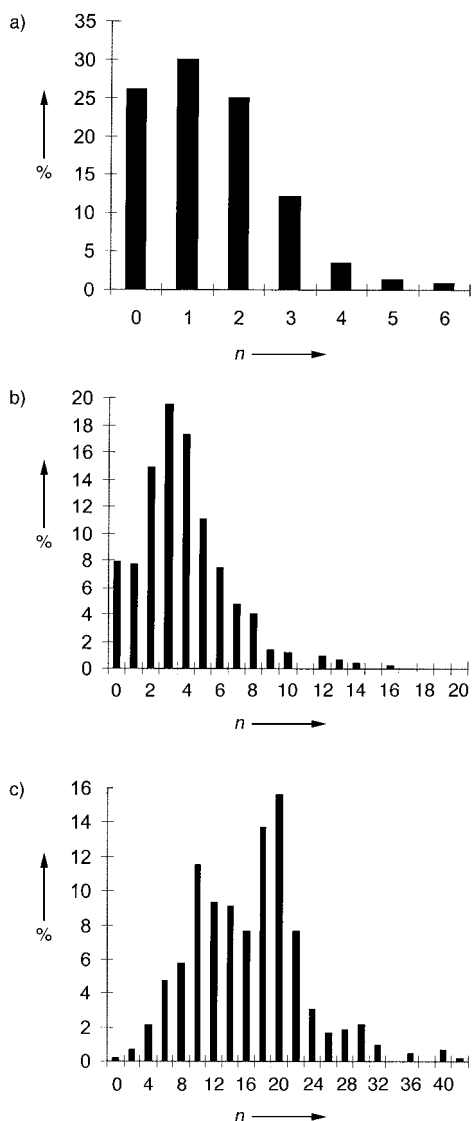


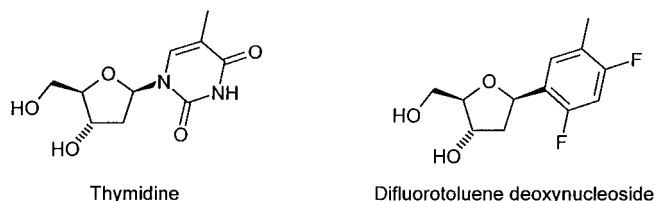
Figure 1. a) Distribution of hydrogen-bond donors in 415 oral drugs listed in the Physicians Desk Reference. b) Corresponding distribution of hydrogen-bond acceptors. c) Corresponding distribution of hydrophobic atoms. Ordinate: frequency in %; abscissa: number n of the donors, acceptors, or hydrophobic atoms.

makes an important contribution to binding, its contribution is nonspecific. Likewise drug–receptor hydrogen bonds are considered as important contributors to binding, and moreover, because of their directionality, important in determining the specificity of drug–receptor binding. These conclusions are reinforced by the large amount of X-ray structural data from protein–inhibitor complexes where hydrogen bonding and charge–charge interactions are clearly visible. But just seeing an interaction tells us nothing about its contribution to binding. In recent years the structures of protein–inhibitor complexes for a series of analogues in the course of investigation of structure–activity relationships have sometimes been used as a drug-design tool, often with surprising

results concerning the relative geometry or orientation of bound ligands. A few examples have been noted previously, but with the rapidly increasing numbers of protein–ligand complexes appearing in the literature^[18] these surprising results are appearing with increasing frequency. In this review we highlight some of these examples and use them to support the hypothesis that the balance of contribution between polar and hydrophobic interactions in molecular recognition may need reevaluation. The importance of hydrophobicity and induced fit in drug–protein interactions is highlighted, together with the deficiencies of the predictive tools presently available to us.

2. Hydrophobic Interactions instead of Hydrogen Bonds

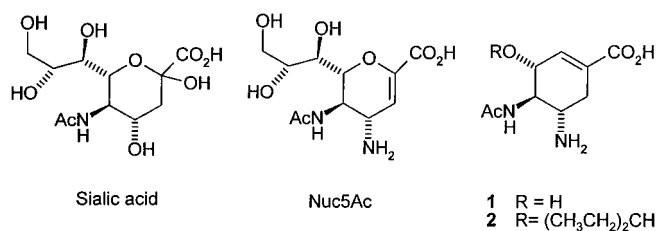
There are many occasions in the literature where affinity is enhanced through hydrophobic interactions, even at the expense of hydrogen bonds. The demonstration that DNA polymerase efficiently and faithfully pairs adenine with difluorotoluene deoxynucleoside when it has been incorporated in a DNA strand provides an elegant demonstration of the importance of shape fit to enzyme–substrate recognition.^[19] The efficiency is only fourfold lower than that observed with thymidine, and the selectivity for adenine is 2.9–4.2 log units with respect to incorporation of C, T, or G.



The current assumption that the number and strength of hydrogen bonds is the prime determinant of efficiency and fidelity in DNA synthesis may require reexamination. The participation of an unconventional hydrogen bond (C–F...H–N) between the fluoro analogue and adenine could not be demonstrated even in favorable solvents such as chloroform. No inherent pairing selectivity was observed in the absence of DNA polymerase. It is interesting to note that the resulting double-stranded helix is actually destabilized by 4–5 kcal relative to thymidine in the same position; thus, hydrogen bonds are important in DNA duplex. However, this work provides evidence that they are much less important in the recognition of bases by DNA polymerase, which is a situation much more analogous to drug–receptor interactions.

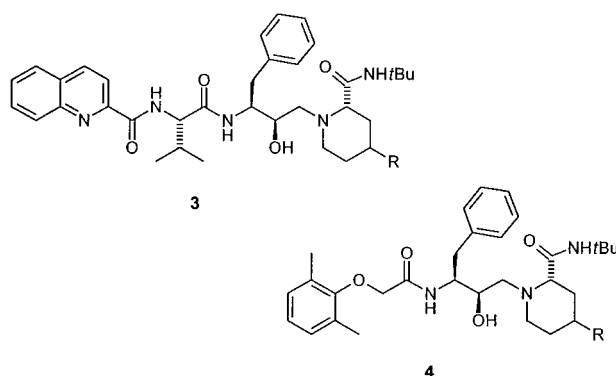
The discovery of inhibitors of influenza neuraminidase (NA) has provided one example where hydrogen-bonding interactions can be replaced by additional hydrophobic binding.^[20] Inhibitors of NA were discovered based upon transition state analogues of sialic acid cleavage from glycoconjugates. X-ray crystallographic studies of Neu5Ac and its analogues with NA revealed that the two terminal hydroxyl groups of the glycerol side chain form a bidentate

hydrogen-bonding interaction with Glu276. Removal of the glycerol side chain and replacement with a hydroxyl group gave **1** ($IC_{50} = 6300 \text{ nM}$), which is in good agreement with data from Fersht et al. for the loss of a hydrogen bond between a

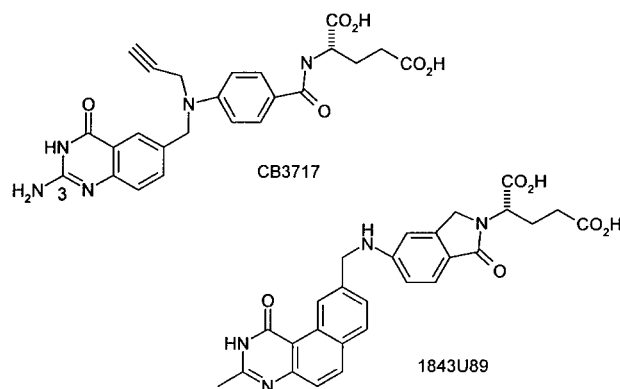


charged and a neutral component.^[6] However, in a series of alkyl analogues, steady increase in inhibitory activity was observed that culminated for **2** ($IC_{50} = 1 \text{ nM}$). X-ray crystallographic analysis of the complex of **2** with NA shows that the 3-pentyl group is bound against a large hydrophobic surface created by the hydrocarbon side chains of polar amino acids Glu276, Arg224, and hydrophobic residues Ala246 and Ile222! The carboxylate group of Glu276, to which the glycerol hydroxyl groups had been bound in Nuc5Ac analogues, was forced outward from the hydrophobic pocket. Interestingly it had previously been suggested^[21] that hydrogen bonds were important in the molecular recognition of all carbohydrates, with one hydrogen bond often associated with each sugar hydroxyl group.

The structure–activity relationships of two series of HIV-2 protease inhibitors of the type **3** and **4** have been described.^[22] They display very similar affinities despite the replacement of the P2 and P3 substituents in **3** by the lipophilic dimethylphenoxy substituent in **4**. X-ray crystallographic analysis of the protein–inhibitor complex of **3** reveals hydrogen bonds between the quinoline amide Asp29' and Gly48'. The complex with **4** shows induced fit to the dimethylphenoxy group through a shift of 1 Å for Asp29' and a shift of 4 Å for the side chain at Asp30'. The flap region (43'–48') also undergoes a conformational change upon binding the more lipophilic inhibitor.



The complexes between thymidylate synthetase (TS) and the inhibitors CB3717 and 1843U89 ($K_d = 0.1 \text{ nM}$) have been compared.^[23] The two inhibitors lie in nearly identical



positions, despite the loss of the hydrogen-bonding guanidine group at position 3 in 1843U89; in CB3717 it was hydrogen bonded to Ala263 (Figure 2). The glutamate attachments occupy quite different positions in the two inhibitor complexes. Induced fit to the receptor is critical since the addition of an extra ring in 1843U89 results in the compound being 4 Å longer. 1843U89 takes up an L-shaped conformation with Ile79 forming a contact to both the isindolinone group and the benzoquinazoline rings from its position on the inside of the “L”. Residue Phe176 contacts the benzoquinazoline group

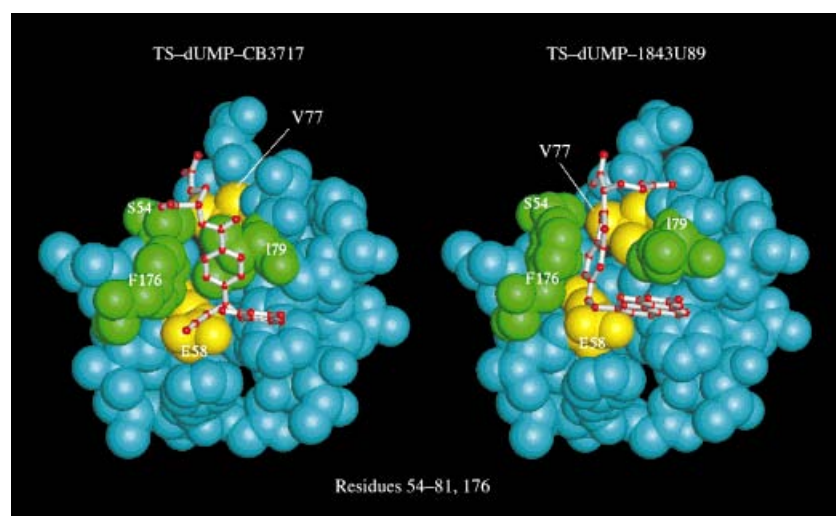


Figure 2. The binding of CB3717 and 1843U89 to thymidylate synthetase. The movement of Ile79 (I79) and Phe176 (F176) are apparent. This figure was produced with permission from Montfort and Weichsel.^[23]

on the outside face. The degree of induced fit caused by 1843U89 is dramatic, and involves roughly half the protein and includes residues on all sides of the binding pocket. The atoms of the main chain of Ile79 shift by approximately 1.5 Å and the side-chain atoms by 2.0–6.6 Å between the two complexes. The authors conclude that the discovery of 1843U89 “has implications for drug design, as 1843U89 could not have been obtained from current structure-based approaches”.^[23]

The complexes of matrix metalloproteinase inhibitors **5** and **6** ($X = \text{CH}$) with stromelysin (MMP-3) have been compared (Figure 3).^[24] Replacement of the P3' N-methyl amide group

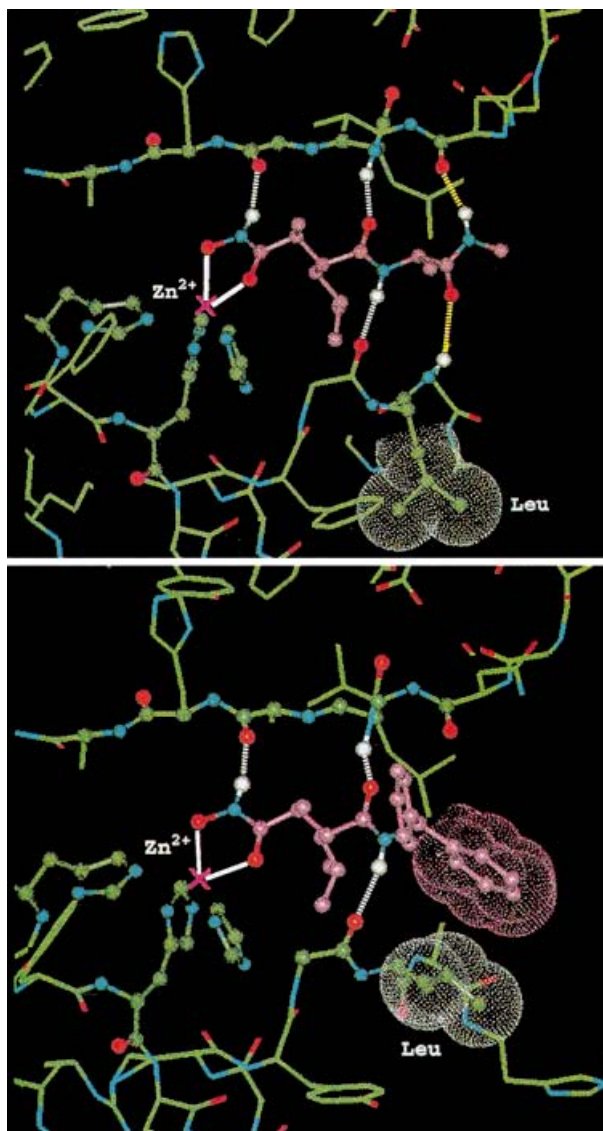
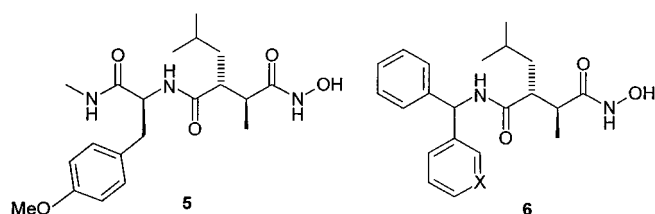
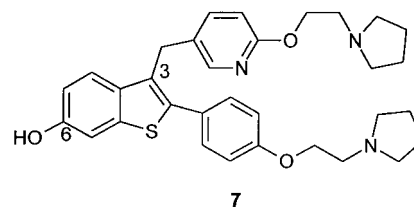


Figure 3. Complexes of **5** (top) and **6** (bottom) with stromelysin. Hydrophobic binding to a leucine residue replaces two hydrogen bonds. This figure was produced with permission from Decicco and DeGrado et al.^[24]

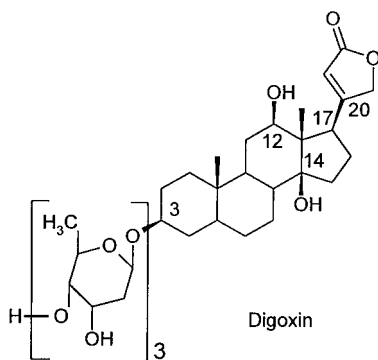
in **5** by a phenyl ring ($\rightarrow$ **6**) proved to be possible despite the loss of two hydrogen bonds. The complex with **6** showed an unexpected shift of a loop (residues 222–231), which allowed a Leu residue to hydrophobically bind to the benzhydryl moiety. Having made this surprising discovery, the group was able to utilize the X-ray data and the newly created hydrophobic environment in this region of the complex to design further improvements to the inhibitors. Thus, by introducing a hydrogen bond acceptor ($X = \text{N}$) a further 16-fold improvement was observed, consistent with the formation of a neutral hydrogen bond.

Several different series of potent, active-site inhibitors of thrombin have been discovered to bind to the enzyme in surprising ways.^[25–27] The dibasic benzo[b]thiophene inhibitors^[25] unexpectedly place the hydrophobic benzo[b]thiophene nucleus in the S_1 pocket of the enzyme, the site which usually accommodates the basic side chain of arginine in the standard D-Phe-Pro-Arg sequence. A hydrogen bond from the hydroxyl group at position C6 of the benzo[b]thiophenes to Asp189 was observed in **7**, with the C6–H compound showing fourfold lower affinity. The relative importance and



specificity of the hydrophobic interaction with the benzo[b]thiophene was demonstrated, however, when replacement by thiophene, benzofuran, indole, or naphthyl rings all resulted in much more dramatic decreases in affinity. High-throughput screening thus provided an opportunity to break out into a new series of inhibitors. The group at Roche^[26] has very ably and amusingly described their attempts to estimate relative affinities of inhibitor analogues based upon X-ray structure determination of the complex between thrombin and representative compounds in a series. Many experienced medicinal chemists will empathize with them for the difficulties they have encountered and agree with their conclusion that “It will continue to be important to critically examine the structure–activity relationships of any class of inhibitor for indications of unusual binding. The enzyme “sees”, binds, and possibly adapts to the outside of the inhibitor folded in some low energy conformation, and is blissfully ignorant of the inhibitor’s internal chemistry. The chemist on the other hand sees a compound as a core structure, decorated on the outside with interesting substituents. It is only fair to say that we must learn to look at inhibitors the way an enzyme does”.

Some drugs possess several hydroxyl and polar groups, which in the many discussions of drug–receptor interactions would be termed “interactive”. However, these assumptions are not always confirmed when the structure of a drug–receptor complex is determined experimentally. One example is the binding of digoxin to 26–10 Fab, a monoclonal antibody^[28] which demonstrates almost exclusively hydrophobic interactions even though these interactive groups are present.

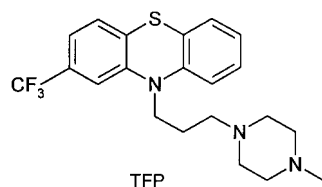


The effectiveness and specificity of shape complementarity and hydrophobic interactions is revealed in this complex. The antibody binds the drug with an affinity of 0.1 nM despite the lack of hydrogen bonds or charged group interactions. Digoxin binds with the lactone ring buried in a deep pocket at the bottom of the combining site and with the carbohydrate groups mostly exposed to external solvent. The lactone ring is flipped by 180° about the C17–C20 bond relative to the structure of unbound digoxin in the crystal. The drug is sandwiched between the aromatic rings of the V_H domain (Tyr33, Tyr47, Tyr50, and Trp100) which form the binding pocket. The surface complementarity is closest at the lactone and the steroid D-ring, decreases towards the periphery of the binding site, and has significant gaps between the surfaces around the hydroxyl groups at C12 and C14. The antibody also binds digitoxigenin, which lacks the 12- β -OH group, with equal affinity.

2.1. The Failure of the “Rigid Receptor” Hypothesis

Hydrophobic interactions are often associated with conformational changes of the receptor. Conformational change in a receptor upon binding a ligand is usually termed induced fit. In theory such changes could be the result of polar or hydrophobic interactions of the receptor with the ligand. However, examples in the literature are overwhelmingly the result of hydrophobic interactions. In these cases they could equally well be thought of as hydrophobic collapse of a receptor around a ligand. The relative preponderance of examples of hydrophobic binding may be a clear indication of the relative strengths of hydrophobic interactions compared to polar interactions.

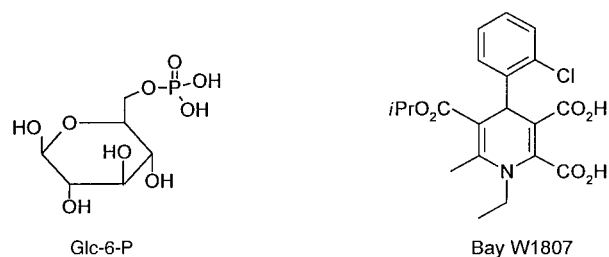
Trifluoperazine (TFP) is an inhibitor of Ca^{2+} -calmodulin (Ca^{2+} -CaM).^[29] Binding by TFP induces a major conformational change in the protein from an elongated dumbbell into a compact molecular form which can no longer interact with its target enzymes. Four TFP molecules per Ca^{2+} -CaM are observed in the X-ray crystal structure. The role of the net



positive charge on the inhibitors is postulated to be stabilization of the resultant globular conformation, rather than providing essential binding capability. Several bind-

ing modes for TFP had previously been predicted, but none agreed with the observed structure for the complex. This was because of the large conformational change upon binding, unexpected hydrophobic interactions between two of the TFP molecules, the involvement of more than one region of the protein in forming a particular TFP binding site, and interaction of many of the involved regions of the protein with more than one TFP.

High-throughput screening has resulted in the discovery of Bay W1807, a potent inhibitor of glycogen phosphorylase (GP; Figure 4).^[30] Bay W1807 binds to an allosteric binding



site which binds a number of endogenous, phosphorylated molecules such as AMP and glucose 6-phosphate (Glc-6-P). Comparisons of the GP complexes of Bay W1807 and Glc-6-P reveal that they both bind their charged groups to the arginine residues of the pocket (Arg309 and Arg310), but that the increased affinity of Bay W1807 (in the nanomolar range) compared to that of Glc-6-P (in the micromolar range) is largely the result of additional hydrophobic interactions.

The chlorophenyl ring is sandwiched between Phe196 and Val45', with the chlorine substituent making contacts with the aliphatic parts of Arg193 and Asp227. Favorable interactions exist between Tyr75 and the N-ethyl group and between the 2-propyl group, Trp67, Ile68, and the aliphatic part of Arg193. Major conformational change of the allosteric binding site is seen on binding of Bay W1807. Shifts in the Phe196 side-chain atoms of up to 2.9 Å occur as well as shifts of 1.2 Å in Val45'. Bay W1807 becomes almost completely buried, leaving only 7% of its surface area accessible to solvent. The authors comment that “Screening for effective ligand binding has identified high-affinity ligands that, by chance, are able to generate conformational changes that lead to high affinity, selective binding. Although such changes are easy to detect and rationalize in terms of the crystal structure they are much less easy to predict in a way that would facilitate structure-based drug design”.

Many peptidic, rationally designed renin inhibitors have been discovered, based upon transition state analogues of the scissile Leu-Val moiety in human angiotensinogen. High-throughput screening^[31] resulted in the discovery of 3,4-disubstituted piperidine **8** ($\text{IC}_{50} = 50 \mu\text{M}$). Optimization resulted in a remarkable improvement in affinity culminating in **9** ($\text{IC}_{50} = 0.47 \text{ nM}$). X-ray crystallographic determination of a series of compounds obtained during this optimization process showed induced fit adaptation of the receptor pocket to accommodate the substituents at positions 3, 4, and 5 of the piperidine ring. The resulting compounds show excellent

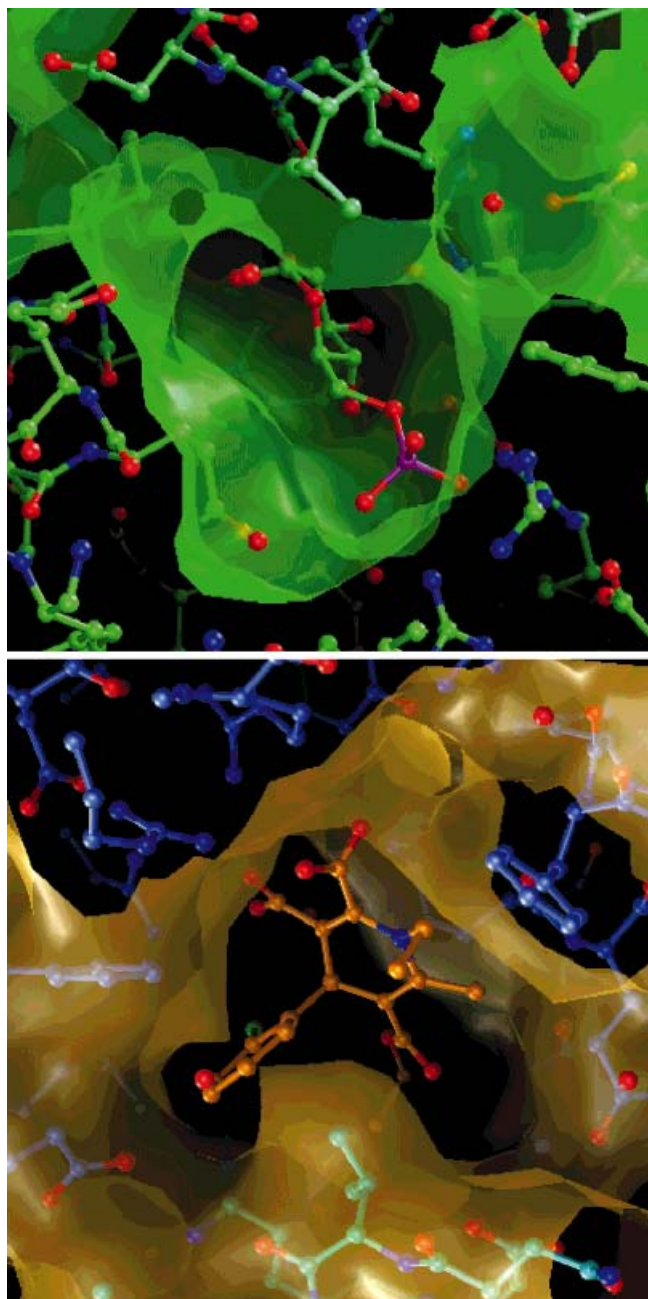
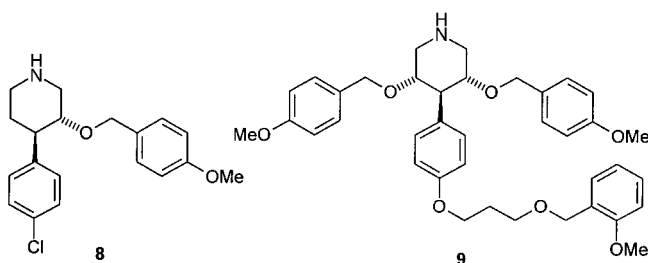


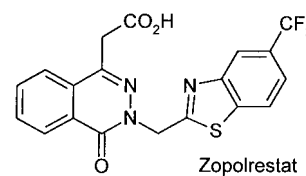
Figure 4. Top: The complex of glycogen phosphorylase b with glucose 6-phosphate. Bottom: The induced fit of the receptor to W1807. This figure was produced with permission from Johnson et al.^[30]



metabolic stability, moderate bioavailability, and good duration of activity in various animal models.

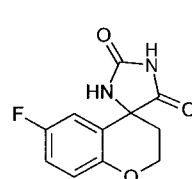
Comparison of the structure of human aldose reductase with and without the 3 nm inhibitor zopolrestat bound reveals a

snugly fitting, hydrophobic pocket for the drug at the active site.^[32] The formation of this pocket is ascribed to hinged-flap motions induced by the ligand. Once bound zopolrestat has an

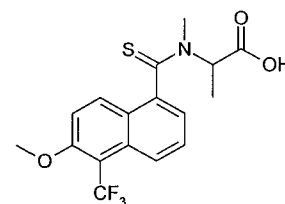


accessible surface area of 12.7 Å², a mere 6.4 % of that for the unbound inhibitor. The benzothiazole ring is sandwiched between Trp111 and Leu300, and the phthalazinone ring between Trp20 and Phe122. Ligand binding is accompanied by displacement of Leu300 towards the pocket and movement of loop 121–135, which enables Phe122 to participate in the binding pocket. The carboxylate group does play some part, being linked to His110 through a salt bridge; one oxygen atom mimics the carbonyl oxygen atom of the substrate. However, the authors comment that “The hydrophobic residues are the major determinants of molecular recognition. The marked preference of the enzyme for hydrophobic substrates (e.g. steroids) is consistent with this mode of binding the inhibitor”. At least six ordered water molecules are also displaced, which may contribute up to 2 kcal mol^{−1} per water molecule to the entropic term in the overall binding energy.^[33]

Crystallization of tolrestat and sorbinil with aldose reductase (AR) reveals that the polar head groups both bind near the coenzyme, but that the orientations of their hydrophobic moieties are at 90° to each other (Figure 5).^[34] Sorbinil binds



Sorbinil



Tolrestat

with few changes in the enzyme structure, but tolrestat binding induces a displacement of Leu300 and Phe122. The observed IC₅₀ values for the two inhibitors are, however, very similar.

Conformational change as a consequence of hydrophobic interactions is observed with cofactors as well as drugs.^[35] The cofactor NAD⁺ induces loop closing in the enzyme isopropylmalate dehydrogenase (IMDH). Five loops from different stretches of primary sequence move up to 2.5 Å to form predominantly hydrophobic interactions with the cofactor. Despite the common prejudice that adenine contains “interactive” nitrogen atoms, this may not always be the case. Determination of the structure of glutamine 5-phosphoribosyl-1-pyrophosphate (PRPP), the regulatory enzyme of de novo synthesis of purine nucleotides, reveals the adenine base of AMP in the C site is sandwiched between the side chains of Tyr242 and Val349 but forms no hydrogen bonds with the protein.^[36]

2.2. Receptor Plasticity and Multiple Ligands

Although the knowledge of several structurally diverse ligands for a given receptor is sometimes taken as sufficient

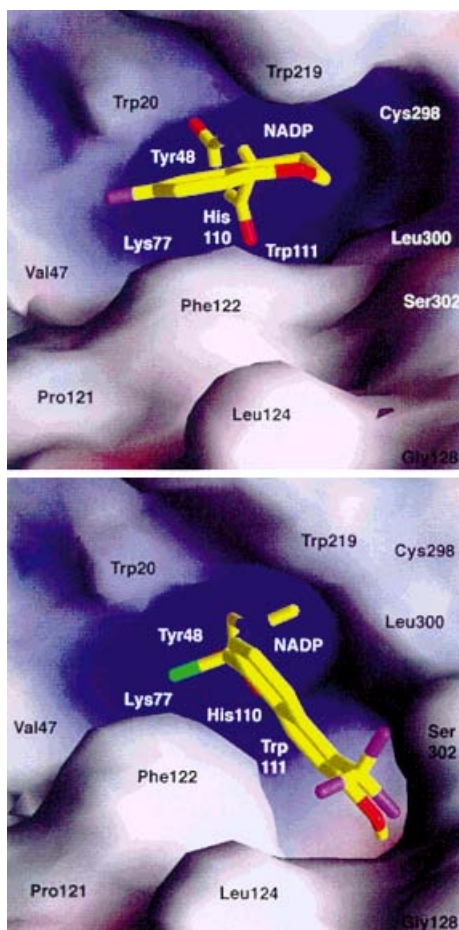
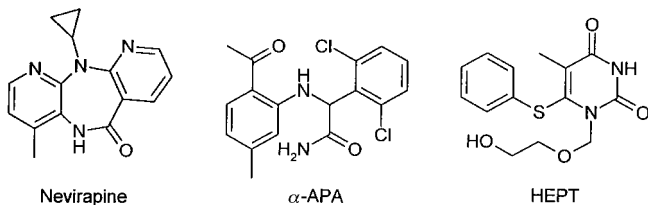


Figure 5. Induced fit of aldose reductase to the inhibitors sorbinil (top) and tolrestat (bottom). This figure was produced with permission from Podjarny et al.^[34]

evidence for several different binding sites, this may not necessarily be the case. A logical consequence of the importance of induced fit and hydrophobic interactions in receptor–ligand binding is that many structurally dissimilar ligands can interact with a biological target in similar orientations at the same binding site. Determination of the structures of HIV reverse transcriptase (RT) complexed with non-nucleoside inhibitors provides one such example (Figure 6).^[37]

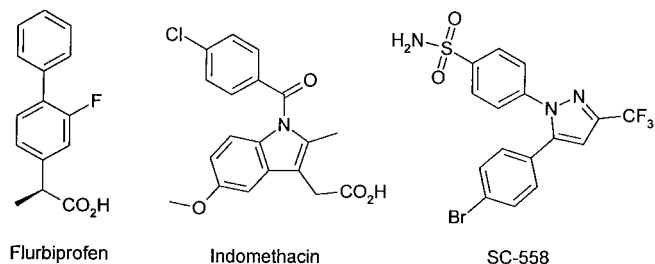
Detailed structural comparisons are made between the complexes of HIV-RT and the four inhibitors nevirapine, a close analogue 1051U91, and the chemically more diverse structures α -APA and HEPT. These compounds share a common mode of binding to RT! The common features of



binding are largely hydrophobic interactions that arise from induced shape complementarity, achieved by reciprocal con-

formational rearrangement of both the enzyme and the compounds. Residues Leu100, Tyr181, and Tyr188 participate in extensive hydrophobic contacts with all four inhibitors. By contrast the binding pocket is “electrostatically bland” (i.e., electrostatic interactions play a minor role), with polar interactions between the receptor and the ligands varying widely. A comparison of the structure with a “consensus” structure shows that none of the *Ca* positions of the enzyme residues are displaced by more than 2.7 Å. However, the changes observed correspond precisely with variation in substituents of the inhibitors. Resistant virus strains have evolved which mostly possess binding site residues with smaller hydrophobic side chains such as Tyr181Cys and Tyr188Cys. These serve to underscore the importance of hydrophobic interactions at the binding site.

Several structurally diverse inhibitors of cyclooxygenase-2 (COX-2) are known to bind to the enzyme in very similar positions.^[38] The distal ring of flurbiprofen overlaps with the bromophenyl ring of SC-558; the pyrazole of SC-558 superimposes on the fluorophenyl ring of flurbiprofen. The distal phenyl ring of flurbiprofen occupies an environment similar to that of the benzoyl ring of indomethacin. The carboxylate salts of flurbiprofen and indomethacin both bind to Arg120.



However, the carboxylate group of flurbiprofen and the hydrophobic trifluoromethyl group of SC-558 bind in the same cavity! The phenyl sulfonamide substituent, which is so crucial to COX-2 selectivity, has its phenyl ring surrounded by hydrophobic residues and the sulfonamide group extending into a relatively polar region near the enzyme surface.

Comparison of the free and bound structures of the enzyme attests to the conformational changes which occur upon binding of the ligands. Many of these changes are explicable by reference to the structure of the enzyme. Kinetic evidence on the slow binding kinetics of inhibitors such as indomethacin had already indicated that large-scale conformational changes accompany binding of ligands. Many drug-discovery programs focus upon selectivity between related receptors as the key issue rather than potency per se. Thus, the challenge for rational drug design is acute, to produce ligands that bind at one site in preference to binding at a structurally closely related site.

3. Incorrect Prediction of Binding Orientation

There are many examples in the literature where emphasis upon the superposition of polar binding sites for putative ligands has resulted in the incorrect prediction of binding orientation. The relative importance of hydrophobic and

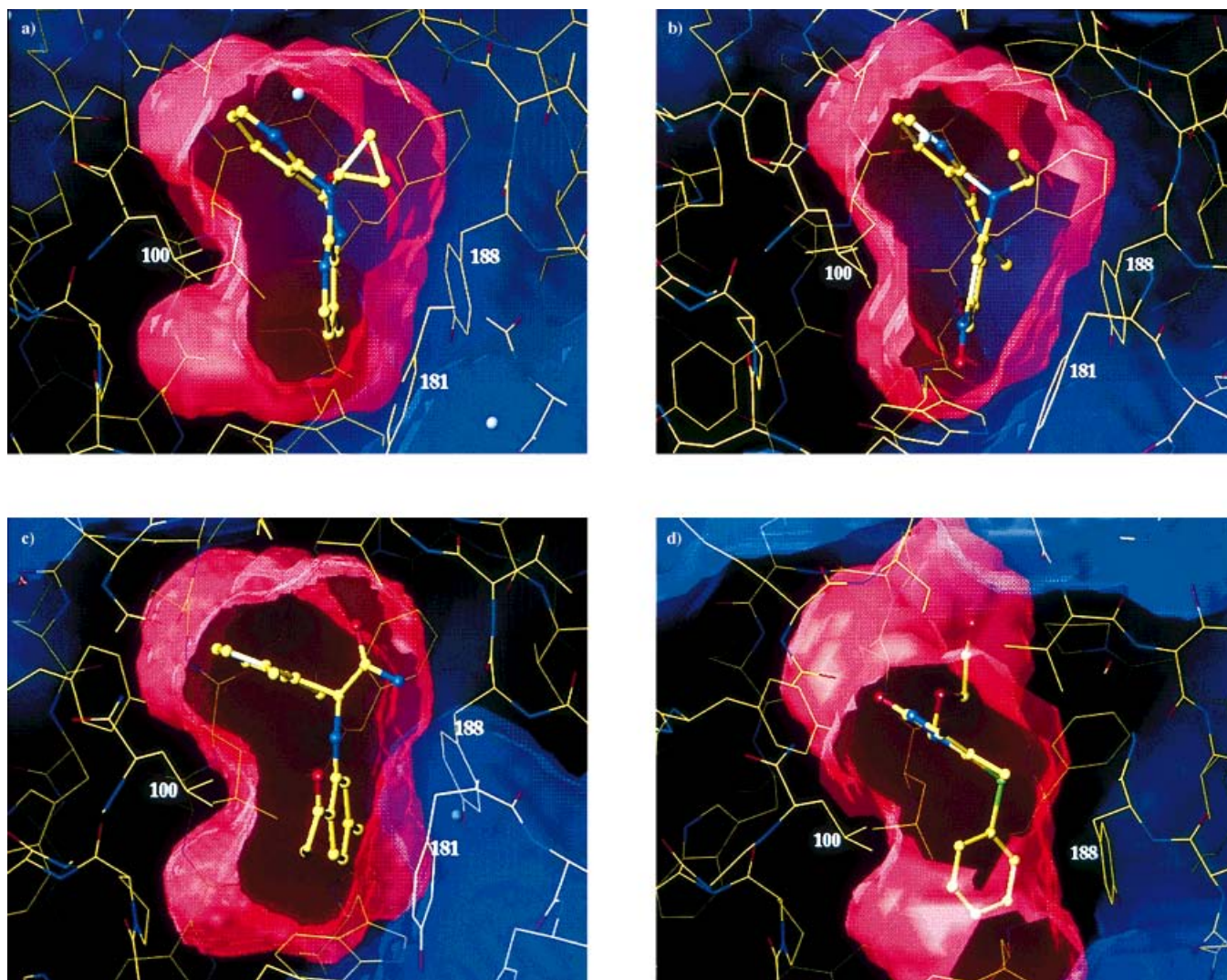
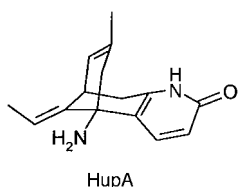


Figure 6. The binding of a) nevirapine, b) 1051U91, c) α -AMP, and d) HEPT to HIV-1 RT. The pink surface delineates the pocket by showing the volume accessible to a small probe (1.4 Å). The adaptations of the pocket to the ligands are clearly visible. This figure was produced with permission from Ren, Stuart, and Stammers et al.^[37]

polar interactions is especially important when attempting to predict the orientation of known ligands for a receptor in the absence of experimental observations. Weaknesses in the modeling programs for dealing with conformational change by the receptor and underestimation of hydrophobic interactions in the available force fields can result in a low level of confidence in the results. The literature is replete with examples of the difficulties encountered.



(-)-Huperazine (HupA) is a potent inhibitor ($K_i = 6 \text{ nM}$) of acetylcholinesterase (AChE). Consideration of its pharmacophoric groups suggested a plausible orientation for HupA parallel to the acetylcholine molecule.^[39] However, subsequent structure determination of the HupA–AChE complex revealed that its orientation in the active site gorge was orthogonal to that which was anticipated. Even though HupA has three potential hydrogen bonding sites only one hydrogen bond was observed. Its binding to AChE is through interaction of the

charged amine group with the aromatic rings of Trp84 and Phe330 together with several hydrophobic contacts to Trp84, His440, and with residues Gly118 through Ser122. Comparison with native AChE reveals conformational changes in the side chains of some of these aromatic residues, especially Trp84 and Phe330. Interestingly HupA lacks potentially complicating muscarinic effects, demonstrating how hydrophobic interactions may be exploited to achieve receptor selectivity.

The importance of conformational induction to signal transduction has been recently described.^[40] 17β -Oestradiol and the oestrogen antagonist raloxifene bind to the same site but in different modes (Figure 7). Distinctly different conformations of the receptor are induced, providing evidence of the mechanism of antagonism. It is tempting to align the phenolic hydroxyl group of the A ring of oestradiol with that on position 6 of the benzothiophene moiety and the hydroxyl group on position 17 with that on the 2-phenyl substituent. However, a comparison of the two complexes shows that the later two are displaced by 5.1 Å. The group at C3 of raloxifene makes extensive hydrophobic contacts as well as a hydrogen

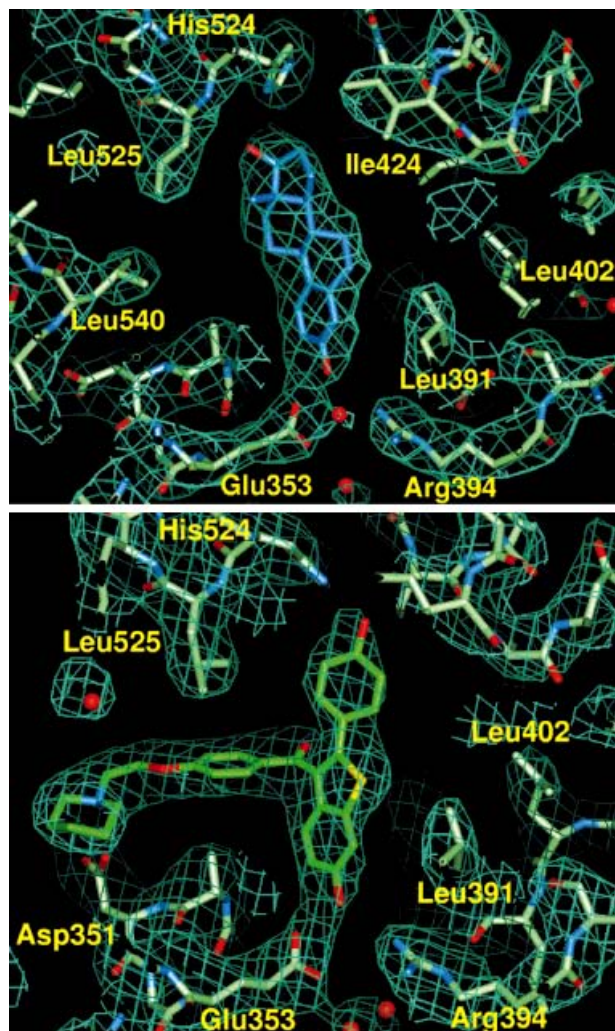
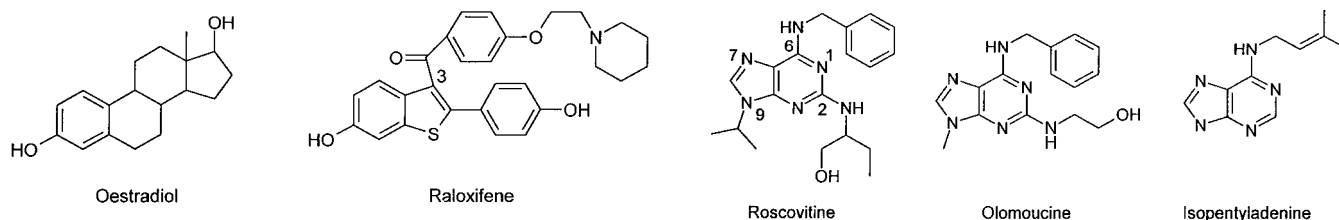


Figure 7. Oestrogen in the ligand binding domain of the oestrogen receptor (top) and the induced fit upon binding raloxifene (bottom). This figure was produced with permission from Pike and Hubbard et al.^[40]

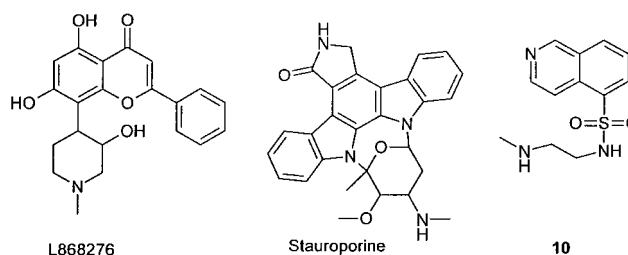
bond between the piperidine nitrogen atom and Asp351. Helix 12 is displaced and the transactivation of genes by the steroid complex disrupted.

The ATP binding sites of kinases provide a striking example of several superficially similar molecules that bind to the adenine site in completely different orientations. Roscovitine binds with the purine ring system flipped over compared to that in the ATP complex.^[41] The atom N7 is close to the position of N1 in the ATP complex. The C2 substituent is placed in the “ribose pocket”. The roscovitine–kinase interaction involves extensive hydrophobic interactions, dominated by contacts to Ile10, Leu83, and Leu134. The N6 benzyl group makes hydrophobic contacts with Ile10, Phe82,

and His84. Ligand-specific differences in induced fit are seen when the two complexes are compared, such as a reorientation of the His84 residue. The reorientation of the purine ring systems results in observations that would be rather surprising in the absence of experimental structure determination. For example, substitution by hydrophobic residues at N9, which bears the ribophosphate in ATP, produces the most active compounds. Olomoucine binds in a very similar orientation to roscovitine.^[42] The side chain of Ile10 is rotated by 120° relative to the conformation of the side chain in the ATP complex. The inhibitor isopentyladenine^[41] shows yet another binding mode with the purine ring rotated by 180° about an axis through N3 and N7, with the substituent at N6 occupying the ribose pocket.

Kinases appear to be very flexible, and the adenine pocket is dominated by hydrophobic interactions. These factors may conspire to produce a permissive binding site which accepts a range of planar heterocyclic systems, many of which have been uncovered by high-throughput screening.

L868276 binds the benzopyran ring in the adenine pocket of CDK2.^[43] The bicyclic ring system is in the same plane as the adenine in the ATP complex, but rotated by 60°. Significant motions of the side chains are seen upon binding L868276, with Asp145 and Lys89 moving away from the pocket and Ala144 moving closer. Many contacts to the pendant phenyl ring are observed, and the possible importance of these to selectivity can be outlined.



Staurosporine uses the indolylcarbazole group in its complexes with cAPK^[44] and CDK2^[45]. The positions of nearly all the residues that interact with staurosporine in its complex with cAPK are altered to accommodate this ligand, which is significantly larger than ATP. A hydrogen bond between Thr183 and N7 of ATP has no equivalent in the staurosporine complex, and the side chain of Phe327 is rotated to make a favorable π – π interaction with one of the indole rings. In the staurosporine–CDK2 complex the side chain of Gln131 is displaced from the binding pocket. The complex of **10** with cAPK^[46] reveals the isoquinoline moiety in the purine pocket with increased numbers of hydrophobic contacts to residues Ala70, Tyr122, Val123, Leu173, and Phe327 when compared to the ATP complex. Only one hydrogen bond acceptor

group, equivalent to N1 of ATP, is conserved in all the inhibitor–kinase complexes determined so far. The presence of receptor flexibility and a permissive lipophilic binding pocket may combine to overcome initial reservations that the common ATP site in kinases would not provide a good target for the discovery of selective inhibitors.

4. Structure-Based Design and Drug–Receptor Interactions

Structure-based design is now an important part of many medicinal chemistry projects. The identification of pharmacophores for a database search or ligand design and the use of experimentally derived or homology-built receptors in ligand docking studies and database searches are two examples. Molecular modeling experts, medicinal chemists, and a number of the docking programs have traditionally put a heavy bias upon the alignment of functional groups for hydrogen bonding and directionality when perceiving pharmacophores.^[47, 48] Although a number of successful docking validations and applications have appeared in the literature,^[47–51] recent work suggests parameterization of some force fields may need to be more weighted towards hydrophobicity relative to hydrogen bonding.^[47, 52, 53] To date the computational expense of an adequate treatment of solvation appears to have been the main drawback for an adequate treatment of hydrophobicity in these force fields. The largest validation so far undertaken for any docking program has been for the program GOLD.^[47] In this study predictions of binding geometry for the redocking of 100 ligands removed from their respective proteins, taken from the Brookhaven crystallographic database, were examined in detail. The program GOLD was able to dock 71 % of the ligands studied with an acceptable agreement to that in the Brookhaven database. Analysis of the success and failure sets highlighted the difficulties faced by many docking programs. The program was successful in docking polar ligands, and tended to fail with more hydrophobic (druglike) ligands. It worked best when the ligand was extensively hydrogen bonded to the receptor site. Although full ligand flexibility is considered by GOLD, only very limited receptor flexibility is allowed (rotation around terminal bonds, for example, rotation of a hydroxyl group to maximize a hydrogen bond). This type of redocking study tends to disguise those examples where the structures of the apo-receptor is grossly different to that of the bound receptor. The assumption often made, that any significant induced fit occurring to the protein upon binding the ligand is likely to be constant across that ligand series, may be more difficult to accept based on some of the examples collected in this review. Recent developments in the program GRID,^[54] which uses small molecular probes to identify binding locations in protein structures, have tried to address these problems by the inclusion of an empirical hydrophobic probe (DRY probe). This is to be used alongside the other molecular probes for hydrogen bonding, ionic interactions, and dipole interactions. Very importantly the latest version of GRID also allows for full flexibility of the receptor side chain.^[55] This is intuitively a major step forward in addressing some of the problems we

have highlighted here. How successful it will be in reality still remains to be seen.

Evidence included in this review demonstrates the importance of induced fit in drug–protein interactions. Induced fit very often seems to be driven by hydrophobicity and can be viewed as hydrophobic collapse of receptors around a ligand. The degree of flexibility is not always that of domain rotations, but sometimes consists of subtle adjustment of specific residue positions to accommodate bulky ligands and achieve hydrophobic contacts. The changes are not always evident from gross measurements of protein shape (circular dichroism spectra). They are sometimes evident from kinetic measurements of the time-course of inhibition. It might be helpful if such time-course experiments were carried out in conjunction with modeling studies; indeed they might be used as a screen for cases which can be effectively modeled. Various methods are available to identify mobile segments of proteins such as determination of thermal *B* factors and NMR relaxation measurements.^[56] It is possible that these could form the basis for incorporating a limited molecular dynamics strategy into a drug design scheme. Such a strategy might target hydrophobic interfaces between mobile portions of the protein. However a distinction should be drawn between the mobility of a protein loop in its native, unligated form and its absolute ability to assume more than one conformation.^[57] The bound conformation may be energetically and kinetically accessible only in the presence of the ligand. Thus induced fit may be a means of selecting a productive mode without the entropic expense of immobilizing a flexible loop.

It should also be remembered that the observed binding affinity is a function of stability of the entire complex relative to ligand and apo-receptor. Thus, formation of new bonds between amino acids of the receptor upon ligand binding may have a substantial effect. This has been observed recently with dihydropyranocarboxyamides related to zanamivir.^[58]

5. Drug Design and the Balance of Interactive Forces

The consequence of this emerging understanding of the relative importance of lipophilicity and induced fit compared to charge-reinforced and neutral–neutral hydrogen bonds may be a different way of thinking about drug design. One possibility is that medicinal chemists may seek increased potency and specificity, not necessarily by incorporating or maintaining hydrogen-bonding functionality and directionality, but by maximizing *specific* hydrophobic interactions. Where the initial ligands are rather polar molecules, the replacement of hydrogen-bonding groups by hydrophobic groups can have a number of beneficial effects. Sialic acid, NADPH, ATP, and most peptidic inhibitors would fall into this class. They tend to be afflicted with problems associated with poor absorption and rapid excretion. Introduction of lipophilic groups often increases potency, removes sites for conjugation, and increases plasma half-life (through increased distribution). Sometimes the journey from a polar starting point to useful drug is protracted, and several companies have revisited areas such as thrombin and renin using starting points generated by high-throughput screening as a means of

addressing these concerns. Compounds uncovered by high-throughput screens often present the medicinal chemist with the opposite problem, that of introducing polar functionality into rather lipophilic molecules. In this case placing polar functional groups where they are tolerated by the receptor is important whether or not an increase in affinity results. In this common medicinal chemical optimization the aim is often to find molecular modifications that reduce the overall bulk lipophilicity ($\lg P$) of the series whilst maintaining the important hydrophobic motifs. For instance, inclusion of an aliphatic ether into a potential drug in place of a methylene group may have several effects. It may alter potency by anything from zero to 15-fold, if it can act as an acceptor to a neutral donor in the receptor. However, the substitution would lower the value of $\lg P$ by two log units, increasing solubility by 100-fold (hence aiding absorption) and improving drug metabolism and pharmacodynamic properties. The observed tendency of present molecular modeling packages to predict the binding of polar molecules better than those with a more druglike balance of polar and lipophilic interactions is very interesting.^[47] This balance of properties is crucial to the drug discovery process. Tight binding of drugs to receptors is achieved through polar interactions and most importantly through the optimization of specific hydrophobic interactions. The bulk properties of drugs, which control solubility, absorption, metabolism, and pharmacokinetic properties, is achieved mainly using polar groups together with sufficient lipophilicity to achieve partition into lipid bilayers. The Rules of 5 ($M_r < 500$, $\lg P < 5$, $\Sigma(\text{donors}) < 5$, $\Sigma(\text{N,O}) < 10$) from Pfizer^[59] elegantly express the interplay between control of the number of hydrogen-bond acceptors and donors and the requirement to limit the overall value of $\lg P$ in order to achieve oral activity by the usual passive mechanisms.

6. Summary and Outlook

The contribution of hydrogen bonds to drug–receptor interactions is highly unpredictable. Charge-reinforced hydrogen bonds can contribute up to 3000-fold in binding. Neutral–neutral hydrogen bonds may contribute anything between zero and 15-fold. Hydrogen bonds can be “seen” and easily described in drug–receptor complexes, and this may lead to overemphasis of their importance relative to other binding interactions. Just “seeing” a hydrogen bond tells nothing about its contribution to binding. Hydrophobic interactions contribute a minimum of 3.2-fold increase in binding per methyl group. They play a major role in the affinity of most drugs for their receptors. This is indicated by counts of hydrophobic groups relative to acceptors and donors in oral drugs from the Physicians Desk Reference. Hydrophobic interactions appear to be the dominant driving force for induced fit of receptors around drugs. This failure of the rigid receptor hypothesis can also be described as hydrophobic collapse of a receptor around a drug. Ligands with increased affinity can sometimes be obtained by utilizing hydrophobic interactions and induced fit even at the expense of removing hydrogen bonds. Induced fit can allow beneficial hydrophobic

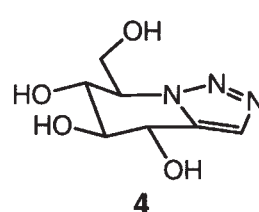
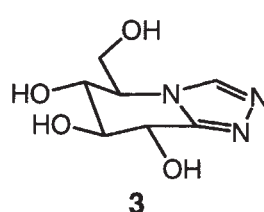
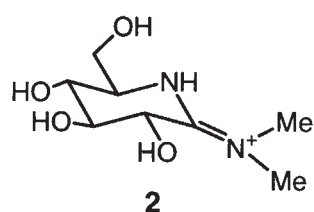
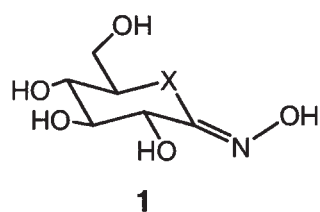
interactions to occur between drugs with different shapes at the same binding pocket in a given receptor. Existing molecular modeling packages have a poor balance between hydrogen bond and lipophilic interactions. At present they do not adequately take account of changes in receptor structure. The exploitation of specific hydrophobic interactions can be a very potent method of increasing affinity and selectivity for a receptor. The above observations also may be seen as potent arguments for retaining consideration of physicochemical properties and serendipity alongside rational design in the drug discovery process.

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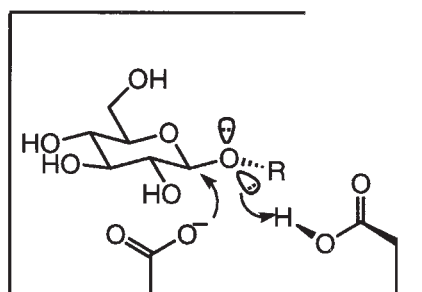
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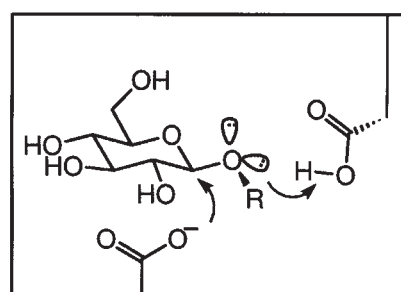


A

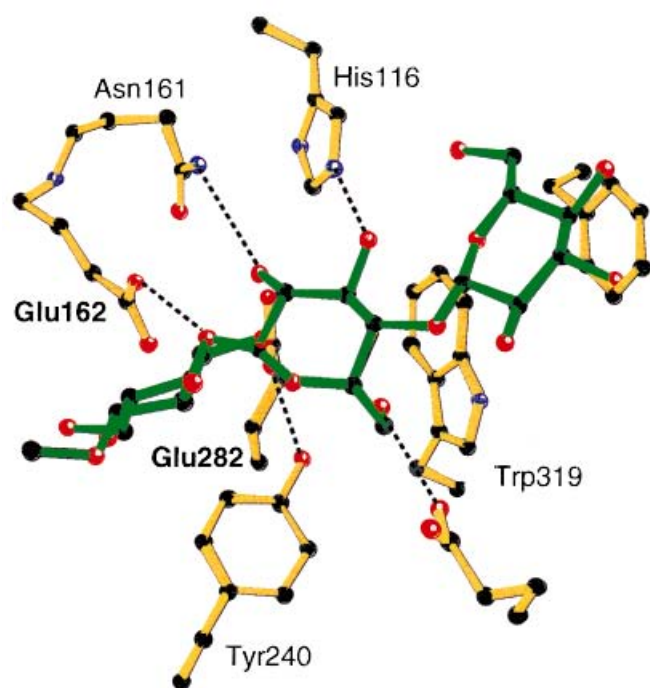


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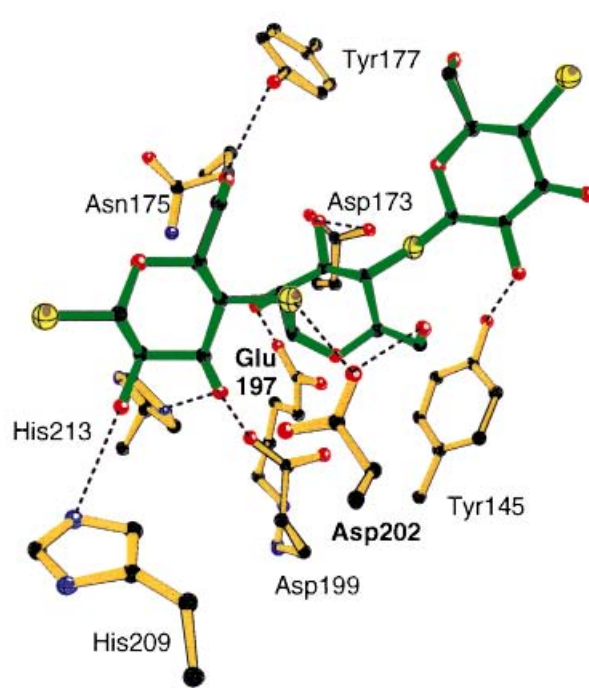
B



syn



Complex of the endocellulase from *Acidothermus cellulolyticus* with cellotetraose



Complex of the cellobiohydrolase from *Fusarium oxysporum* with a thioglycoside

Insights into the detailed mechanism of action of glycosidases have been gained by combining the study of the kinetics of inhibitors such as **1**–**4** with the analysis of crystal structures of glycosidases and glycosidase–ligand complexes. Configuration-retaining β -glycosidases do not protonate their substrate “from above”, but “from the side”, either *anti* (**A**) or *syn* to the endocyclic C1–O bond (**B**).

Recent Insights into Inhibition, Structure, and Mechanism of Configuration-Retaining Glycosidases

Tom D. Heightman and Andrea T. Vasella*

Dedicated to Professor Duilio Arigoni on the occasion of his 70th birthday

Until recently, the design of highly potent, selective glycosidase inhibitors has been patterned upon the analogy with the structure of natural products with established activity as glycosidase inhibitors or with the structure of the hypothetical reactive intermediate closest to the transition state, that is, an oxycarbenium cation. Relationships between the structure of the inhibitors and the kinetic data have rarely been examined in a systematic way, and there are no standards universally adopted for the chromogenic substrate, the pH value, the buffer, and the enzymes analyzed. However, the systematic variation of key structural parameters of inhibitors, combined with the interpretation of standardized

kinetic data, is a powerful method for the analysis of the enzymic mechanism. The combination of these data with the information available from the growing number of crystal structure analyses of glycosidases, in some cases in complexes with ligands, provides the opportunity to correlate the inhibition with the structure of both the inhibitor and the enzyme. In view of the relationship between the structures of glycosidases for which X-ray data are available, and the structures of glycosidases of the same family of which only the amino acid sequence is known, such correlations acquire an exemplary and thus particularly valuable character; they can be readily tested as the number of protein X-ray

analyses increases. This information provides a detailed rational basis for the design of selective and potent inhibitors and for the interpretation of subtle differences of reaction mechanism. This is itself of interest, even disregarding the potential applications of strong and selective inhibitors as antiviral, antidiabetic, and antifungal agents. Our review describes recent progress in the design, synthesis, and characterization of inhibitors of O-pyranosidases in light of the increasing availability of enzymic structural information.

Keywords: azoles • enzyme inhibitors • glycosidases • lactams • protonations

1. Introduction

Glycosidases and their inhibitors have enjoyed much research interest in the last decade, much of which has been reviewed.^[1–22] The application of naturally occurring glycosidases in enzyme replacement therapy; in textile, food, and pulp processing; and as catalysts in oligosaccharide synthesis has encouraged the engineering of proteins with improved catalytic properties and stability. Inhibitors of glycosidases, already used or tested in the treatment of diabetes and HIV infection and as antifungal agents, are expected to arouse increasing interest as therapeutic agents as our understanding of the role of glycosidases in recognition processes improves.

The enzymic cleavage of the glycosidic bond liberates a sugar hemiacetal with either the same configuration as the substrate (retention) or, less commonly, the opposite configuration (inversion). The catalytic mechanism for configuration-retaining glycosidases proposed by Koshland in 1953 has, despite refinement in many details, stood the test of time.^[23] In the currently accepted form of this mechanism (Figure 1 a), the aglycon is activated as a leaving group by coordination of the exocyclic oxygen atom with an enzymic acidic function (catalytic acid HA; partial protonation by a carboxyl group).^[24, 25] More or less concerted attack of a carboxylate group (catalytic nucleophile B[−]) located on the opposite side of the plane of the pyranose ring leads to a glycosyl ester intermediate.^[26, 27] The degree of concertedness of bond breaking and bond making is unclear, although both extremes (S_N2 or S_N1) are unlikely.^[28] The aglycon diffuses away from the active site and is replaced with a water molecule; the reverse process takes place: this water molecule is partially deprotonated by the conjugate base of the catalytic acid, attacks the anomeric carbon, and cleaves the newly formed

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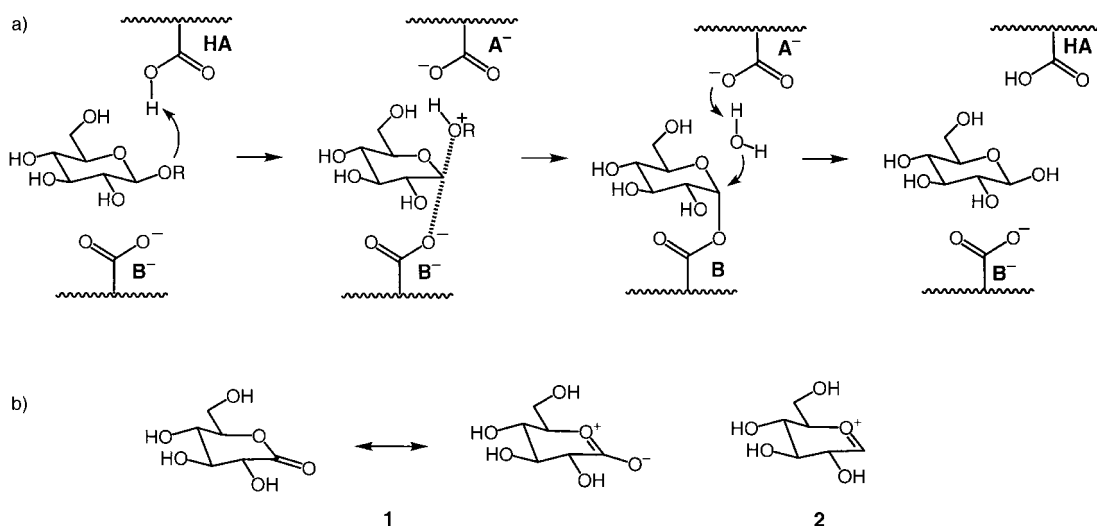


Figure 1. a) Catalytic mechanism for configuration-retaining glycosidases according to Koshland.^[23] b) Glucono-1,5-lactone **1** as a transition state analogue.

glycosyl ester bond. The double inversion leads to overall retention of configuration. This review will highlight the essential data and interpretations that have changed our understanding of aspects of the mechanism of configuration-retaining glycosidases in recent years.

2. Lactones and Piperidines: Neutral and Basic Inhibitors

2.1. Shape or Charge?

The observation that glyconolactones such as **1** (Figure 1b, Table 1) are relatively strong inhibitors of β -glycosidases was first made in 1940 by Ezaki^[33] and confirmed by Horikoshi in 1942, who studied the β -glucosidases from *Aspergillus* and from almonds.^[34] Levvy and Conchie later made the same observation with other glycosidases.^[35–37] Leaback pointed to the “stereochemical and conformational similarities between the lactone and the transition state in the enzyme-catalyzed

Table 1. K_i values [μM] of the lactone **1** and the piperidines **3**, **4**, and **5**.

Enzyme (source)	1	3 ^[29]	4 ^[30, 31]	5 ^[32]
α -glucosidase (brewers' yeast)	2000	330	14.6	> 1500
β -glucosidase (almonds)	400	2.8	18	1.5
β -glucosidase (<i>Agrobacterium faecalis</i>)	1.4	–	–	–

pyranoside hydrolysis”^[38] and Reese et al.^[39] added that the polar oxy group also partially mimics the positive charge of the oxycarbenium ion intermediate **2** (Figure 1b). The relative importance of these two features, shape and charge,^[40] has remained the subject of much debate, much more so than their correlation with details of the enzymic mechanism.^[41–45] Pauling had emphasized the importance of “shape” when he expounded that an enzyme should bind the transition state more strongly than the substrate in order to effectively catalyze a reaction, and thus transition state analogues should be potent inhibitors.^[41] The transition state for glycoside hydrolysis must have some considerable resemblance to the hypothetical oxycarbenium ion intermediate, leading

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A. T. Vasella

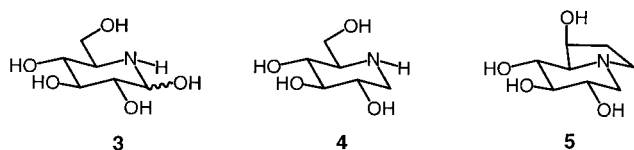


T. D. Heightman

Leacock and Reese to suggest that the lactones are analogues of the reactive intermediate and, hence, of the transition state.

The importance of charge was emphasized by the modeling of substrates and inhibitors in the active site of lysozyme based on X-ray structure analysis, suggesting that a carboxylate group was implicated in stabilizing the oxycarbenium ion intermediate without the formation of a glycosyl ester.^[46] It was generally agreed that the analogous interaction of the catalytic carboxylate group with the dipole of the lactone contributed to its strong inhibition of lysozyme.^[47–50]

The catalytic carboxyl and carboxylate groups were later shown—by a combination of irreversible inhibitor labeling, site-directed mutagenesis, and sequence alignments—to be almost ubiquitous in glycosidases.^[51, 52] The protonated form of naturally occurring basic inhibitors, including nojirimycin (**3**), deoxynojirimycin (**4**), and castanospermine (**5**), must be bound by charge–charge interactions.^[53] Certainly, with their



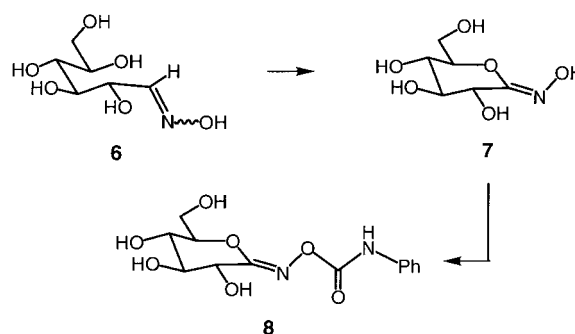
sp³-hybridized anomeric carbon atom and chair conformation they resemble the shape of the oxycarbenium ion less than the lactone. Interaction with the catalytic carboxyl/carboxylate groups thus provided a rational basis for the design of new inhibitors, although it was not clear whether the interaction was with the catalytic nucleophile or the carboxylate derived from deprotonation of the catalytic acid by the basic inhibitor.^[30, 54]

Many researchers have asked the questions: why is the neutral lactone **1** as good a β -glycosidase inhibitor as the positively charged piperidines **3–5**, and why, in contrast, is the lactone so much weaker in its inhibition of α -glycosidases, whereas the piperidines are stronger (Table 1)? Which factors are more important for inhibition of a given type of glycosidase: charge or the shape dictated by hybridization and the conformation of the pyranose or piperidinose ring? Are lactones and piperidines (and other basic inhibitors) really transition state analogues? Only if this is so will their study contribute in a rational way to a deeper understanding of the mechanism of action of glycosidases. The following describes, from the perspective of the reviewers, recent synthetic, kinetic, and structural research attempting to answer these and other questions.

2.2. Synthesis and Evaluation of Lactone Analogues

A disadvantage of the glycono-1,5-lactones is their tendency to undergo hydrolytic ring opening under mildly basic conditions and conversion into (inactive) 1,4-lactones under mildly acidic conditions, complicating the measurement of inhibition constants,^[39, 55] and reducing the chances of obtaining crystalline enzyme–inhibitor complexes for X-ray analysis.^[56] This may account for the fact that the transition state character of lactones has remained speculative. For these

reasons, we turned to the study and design of lactone analogues. A first lactone analogue synthesized and studied by our group is the hydroximolactone **7**, prepared by oxidation of the hydroxy oximes **6**, which are in equilibrium with the corresponding cyclic hydroxylamines.^[57] Like the lactone, **7** has a flattened chair conformation, but is stable to basic and



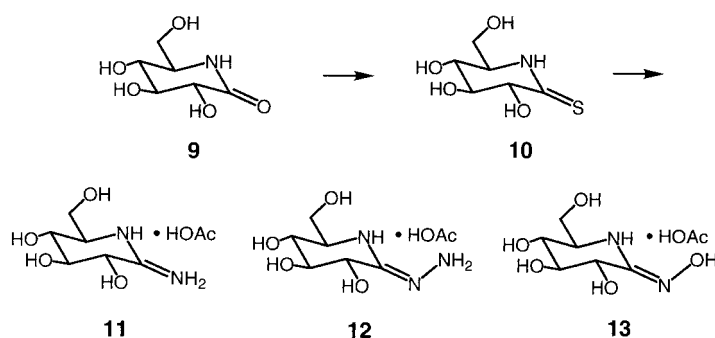
mildly acidic hydrolysis and ring conversion. The additional hydroxyl group allows the introduction of substituents, potentially mimicking the aglycon. The unsubstituted hydroximolactone inhibits the sweet-almond β -glucosidases ten times more weakly than the lactone **1** (Table 2), but the phenylurethane **8** proved stronger, as would be anticipated

Table 2. K_i values [μM] at pH 6.8 of the lactone oximes **7** and **8**, the amidine **11**, the amidrazone **12**, and the lactam oxime **13** and at pH 4.5 of the lactone oximes **7** and **8**.

Enzyme (source)	7	8	11 ^[58]	12 ^[58]	13 ^[58]
α -glucosidase (brewers' yeast)	6800	–	–	–	2.9
β -glucosidase (almonds) at pH 6.8	4300	43	10	8.4	14
β -glucosidase (almonds) at pH 4.5	98	2.3	–	–	–
β -glucosidase (<i>Agrobacterium faecalis</i>)	30	1.4	–	–	0.6
β -galactosidase (bovine liver)	–	–	–	19	–
α -mannosidase (jack beans)	–	–	9.0	3.1	–

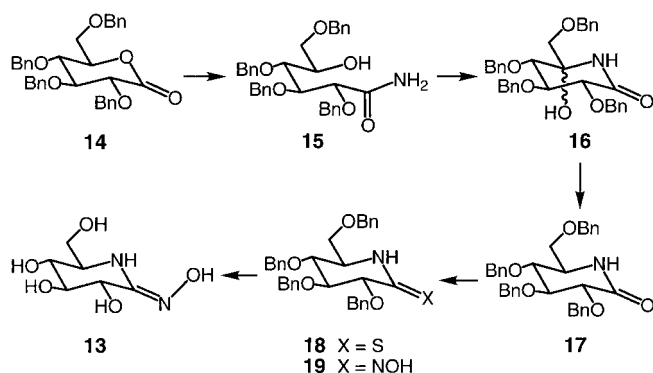
from the preference of the almond enzymes for aromatic glycosides.^[59] The transition state analogue character, however, remained speculative.

The next types of lactone analogues were designed to combine the sp²-hybridized anomeric center of the lactone with the positive charge of the piperidines. The Ganem group synthesized the glyconoamidine **11** and the analogous lactam hydrazone **12** and hydroximolactam **13** by oxidation of the naturally occurring nojirimycin and activation of the resulting lactam **9** as the thionolactam **10**, allowing the introduction of nitrogen nucleophiles.^[44, 58, 60]



All three lactone analogues **11–13** proved micromolar inhibitors of several α - and β -glycosidases (Table 2), leading Ganem et al. to call them “broad spectrum transition state analogues”.^[44] The latter contention was based on their proposal that these lactone analogues should be isomers of **11–13** with an endocyclic double bond, possess a half-chair conformation, and thus resemble the oxycarbenium ion intermediate (and thereby the transition state) more closely than the lactone **1** or the hydroxylactone **7**. The inhibition by the permanently protonated amidine **11** and lactam hydrazone **12** was shown to be pH independent, whilst that of the less basic hydroxylactam **13** was pH dependent, becoming weaker at pH values above 6. The inhibition results were interpreted in a fruitfully challenging way. The configuration was considered relatively unimportant. In spite of the high K_i value of **13** at pH 7, it was also concluded that, since the K_i values of **11–13** are similar (at pH 5) despite large differences in their pK_a values, “Mimicking the incipient charge buildup proved less important for enzyme inhibition than a flattened chair conformation, since even the relatively neutral amidoxime effectively blocked glycoside hydrolysis”.^[58] By interpolation (similar inhibition by the amidinium salt and the neutral **13**) this would mean that proton transfer to the inhibitor cannot be important.

We were challenged by the contention that these inhibitors are “broad spectrum transition state analogues”: firstly because many glycosidases are configurationally selective, and their discrimination should be most pronounced in the transition state; and secondly because Ganem et al. considered enzymic protonation of the inhibitor as a relatively unimportant factor, whilst the substrate must be at least partially protonated by the enzyme in the transition state. Clearly the structure, pK value, and inhibitory activity of the hydroxylactam **13**—and the choice of the glycosidases^[61]—were crucial to this interpretation. We therefore sought a practical synthesis of **13** and its two monolabeled ^{15}N isotopomers to verify its structure. The benzylated D-glucolactam **17** (Bn = benzyl) was prepared in an overall yield of 70 % from the corresponding lactone **14** by ammonolysis to the hydroxyamide **15** followed by an oxidoreduction.^[62] Conversion of **17** into the hydroxylactam **13** was then



carried out via the thionolactam **18**.^[63] ^1H and ^{15}N NMR spectroscopy and X-ray structure analysis of **13** clearly demonstrated that the double bond is exocyclic, and the ring a flattened chair (Figure 2). Upon protonation, **13** undergoes

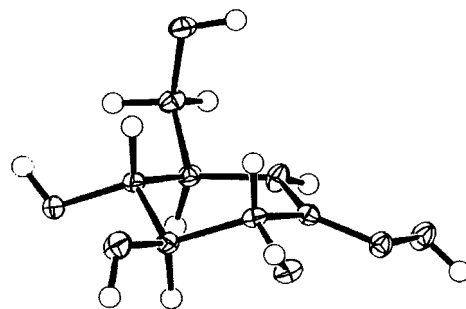


Figure 2. X-ray structure of **13**.

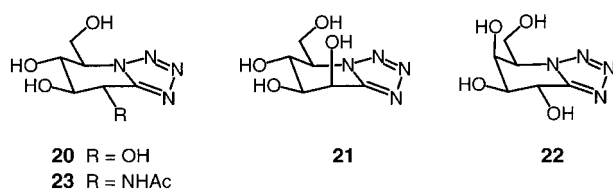
a conformational change to a half-chair, suggesting that protonation by the glycosidase is an important factor in the binding of **13**. The weaker inhibition of the hydroxylactone **7** as compared with that of the hydroxylactam **13** would then reflect its lower basicity. In keeping with this hypothesis, **7** proved a stronger inhibitor at pH 4.5 than at pH 6.8 (Table 2).

The binding of the basic derivatives **11** and **12**, which are protonated at pH values at least up to 7, is most probably driven by electrostatic interactions, as with the piperidines, and likewise results in poor configurational selectivity. The low pH dependency indicates that the main electrostatic interaction is with the catalytic nucleophile, which remains deprotonated throughout the pH range studied. The lack of configurational selectivity indicates that these inhibitors are at best partial transition state analogues. In addition, the selectivity of the inhibitor must be compared to the selectivity of the enzyme. It is known that the β -glucosidases from sweet almonds and the β -galactosidases from bovine liver, both used by Ganem et al., hydrolyze *gluco*-, and *galacto*-configured substrates about equally well.^[64, 65] For the almond glycosidase the ratio of $V_{\text{max}}K_M^{-1}$ (glucoside) to $V_{\text{max}}K_M^{-1}$ (mannoside) is greater than 300,^[30] whilst the inhibition by the *manno*-amidrazone and *manno*-hydroxylactam is only 24 and 15 times weaker, respectively, than that by the corresponding *gluco* analogues, which also renders an analogy between these inhibitors and the transition state unlikely.

2.3. Tetrazoles: Configurationally Selective Transition State Analogues

These results led us to ask the questions: are neutral, lactone-type inhibitors configurationally selective, and are they indeed transition state analogues? What is the effect of the ring conformation (chair versus half-chair) on the inhibitory potency and selectivity?

We designed the tetrazole **20** as a neutral, stable lactone analogue, the six-membered ring of which presumably having a half-chair conformation as a result of its fusion with the aromatic ring. The key step in the first synthesis of the tetrazole was the intramolecular 1,3-dipolar cycloaddition of a protected 5-azido-glucononitrile.^[66] This was prepared by transforming tetrabenzyl glucose into the open-chain glucononitrile, inverting the configuration at C5 by oxidoreduction, and introducing the azido group with a second inversion. The



manno-, *galacto*-, and *N*-acetylglucosamine-derived tetrazoles **21**, **22**, and **23** were prepared similarly.^[67, 68] More recently, we have transformed protected glyconolactams in one step to these and similar tetrazoles by treatment with sodium azide and trifluoromethanesulfone (triflic) anhydride.^[69]

These tetrazoles are indeed half-chairs in D₂O. For **20** and **21**, a half-chair to sofa conformation has also been established by X-ray analysis (Figure 3). These tetrazoles possess an

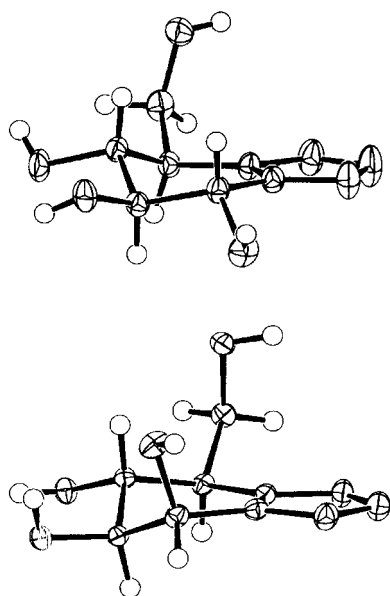


Figure 3. X-ray structures of **20** (top) and **21** (bottom).

excellent shelf life and are stable in solutions over a pH range of 1–14. Their inhibition of α - and β -glycosidases (Table 3) is similar to that of glucono-1,5-lactone **1** (Table 1) and of mannono-1,5-lactone (1.7 μ M against snail β -mannosidase^[70]).

To explore the question of their configurational selectivity, the inhibition by the *gluco*- and *manno*-tetrazoles **20** and **21** was tested by Withers et al. against three mannosidases and three glucosidases.^[67] Each of these glucosidases and mannosidases hydrolyzes both glucosides and mannosides, though there is a much greater activity with the correct substrate, as quantified by $V_{\max}K_M^{-1}$ values. Correspondingly, all these

Table 3. K_i values [μ M] of the *gluco*- and *manno*-tetrazoles **20** and **21**.

Enzyme (source)	20	21
α -glucosidase (yeast)	1300	100 000
β -glucosidase (almonds)	200	–
β -glucosidase (<i>Agrobacterium faecalis</i>)	1.4	6000
β -galactosidase (bovine liver)	1.5	14 000
α -mannosidase (jack beans)	8500	180
α -mannosidase (almonds)	13 800	700
β -mannosidase (snail)	47 000	160

enzymes are inhibited by both tetrazoles, but the correct tetrazole is considerably more effective than the incorrect one. Thus, β -glucosidases are inhibited more strongly by **20** than by **21**, and the β -mannosidase more strongly by **21** than by **20**. There is some inhibition of the α -glycosidases, but only in the millimolar range; again, there is discrimination of the configuration at C2. This demonstrates the configurational selectivity of these glycosidases and inhibitors.

To settle the question of the transition state analogue character of the tetrazoles appeared all the more important since it had never been demonstrated for the glyconolactones. Two types of experiments were performed. First, the activation free energy ($V_{\max}K_M^{-1}$) for each substrate and corresponding enzyme was plotted against the free energy of binding of the inhibitor (K_i^{-1} ; Figure 4).^[67] Rate enhancement

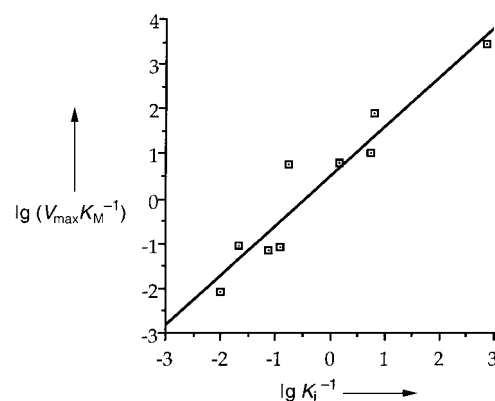


Figure 4. Plot of inhibition constants K_i of tetrazoles versus $V_{\max}K_M^{-1}$ of the substrate.

for the transformation of the substrate reflects transition state binding of the substrate, and that should correspond to the binding of transition state analogue inhibitors, but not to ground state substrate binding. This plot for the binding of substrates and inhibitors to these enzymes is characterized by a slope of 1.1 and a correlation coefficient of $r=0.9$, as expected for a transition state mimic, while there was no correlation with K_M . The good correlation obtained, despite the enzymes belonging to different families, must mean that the tetrazoles are complementary to a common aspect of the transition state structure of these enzymes.

Further evidence for the transition state character of the tetrazoles comes from studying a mutant of the *Agrobacterium faecalis* β -glucosidase where glutamic acid 358 has been replaced by aspartic acid.^[71] Glutamic acid 358 is the catalytic nucleophile; thus it plays a major role in transition state binding, but a minimal one in ground state binding. This mutation results in a decrease in rate constants of about 10 000-fold (corresponding to a $\Delta\Delta G$ value of ca. 5.6 kcal mol⁻¹), while the binding of substrate (ground state) analogues is weakened only 5-fold ($\Delta\Delta G$ ca. 1 kcal mol⁻¹) and of the *gluco*-tetrazole **20** some 200-fold ($\Delta\Delta G$ ca. 3.2 kcal mol⁻¹). These results show the tetrazole to be a transition state analogue, albeit an imperfect one. This finding may be extrapolated to glyconolactones, glyconolactams, and their oximes.

These experiments show that the configuration of the inhibitor is relatively unimportant only for transition state analogue inhibitors of configurationally unselective enzymes.

2.4. Enzymic Protonation of Inhibitors

2.4.1. C-Glucosyl Imidazoles

The inhibition of the sweet-almond β -glucosidases by several imidazole derivatives, such as 4-phenylimidazole, reported by Li et al. and Field et al.^[72, 73] suggested that protonation by the enzyme is indeed important for the activity of glycosidase inhibitors. The inhibition by the imidazoles was readily interpreted on the basis of the generally accepted mechanism. Imidazoles may interact synergistically with an acid and a base (Figure 5a). According to molecular mechanics calculations, the imidazole ring of the C-glucosyl imidazole **27** should be more or less perpendicular to the plane of the pyranose ring. Thus, it should be ideally disposed to interact with the catalytic acid and the catalytic nucleophile, disposed on opposite sides of the average plane of the pyranose ring^[74] (Figure 5b).

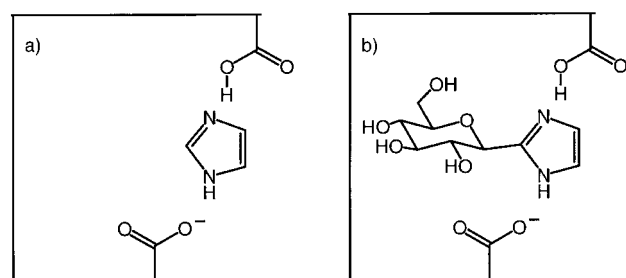
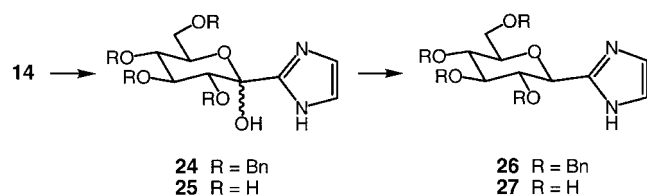


Figure 5. Mechanism of inhibition of glycosidases by imidazole (a) and β -D-glucopyranosylimidazole (b).

The C-glucosyl imidazoles **25** and **27** were made by addition of an N-protected 2-lithioimidazole to tetrabenzyl gluconolactone **14**, reduction of the resulting hemiacetal **24** to the diol, and selective acylation followed by base-promoted cyclization to **26**.^[74] To our surprise, both the hemiacetal **25** and the



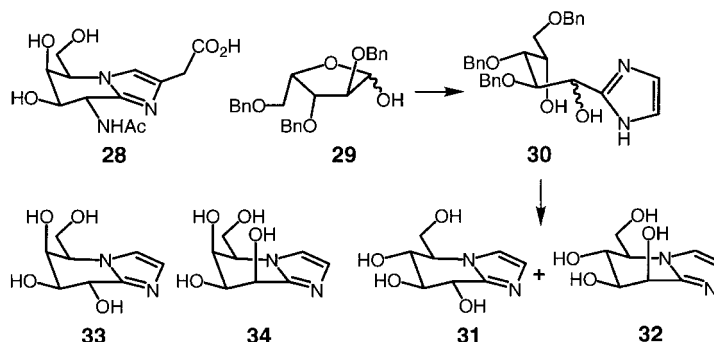
C-glycoside **27** turned out to be weak inhibitors of the β -glucosidases from sweet almonds (Table 4). Why? Which factor is responsible for the weak inhibition—conformation or the greater distance of the basic nitrogen atom from the anomeric center?^[75, 76]

Table 4. K_i values [μ M] of the C-glucosyl imidazoles **25** and **27**.

Enzyme (source)	25	27
β -glucosidase (almonds)	790	640

2.4.2. Annulated Pyrroles and Imidazoles

During our work on these inhibitors, Aoyagi, Aoyama, and co-workers published the structure of the natural product nagstatin (**28**), a very potent inhibitor of some glucosaminidases with a K_i value of 4 nM for the porcine kidney enzyme.^[77, 78] Tatsuta et al. synthesized the *gluco*-, *manno*-, *galacto*-, and *talo*-imidazoles **31**, **32**, **33**, and **34** by addition of



lithiated *N*-tritylimidazole to protected L-pentofuranoses, such as **29**, and cyclization of the resulting diastereoisomeric diols.^[79–82] Although the IC_{50} values of these imidazoles were not determined under the same conditions as the K_i values of the tetrazoles, the imidazoles are clearly more potent inhibitors of β -glucosidases, and show little activity towards α -glucosidases (Table 5). Thus, the inhibitory activity of nagstatin is mainly associated with the imidazole ring and not with the carboxymethyl substituent.

Table 5. IC_{50} values [μ M] of the annulated imidazoles **31**–**33**. Values in μ M^[80] have been transformed in μ M.

Enzyme (source)	31	32	33
α -glucosidase (bakers' yeast)	105	> 500	> 500
β -glucosidase (almonds)	0.7	17.5	0.5
β -mannosidase (snail)	300	0.11	370
β -galactosidase (<i>E. coli</i>)	> 500	> 500	0.008

The tetrazole **20** and the imidazole **31** possess a very similar shape; hence, the imidazole ought also to be a transition state analogue. Transition state analogues should be protonated in the same way as the substrate on the way to the transition state. The Koshland–Phillips–Vernon mechanism implies that proton transfer from the catalytic acid to the inhibitor occurs in the π plane of the azole. This would lead to C-protonation of the imidazole (but hardly the tetrazole) and loss of aromaticity (Figure 6), as has been observed in

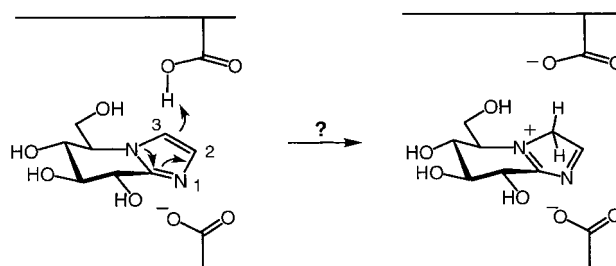
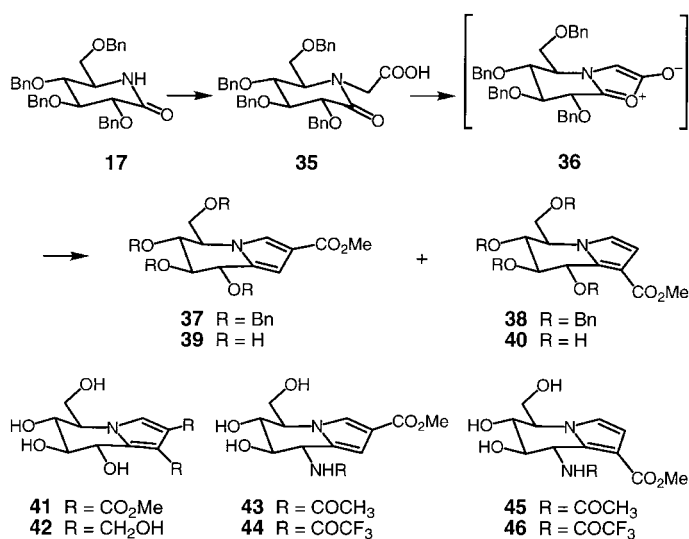


Figure 6. Application of Koshland's mechanism^[23] to the inhibition of glycosidases by the annulated *gluco*-imidazole **31**.

histidine ammonia lyase.^[83, 84] If this should be so, one expects the analogous pyrrole to be a stronger inhibitor than the imidazole.

The pyrroles **39** and **40** were prepared from the gluconolactam **17** that again proved its value as an advanced intermediate. The key step is the 1,3-dipolar cycloaddition



of the münchnone **36** to methyl propiolate.^[85] Alkylation of the lactam **17** with ethyl iodoacetate, saponification, and dehydration of the substituted glycine **35** in the presence of propiolate gave the regioisomeric esters **39** and **40** in good yields. The deprotected pyrroles possess a half-chair conformation in solution and in the solid state (Figure 7). These compounds were very weak inhibitors, as were the analogous diester **41** and diol **42** derived from the cycloaddition of **36** to dimethyl acetylenedicarboxylate (Table 6). For the preparation of the analogous *N*-acetyl and *N*-trifluoroacetyl glucos-

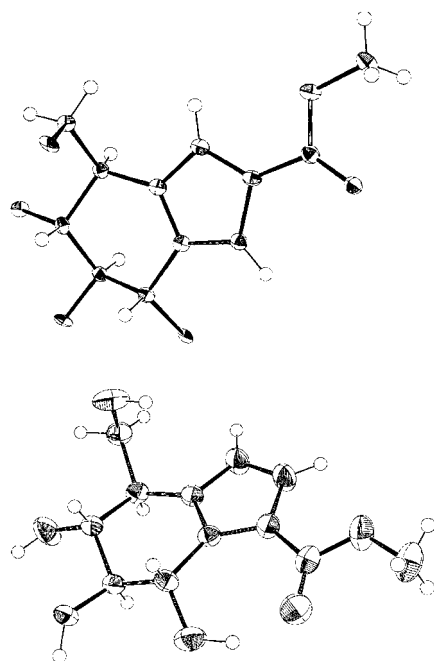


Figure 7. X-ray structures of **39** (top) and **40** (bottom).

Table 6. *K_i* values [μM] of the annulated pyrroles **39**–**42** derived from glucose.

Enzyme (source)	39	40	41	42
β-glucosidase (almonds)	6000	300	25 000	14 000

amine-derived pyrroles **43**–**46**, we treated **37** and **38** with Me₃SiN₃ and a Lewis acid, introducing the azide group by regioselective elimination followed by addition to the intermediary azoniatulenes.^[86] The *N*-acetylglucosamine-derived pyrroles were moderately strong inhibitors of bovine β-*N*-acetylglucosaminidase, but, again, they were significantly weaker than the corresponding tetrazole (Table 7).

Table 7. *K_i* values [μM] of the annulated pyrroles **43**–**46** and the tetrazole **23** derived from GlcNAc.

Enzyme (source)	43	44	45	46	23
β-glucosaminidase (bovine kidney)	19	10	20	13	0.2

3. A Modified Hypothesis: In-Plane or “Lateral” Protonation

These results and the hypothesis that proton transfer to the inhibitor is important could only be reconciled by postulating that protonation of the substrate and of a transition state analogous inhibitor takes place in a different way from that generally assumed. According to the original interpretation of the X-ray analysis of crystalline lysozyme, the two catalytic carboxyl residues are located “on each side of the center of formal substitution”;^[24, 87] that is, the catalytic acid/base is above the mean plane of the pyranoside ring, and the nucleophile below it, as depicted in Figure 1a. This finding has tacitly been generalized to other β-glycosidases.^[88]

The glycosidic oxygen atom has two doubly occupied nonbonding orbitals by which it can interact with a proton donor. In the preferred conformation (rationalized by the *exo* anomeric effect^[89]), one of them is oriented more or less in the plane of the pyranoside ring, and the other perpendicular to it (Figure 8a). All lactone-type inhibitors possess a heteroatom corresponding to the glycosidic

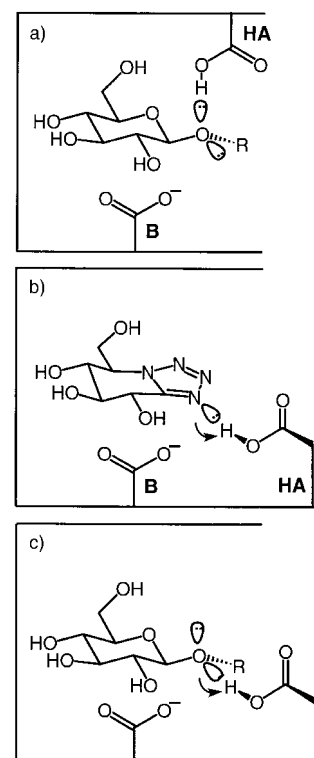
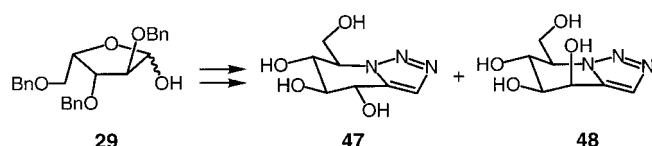


Figure 8. Proposed direction of protonation a) of a glucoside perpendicular to the plane of the ring and b) and c) of gluco-tetrazole **20** and a glucoside in the plane of the ring.

oxygen atom of the substrate. This heteroatom possesses a protonable lone pair only in the mean plane of the ring. Since proton transfer to the tetrazole **20** and related lactone-type inhibitors can only occur in the plane of the ring,^[90] the same must be valid for the substrate; that is, the proton donor must be close to the mean plane of the ring, and not perpendicular to it (Figure 8b, c). This places the catalytic acid much closer to the catalytic nucleophile than would be possible if they were on opposite sides of the mean plane of the pyranose ring, in agreement with the effect of the formation of the intermediate glycosyl ester on the pK_a value of the catalytic acid.^[91]

3.1. Synthesis of Annulated 1,2,3-Triazoles

To confirm the hypothesis of in-plane protonation we decided to compare the inhibition of β -glycosidases by the tetrazole **20** with that by the analogous 1,2,3-triazole **47**. The 1,2,3-triazole must possess a very similar shape to the tetrazole, but proton transfer is no longer possible as the position corresponding to the glycosidic oxygen is occupied by a CH group.



The *gluco*- and *manno*-1,2,3-triazoles **47** and **48** were prepared by intramolecular 1,3-dipolar cycloaddition of azidoalkynes, which are readily available from the tri-*O*-benzyl-L-xylose **29** by addition of an alkynyl cerium, regioselective protection, and substitution at C5 with azide.^[92] The benzylated *gluco*- and *manno*-1,2,3-triazoles were readily separated and deprotected to **47** and **48**.

These triazoles possess very similar conformations to their tetrazole analogues, both in solution and, for **47**, in the solid state (Figure 9), but a dramatically different inhibitory

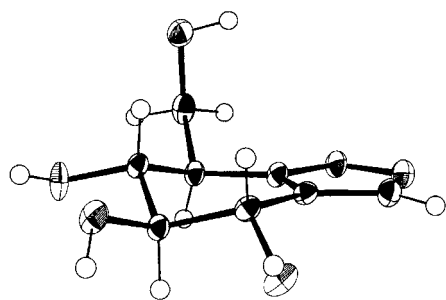


Figure 9. X-ray structure of **47**.

potency (Table 8). The triazole **47** is inactive at a concentration of 8 mM against the β -glycosidases from sweet almonds and bovine liver, and against the β -glucosidase from *C. saccharolyticum* it is 400 times weaker than the tetrazole **20**. The *manno*-triazole **48** is inactive at 8 mM against snail β -mannosidase. This is equivalent to a loss of binding energy of

Table 8. IC_{50} values [μM] of the *gluco*- and *manno*-tetrazoles **20** and **21** and the *gluco*- and *manno*-1,2,3-triazoles **47** and **48**.

Enzyme (source)	20	47	21	48
β -glucosidase (almonds)	200	> 8000	–	–
β -glucosidase (<i>Caldocellum saccharolyticum</i>)	5	2000	–	–
β -mannosidase (snail)	–	–	150	> 8000
β -galactosidase (bovine liver)	1.5	> 8000	–	–

3.3–5.0 kcal mol^{–1}, in keeping with the hypothesis of a lateral protonation at N1 of the tetrazole **20**.

3.2. Hydrogen Bonding versus Charge–Charge and Charge–Dipole Interactions

The in-plane protonation explains the difference between the inhibition of the tetrazole and 1,2,3-triazole. It does not, however, explain why Ganem's dimethylamidinium salt **49**, which cannot form a hydrogen bond in the plane of the ring (Figure 10a), with a K_i value of 83 μM against the sweet-almond β -glucosidases^[58] is about as good an inhibitor as the tetrazole **20** ($K_i = 150 \mu M$ ^[67]). The binding of **49** must be favored by a charge–charge interaction with the catalytic nucleophile, which suggests an analogous charge–dipole interaction for neutral lactone analogues (Figure 10b). This recalls the explanation by Reese et al. for the inhibition by glyconolactones, that is, the interaction between the negative charge of the catalytic nucleophile and the dipole of the C=O bond.^[39] This charge–dipole interaction is consistent with the stronger inhibition by the more electro-negative tetrazole as compared with the 1,2,3-triazole.

Conceivably, the importance of this charge–dipole interaction might be investigated by systematically modifying the number and position of the nitrogen atoms in the azole ring. For the in-plane (i.e., “lateral”) protonation one expects a correlation between inhibitory power and basicity of the azole. The inhibitory power should thus increase in the series tetrazole < 1,2,4-triazole < imidazole. If the charge–dipole interaction were decisive, the tetrazole should be the strongest and the imidazole the weakest inhibitor. However, the two factors—protonation and the interaction with the catalytic nucleo-

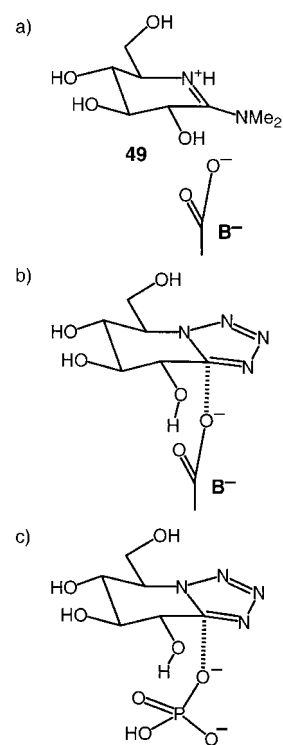
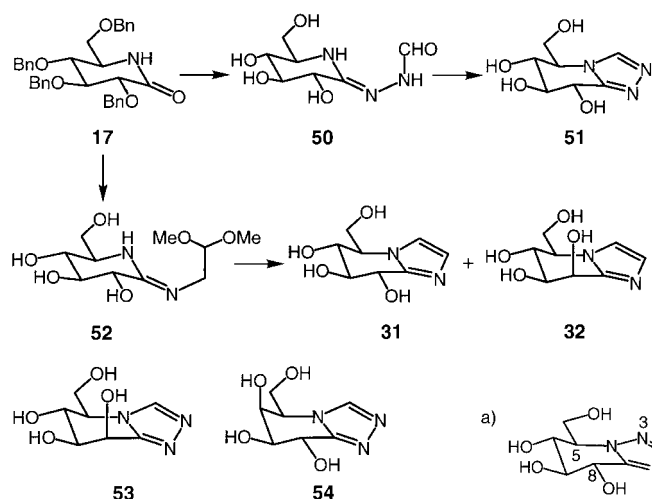


Figure 10. Charge–charge or charge–dipole interaction in β -glycosidases between the catalytic nucleophile and a) the amidinium ion **49** or b) the *gluco*-tetrazole **20** and c) in glycogen phosphorylase b between the catalytic nucleophile and the *gluco*-tetrazole **20**.

phile—are not independent of each other. Progressive proton transfer to the azole in theory leads ultimately to an azolium cation, and thereby to a salt bridge and a charge–charge interaction with the catalytic nucleophile that should be stronger than any charge–dipole interaction, although the expected small pH difference between the catalytic acid and the imidazole, both around pH 7, makes a complete proton transfer unlikely, even considering the favorable entropic effects.^[93, 94] To evaluate the contribution of the charge–dipole interaction independently from a (partial) proton transfer, we considered using the known interaction of hydrogen phosphate or dihydrogen phosphate with the tetrazole **20** in glycogen phosphorylase b^[95] (Figure 10c), which lacks a catalytic acid in the active site.^[96, 97] We will return to these questions after discussing the inhibition of the β -glycosidases by the various azoles.

3.3. Annulated 1,2,4-Triazoles and Imidazoles: Synthesis and Inhibition

As mentioned in Section 2.4.2, the inhibition of the imidazole **31**^[79] and the tetrazole **20** was measured under different conditions. To allow a valid comparison, we devised a new synthesis of the imidazoles **31** and **32** and the 1,2,4-triazoles **51** and **53**.



The gluconolactam **17** again served as an advanced common intermediate. Conversion into the thionolactam **18**, addition of *N*-formylhydrazine, cyclization under acidic conditions, and deprotection gave the 1,2,4-triazole **51** in 49% yield (from **17**).^[98] The use of aminoacetaldehyde dimethyl acetal in place of formylhydrazine yielded the amidine **52**, which was cyclized under acidic conditions to give, after deprotection, the *gluco*- and *manno*-imidazoles **31** and **32** (60% from **17**).^[98] The *galacto*-imidazole **33** (see Section 2.4.2) was prepared similarly in 60% yield from tetra-*O*-benzyl-1,5-gal-

actonolactam.^[99] These 1,2,4-triazoles and imidazoles possess very similar conformations to the 1,2,3-triazoles and tetrazoles in solution and, for the hydrochloride of **31**, in the solid state (Figure 11).

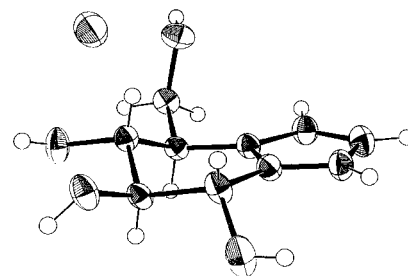


Figure 11. X-ray structure of **31**·HCl.

Tatsuta et al. had previously synthesized the *manno*- and *galacto*-1,2,4-triazoles **53** (27% yield from 2,3,5-tri-*O*-benzyl-L-xylose) and **54** (24% yield from 2,3,5-tri-*O*-benzyl-L-ribose) using lithiated azoles that allowed rapid access to the imidazoles **31/32** and **33/34**.^[100]

Among the *gluco*-, *manno*-, and *galacto*-azoles, the imidazoles proved consistently stronger β -glycosidase inhibitors than the 1,2,4-triazoles, which, in turn, are stronger inhibitors than the tetrazoles. The K_i values reported in Figure 12 are consistent with the dominant influence of the proposed in-plane protonation.

3.4. Glycogen Phosphorylase: Further Clues to Inhibitor Binding in Glycosidases

As mentioned in Section 3.2, the contribution to the inhibition of the charge–dipole interaction between the azole and the catalytic nucleophile B[−] might be quantified by studying glycogen phosphorylase b (GPb). GPb phosphorytically cleaves the α -glycosidic bonds of glycogen, but in contrast to the glycosidases possesses no catalytic proton

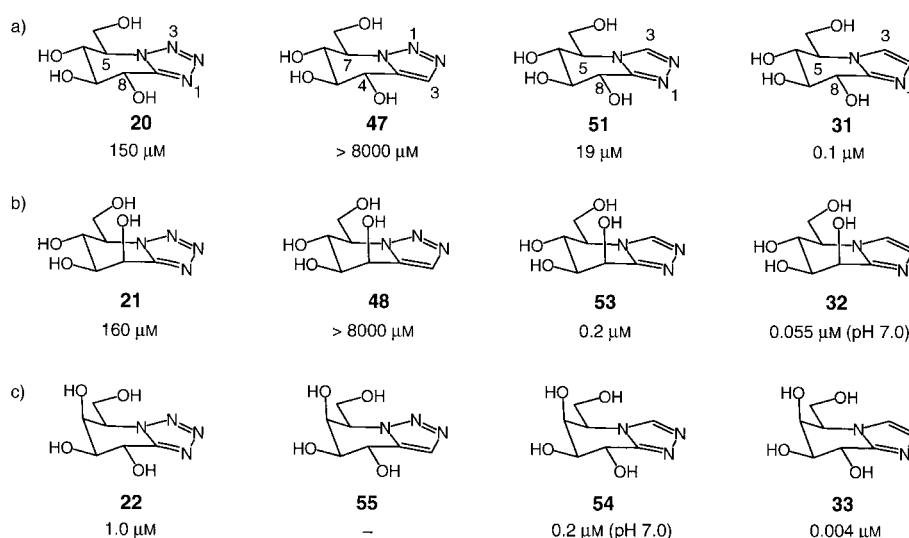


Figure 12. K_i values of *gluco*-, *manno*-, and *galacto*-configured tetrahydropyridoazoles against a) almond β -glucosidase, b) snail β -mannosidase, and c) *E. coli* β -galactosidase.

donor; both the function of the catalytic proton donor and of the catalytic nucleophile are performed by hydrogen phosphate or dihydrogen phosphate.^[96, 97] It had been found^[95] that the tetrazole **20** inhibits GPb ten times more strongly in the presence of phosphate than in its absence. An X-ray structure of the GPb–phosphate–tetrazole complex showed that the hydrogen phosphate or dihydrogen phosphate ion is located beneath the inhibitor, with the proximal oxygen atom 3.1 Å removed from the “anomeric” carbon atom^[95] (see Figure 10c), presumably interacting with the azole ring by a charge–dipole interaction. The position of the hydrogen phosphate or dihydrogen phosphate ion with respect to the tetrazole strongly resembles that expected for the catalytic carboxylate in β -glycosidases (see Figures 1, 10b, and 10c). In GPb, however, there is no hydrogen bond to the nitrogen atom of the tetrazole corresponding to the glycosidic oxygen atom. These properties make GPb a suitable model to investigate the interaction between an anion corresponding to the catalytic nucleophile and the tetrazole **20**, or the 1,2,3-triazole **47**, without any interference from protonation of the azole.

In the presence of phosphate, the 1,2,3-triazole **47** binds ten times more weakly to GPb than the tetrazole **20**, which is equivalent to a difference in binding energy of about 1.4 kcal mol^{−1} at 25 °C (Table 9). A comparison of the crystal

Table 9. K_i values [μ M] of tetrazole **20**, the 1,2,4-triazole **51**, the 1,2,3-triazole **47**, and the imidazole **31** against almond β -glucosidase and glycogen phosphorylase b (GPb).

Inhibitor	K_i (β -glucosidase), mode of inhibition	K_i (GPb/phosphate), mode of inhibition	K_i (GPb/Glc-1-P), mode of inhibition
20	150, competitive	53, competitive	700, competitive
51	19, competitive	8100, competitive	no inhibition ^[a]
47	> 8000, no inhibition	540, competitive	2500, competitive
31	0.35, competitive	9700, competitive	no inhibition ^[a]

[a] At concentrations of inhibitors from 0.1 to 5 mM in the presence of 3 mM Glc-1-P, 1 mM AMP, and 1 % glycogen.

structure of the GPb–phosphate–triazole and GPb–phosphate–tetrazole complexes shows that (within the resolution limits) the tetrazole **20** and triazole **47** are bound in the same way (Figure 13). Thus the strength of the interaction between the azole and the hydrogen phosphate or dihydrogen phosphate must be the dominant factor contributing to the difference in binding energy.^[101]

The 1,2,4-triazole **51** and imidazole **31** are not bound significantly at the active site of GPb (Table 9). The structure of the ternary complexes of the tetrazole or triazole indicates that replacement of N3 of the tetrazole by CH must interrupt a hydrogen bond with an enzymic main chain NH group (Leu 136), thus lowering the binding affinity (see Figure 13). Hence, the evaluation of the oxanion–azole interaction with the phosphate ion had to be restricted to **20** and **47**.

The relatively small difference in binding energy for the tetrazole **20** and 1,2,3-triazole **47** to GPb suggests that the oxanion–azole interaction is not the main factor responsible for the large difference in binding energy of these azoles to β -glycosidases. However, for β -glycosidases one expects partial protonation of the azoles to raise their electronegativity, and

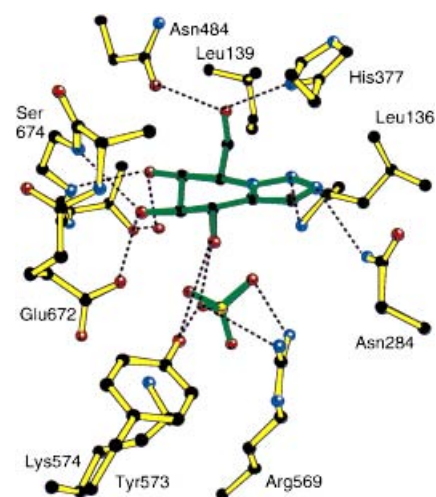


Figure 13. Structure of the complex made up of GPb, 1,2,3-triazole **47**, and phosphate.

to increase the strength of the interaction of the azole (or azolium cation) with the catalytic nucleophile; the two interactions—protonation and interaction with the catalytic nucleophile—should be synergistic. The synergism, expressed in Figure 8b, means that the individual contributions to the inhibition of the lateral protonation and of the interaction with the catalytic nucleophile are not experimentally accessible. However, the experimental difference for the interaction between phosphate and the 1,2,3-triazole **47** or the tetrazole **20** in the active site of GPb may be used to validate semiempirical (AM1) calculations of this energy difference, which may then be extended with reasonable confidence to quantify the interaction between the azole inhibitors and the catalytic nucleophile of β -glycosidases.

The interaction between the gluconotetrazole **20**, the 1,2,3-triazole **47**, the 1,2,4-triazole **51** or the imidazole **31**, and either hydrogen phosphate or dihydrogen phosphate was determined by calculating a range of heats of formation for each complex, reducing the distance between the proximal phosphate oxygen atom and the “anomeric” carbon atom by increments of 0.1 Å to give a potential energy curve.^[101] As shown in Figure 14, the oxanion–azole interaction for either a hydrogen phosphate or dihydrogen phosphate ion increases with the number of nitrogen atoms in the azole ring (i.e., it correlates inversely with the energy of the LUMO). The ΔK_i -derived energy difference for the phosphate–tetrazole and phosphate–1,2,3-triazole interaction in GPb (1.4 kcal mol^{−1}) and the crystallographic data agree with the calculated difference between the energy minima ($\Delta E_{\min}$) of 1.1 kcal mol^{−1} and the geometry for the complexes with the dihydrogen phosphate rather than with the hydrogen phosphate ion. On the basis of this close correspondence, we similarly calculated the heats of formation for the azole–carboxylate complexes, maintaining the conformation of the HO group at C2 that is required for the important hydrogen bond^[102] between this group and the catalytic nucleophile. A similar dependence of the energy of interaction and the number of nitrogen atoms of the azoles was observed, as before (Figure 14), with a calculated energy difference of

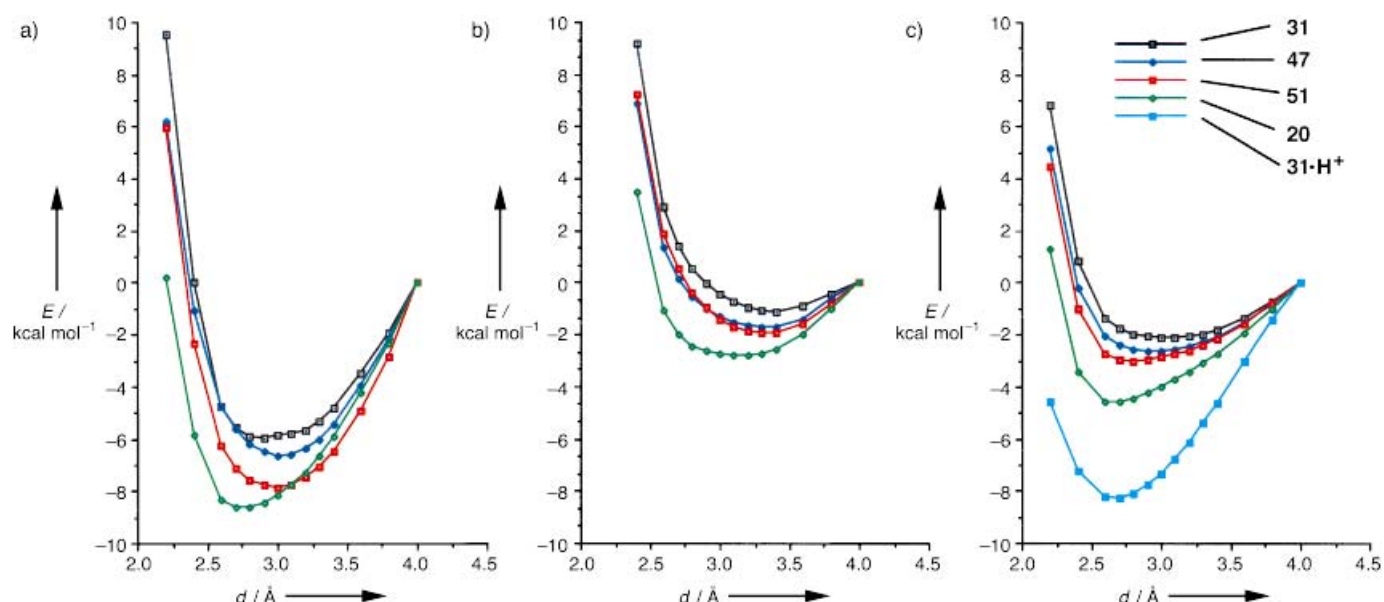


Figure 14. Results of AM1 calculations: plots of potential energy E versus the distance d between the oxygen atom of a) HPO_4^{2-} , b) H_2PO_4^- , and c) CH_3CO_2^- and the “anomeric” carbon atom of the azole inhibitors **20**, **51**, **47**, and **31**.

2.0 kcal mol⁻¹ for the tetrazole and 1,2,3-triazole complexes. This compares to an experimental difference of binding energy between **20** or **47** and the β -glycosidases listed in Table 8 of at least^[103] 2.4 to 5.3 kcal mol⁻¹. This means that the interaction with the catalytic nucleophile contributes only about half of the binding energy; the other half must derive from the interaction with the catalytic acid.

The strength of the calculated azole–oxanion interaction correlates with the inhibition of GPb, but with a trend that is opposite for the inhibition of β -glycosidases by these azoles. In agreement with the synergism between the two interactions, the calculations showed that protonation of the imidazole **31** dramatically increases the energy of the interaction with the catalytic nucleophile, by as much as 6 kcal mol⁻¹.

3.5. Insights from X-ray Structures and Molecular Modeling

3.5.1. In-Plane Protonation

The hypothesis of in-plane protonation was initially checked by docking the *gluco*- and *galacto*-tetrazoles **20** and **22** into the active site of the three configuration-retaining *exo*- β -glycosidases, for which structures were then available from X-ray analysis.^[92] Two features facilitated docking of the inhibitors. Firstly, *exo*-enzymes cleave the terminal pyranoside residue from the nonreducing end of an oligosaccharide or glycoside, so the fissile glycosidic bond must point towards the entrance of the binding pocket. Secondly, the catalytic nucleophile must be located on the α face of the pyranoside ring, about 3 Å beneath the anomeric carbon in a position to attack the incipient oxycarbenium ion.

Although belonging to two different families according to Henrissat's amino acid sequence classification,^[51, 52] the *E. coli* β -galactosidase (family 2),^[104] white clover cyanogenic β -glucosidase,^[105] and *L. lactis* 6-phospho- β -galactosidase (both

family 1)^[106] show very similar arrangements of polar and hydrophobic side chains in their active sites. Additional information relevant to the docking of inhibitors into these glycosidases was provided by the structure of a *Cellulomonas fimi* cellulase by White et al.^[107] Although it is an *endo*-glycosidase, and belongs to family 10, the arrangement of amino acid side chains responsible for recognition of the pyranoside ring and for catalysis is very similar to that in the active site of the above three *exo*-glycosidases. In the *C. fimi* cellulase structure, the catalytic nucleophile is covalently bonded to a 2-deoxy-2-fluorocellobiosyl moiety (Figure 15), which strongly supports the long-standing hypothesis that, at least in some glycosidases, hydrolysis proceeds via a glycosyl ester.^[23, 108]

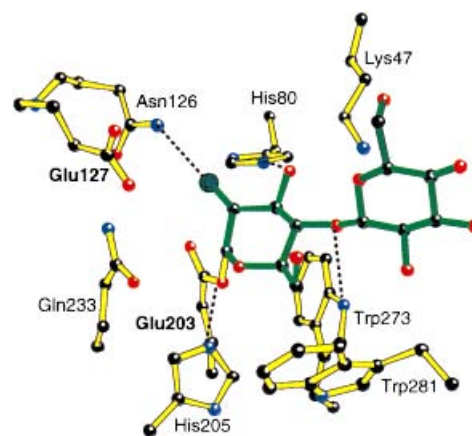


Figure 15. Structure of *C. fimi* endocellulase (family 10) covalently bonded to a 2-deoxy-2-fluoro-cellobiosyl moiety.^[107]

The structures which resulted from manual docking of the tetrazoles **20** or **22** into the active site of the three *exo*-glycosidases are strikingly similar (Figure 16a–c).^[92] Energy minimization, using fixed enzyme coordinates and starting from the geometry of the complexes resulting from manual

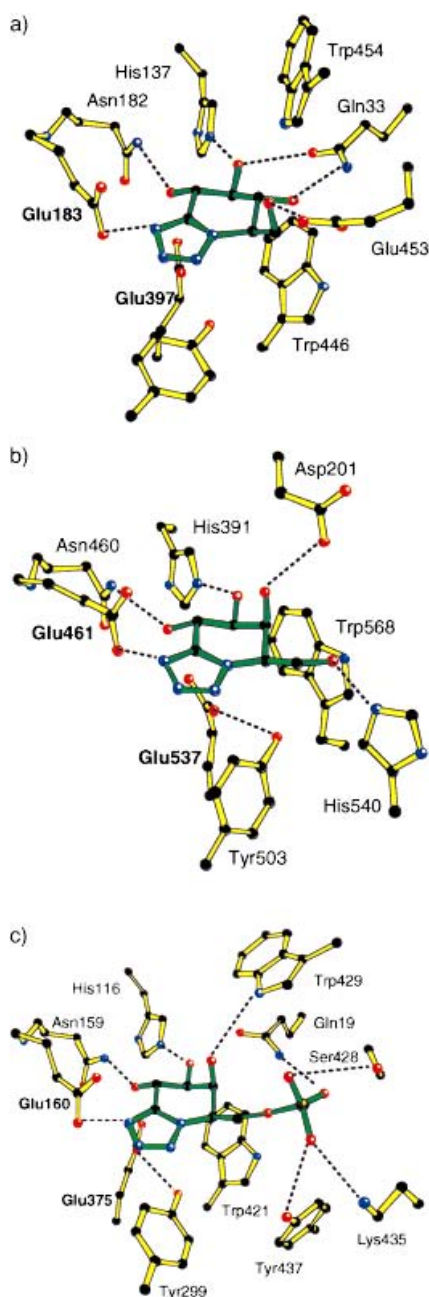


Figure 16. Modeled structures of complexes a) between *E. coli* β -galactosidase (family 2) and galacto-tetrazole **22**, b) between white-clover β -glucosidase (family 1) and gluco-tetrazole **20**, and c) between *L. lactis* 6-phospho- β -galactosidase (family 1) and phosphate derived from galacto-tetrazole **22**.^[92]

docking, suggested that the resulting geometry reflects the optimal inhibitor binding orientation. Potential hydrogen bonds are observed between the glycosidic heteroatom and the carboxyl group of the conserved catalytic acid Glu, between HO-C2 and the conserved Asn preceding the catalytic acid, and between HO-C3 and a conserved His. The models also indicate a hydrophobic stacking at the α face, between the pyranoside ring and a conserved Trp residue, typical in carbohydrate-binding proteins.^[109] The inescapable conclusion is that the catalytic proton donor is indeed located close to the mean plane of the pyranoside ring; rotation of the inhibitor, or movement of the catalytic proton donor, so that

protonation could occur from above the ring is unfeasible. Sakon, Karplus et al. determined the structure of a cellotetraose substrate bound to the active site of a family 5 endocellulase, in which they observed a very similar array of hydrogen bonds and hydrophobic interactions (Figure 17a).^[110] The authors commented that the location of the catalytic groups with respect to the fissile glycosidic bond

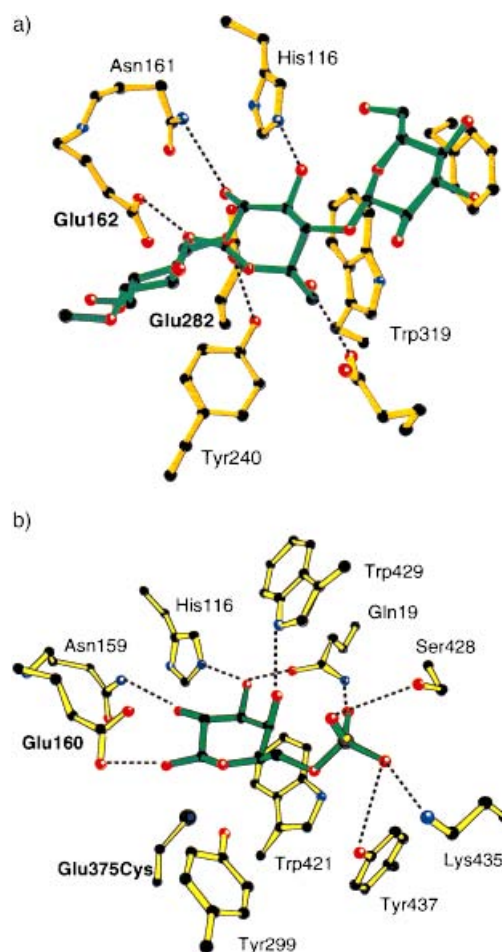


Figure 17. Structures of complexes between a) *A. cellulolyticus* endocellulase (family 5)^[110] and substrate and b) *L. lactis* 6-phospho- β -galactosidase (family 1)^[111] and product.

contrasts with the “textbook paradigm” since these groups are not on opposite sides of the glycon ring plane, but the proton donor is located close to the mean plane of the pyranoside ring and close to the catalytic nucleophile. Wiesmann et al. subsequently determined the crystal structure of a mutant of 6-phospho- β -galactosidase complexed with 6-phospho- β -galactose^[111] (Figure 17b), confirming all the interactions proposed in our inhibitor model^[92] and again showing that the proton donor is located close to the ring plane.

3.5.2. Generality of In-Plane Protonation—Classification of Glycosidases according to the syn or anti Protonation Trajectory

In-plane protonation thus appeared fairly well established, at least for the β -glycosidases we had examined. This raises the question of whether in-plane protonation is a general feature of glycosidases.

There is now a significant body of data available from crystal structure analyses of configuration-retaining β -glycosidases complexed with substrates, products, or inhibitors.^[104, 105, 107, 110–144] In all of the complexes, the proton donor is located close to the mean plane of the pyranoside ring of the substrate or an inhibitor (Table 10). These structural data also reveal that there are two types of β -glycosidases. In the first type, the proton donor is *anti* to the pyranoside ring oxygen atom with respect to the plane defined by O1, C1, and H1, as in the structures described above (i.e., *anti* to the (C5)O–C1 bond; Figure 18a). This corresponds to the orientation of one of the doubly occupied, nonbonding orbitals on the glycosidic oxygen atom when the aglycon is in the orientation favored by the *exo* anomeric effect. In the second type, the proton donor is located *syn* to the ring oxygen atom, requiring a conformation which is not favored by the *exo* anomeric effect

(Figure 18b). Table 10 shows the considerable number of glycosidase structures which are compatible with an *anti* or *syn* in-plane protonation trajectory. A small number of glycosidases do not show sufficient structural homology to assign them as *anti* or *syn* protonators. Their assignment will be assisted by the analysis of crystalline ligand complexes.

There is, perhaps unsurprisingly, a correlation between the glycosidase families^[51, 52] defined by the amino acid sequence and the *syn* or *anti* protonation trajectory. The similarity of the structure of the active site for enzymes of families 1, 2, 5, and 10,^[56, 104, 105, 107, 110, 112, 113, 119, 120, 122] which all belong to the GH-A clan, defines them as *anti* protonators. A similar *anti* orientation is also observed in a family 20 chitobiase;^[118] *syn* protonators are found in families 7, 9, 11, 22, 23, and 45.^[123, 125, 127, 129, 130, 145]

Table 10. Classification of glycosyl hydrolases according to *anti* or *syn* protonation trajectory.

Enzyme	Source	Conf. ^[c]	Family	PDB code	Ligand	Acid/base ^[a]	Nucleophile ^[a]	Ref.
<i>anti</i> protonators								
6-phospho- β -galactosidase	<i>Lactococcus lactis</i>	β – β	1	4pbg	product	Glu160	Glu375	[111]
myrosinase	<i>Sinhalpa albis</i>	β – β	1	2myr	2-F-glycosyl ^[f]	Gln187 ^[b]	Glu409	[112]
cellulase C	<i>Clostridium thermocellum</i>	β – β	5	1cen	product	Glu140	Glu280	[113]
cellulase	<i>Acidothermus cellulolyticus</i>	β – β	5	1ece	substrate	Glu162	Glu282	[110]
cellulase	<i>Bacillus agaradherans</i>	β – β	5	–	2-F-glycosyl	?	?	
cellulase	<i>Cellulomonas fimi</i>	β – β	10	1exp	2-F-glycosyl	Glu127	Glu233	[107]
amylase I	porcine pancreas	α – α	13	1ppi	acarbose	Glu233	Asp197	[114]
amylase II	porcine pancreas	α – α	13	1ose	acarbose	Glu233	Asp197	[115]
amylase	<i>Aspergillus oryzae</i>	α – α	13	7taa	acarbose	Glu230	Asp206	[116]
CGTase ^[g]	<i>Bacillus circulans</i>	α – α	13	1cxg	acarbose	Glu257	Asp229	[117]
chitobiase	<i>Serratia marcescens</i>	β – β	20	1qbb	product	Glu540	– ^[c]	[118]
<i>anti</i> by similarity (in amino acid sequence)								
β -glucosidase	white clover	β – β	1	1cbg	–	Glu183	Glu397	[105]
β -glycosidase	<i>Sulfolobus solfataricus</i>	β – β	1	1gow	–	Glu206	Glu387	[119]
β -galactosidase	<i>Escherichia coli</i>	β – β	2	1bgl	–	Glu461	Glu537	[104]
β -glucuronidase	human	β – β	2	1bhg	–	Glu451	Glu540	[120]
xylanase	<i>Clostridium thermocellum</i>	β – β	10	1xyz	–	Glu645	Glu754	[121]
xylanase A	<i>Pseudomonas fluorescens</i>	β – β	10	1clx	–	Glu127	Glu246	[122]
<i>syn</i> protonators								
cellulase	<i>Fusarium oxysporum</i>	β – β	7	1ovw	thioglycosides	Glu202	Glu197	[123]
cellulase	<i>Thermomonospora fusca</i>	β – α	9	3tf4, 4tf4	product	Glu424	Asp55, Asp58	[124]
xylanase	<i>Bacillus circulans</i>	β – β	11	1bcx, 1xnb	substrate	Glu172	Glu78	[125]
lysozyme	hen egg white	β – β	22	1lyz, 9lyz, 1lsz	substrate	Glu35	Asp52	[126–128]
lysozyme	goose egg white	β – β	23	154l	substrate	Glu73	– ^[c]	[129]
endoglucanase V	<i>Humicola insolens</i>	β – α	45	4eng	product	Asp121	Asp10	[130]
<i>syn</i> by similarity (in amino acid sequence)								
cellobiohydrolase I	<i>Trichoderma reesei</i>	β – β	7	1cel	product	Glu217	Glu212	[131]
endoglucanase	<i>Trichoderma reesei</i>	β – β	7	1eg1	–	Glu201	Glu196	[132]
cellulase D	<i>Clostridium thermocellum</i>	β – α	9	1clc	–	Glu555	Asp201	[133]
xylanase I	<i>Trichoderma reesei</i>	β – β	11	1xyn	–	Glu164	Glu75	[134]
xylanase II	<i>Trichoderma reesei</i>	β – β	11	1red, 1ree, 1ref	epoxyalkylglycosides	Glu177	Glu86	[135]
1,3–1,4-glucanase	<i>Bacillus macerans</i>	β – β	16	1byh, 1gbg	–	Glu109	Glu105	[136]
1,3–1,4-glucanase	<i>Bacillus macerans</i>	β – β	16	1mac	–	Glu107	Glu103	[137]
chitinase A	<i>Serratia marcescens</i>	β – β	18	1ctn	–	Glu315	Asp391	[138]
endochitinase	<i>Hordeum vulgare</i>	β – α	19	2baa	–	Glu67	Glu89	[139]
unclassified								
cellobiohydrolase II	<i>Trichoderma reesei</i>	β – α	6	1cb2	–	Asp221	Asp263	[140]
cellulase A	<i>Clostridium thermocellum</i>	β – α	8	1cem	–	Glu95	Asp152	[141]
xylanase	<i>Streptomyces lividans</i>	β – β	10	1xas ^[d]	–	Glu128	Glu236	[142]
1,3–1,4-glucanase	<i>Hordeum vulgare</i>	β – β	17	1ghr	–	Glu288, Glu280	Glu232	[143]
endochitinase H	<i>Streptomyces plicatus</i>	β – β	18	1edt	–	Glu132	Asp130	[144]

[a] Acid/base and nucleophile assignments are taken from the accompanying references and in some cases may not be unambiguous. [b] Myrosinase has no catalytic proton donor; Gln187 occupies the same position as the isosteric, catalytic Glu in other family 1 glycosidases. [c] The *N*-acetamido group of the substrate acts as the nucleophile. [d] The PDB file gives only the coordinates of the peptide backbone. [e] Anomeric configuration of substrate and product. [f] Covalently bound 2-deoxy-2-fluoro substrate. [g] CGTase = cyclodextrin glycosyltransferase.

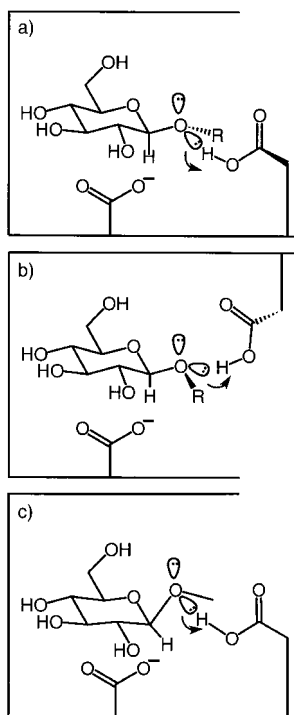


Figure 18. a) *anti* Protonation and b) *syn* protonation of a β -glycosidase; c) lateral protonation moves the fissile C–O bond into a pseudoaxial position.

electronic considerations, as expounded by Deslongchamps,^[143] require that protonation of the glycosidic oxygen atom be accompanied by a lengthening of the fissile C–O bond in a pseudoaxial direction as the incipient oxycarbenium ion develops. This is supported by two high-resolution crystal structures of the cellulases from *Fusarium oxysporum*^[123] (family 7, *syn* protonation; Figure 19) and *Bacillus agaradherans*^[147] (family 5, *anti* protonation) by Davies and co-workers. They show that the pyranoside ring is distorted to a skew conformation which places the glycosidic bond in a pseudoaxial orientation. A similar observation has been reported for

Neither of these protonation trajectories corresponds to the lysozyme paradigm in which the catalytic residues were proposed to be located on “opposite sides” of the fissile glycosidic bond.^[24, 25] However, recent high-resolution crystal structure analyses of inhibitor complexes show that lysozyme is a *syn* in-plane protonator.^[128, 129] We have found no example of a configuration-retaining β -glycosidase in which the proton donor is not either *anti*- or *syn*-periplanar to the ring oxygen atom with respect to the above-defined plane.

It makes sense that the catalytic carboxy group is located close to the mean plane of the pyranoside ring and not above it. The departure of the aglycon in a pseudoaxial direction would be restricted if the carboxy group were located above the plane of the ring (Figure 18c). Stereo-

two chitin-degrading enzymes of families 18 and 20 by Tews et al.^[148] Similarly, the water molecule which subsequently attacks the glycosyl ester intermediate must have access from the pseudoaxial direction.

The *syn* and *anti* protonation trajectories provide an important criterion for the design of selective inhibitors. Among the lactone-type inhibitors, only the lactone itself possesses a lone pair in a position suitable to accept a hydrogen bond from a *syn*-protonating catalytic acid; such a protonation is blocked in the azole inhibitors and in the hydroximolactams, which should be selective inhibitors of *anti*-protonating glycosidases. The testing of this hypothesis and the design and synthesis of selective inhibitors of *syn*-protonating glycosidases are in progress.

3.5.3. Configurational Selectivity

3.5.3.1. Inhibition of α - and β -Glycosidases

The results discussed in Section 3.5.2 indicate that the transition state for glycoside hydrolysis is characterized by a pseudoaxial orientation of the fissile C–O bond and a skew conformation. This appears to counter the evidence that lactone-type inhibitors are transition state analogues, since, in the ground state conformation, the carbonyl group accepting the hydrogen bond from the catalytic acid is in a pseudo-equatorial orientation. However, it is not clear to what extent partial protonation and interaction with the catalytic nucleophile of a lactone or some of the lactone analogues will lead to rehybridization and to a change of conformation in the enzyme active site, so that the heteroatom attached to C1 is placed above the mean ring plane as shown in Figure 18c. Certainly, lactones easily adopt a boat or skew-boat conformation.^[149] It seems likely, however, that lactone-type inhibitors mimic a structure corresponding to an early region of the reaction coordinate.

The active sites of configuration-retaining α - and β -glycosidases show similar locations for the hydrophilic and hydrophobic amino acid side chains responsible for recognition of the pyranoside ring. This suggests that the main differences between the two enzyme classes are the positioning of the catalytic nucleophile and of the catalytic proton donor. The catalytic nucleophile is for obvious reasons located close to the α face of the pyranoside ring in β -glycosidases, and to the β face in α -glycosidases. As discussed above, the proton donor is located in a plane close to the pyranoside ring plane in β -glycosidases; in α -glycosidases of family 13 (*anti* protonators) the proton donor is also to one side of the fissile bond, but in a plane about 1 Å below that of the mean ring plane,^[114, 115, 150] corresponding to the position of the α -glycosidic oxygen atom.

As discussed in Sections 3.1–3.4 lactone-type inhibitors may be bound by a hydrogen bond with the catalytic proton donor, by a charge–charge (or charge–dipole) interaction with the catalytic nucleophile, or by a combination of these. Permanently protonated analogues such as the amidine **11** and the amidrazone **12** (see Section 2.2) are bound by charge–charge interactions, which are strong and relatively non-directional and may allow (minor) adjustments of the

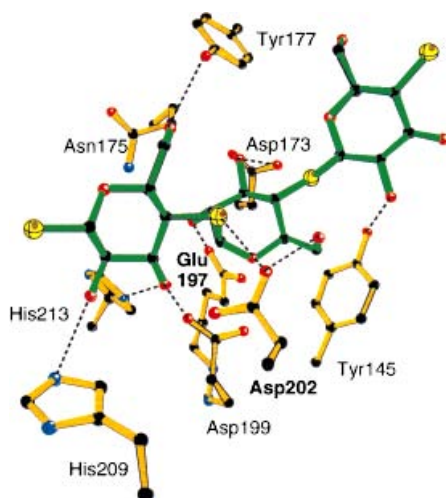


Figure 19. Structure of the complex between *F. oxysporum* cellobiohydrolase (family 7) and a thioglycoside.

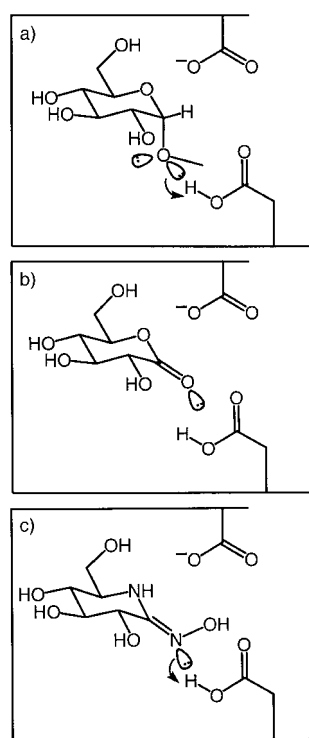


Figure 20. Interaction of the catalytic acid of α -glycosidases with a) an α -glycoside, b) the gluconolactone **1**, and c) the lactam oxime **13**.

using more strongly basic lactone analogues? It appears to be so, as the hydroximolactam **13** (pK_a 4.8–5.2) inhibits both α - and β -glycosidases with similar potency (Figure 20c).^[151] Such analogues mark the continuity between neutral lactone-type inhibitors (weak hydrogen-bond acceptors) and strongly basic, fully protonated inhibitors. The K_i values of strongly basic inhibitors such as the amidine **11** and amidinium **12** reflect (partial?) desolvation upon binding to the enzyme, and presumably the absence of a salt bridge to the catalytic acid. The ideal inhibitor will thus be neutral in solution, and well protonated by the enzyme.

The well-established effect of directionality in the strength of salt bridges^[152] may play a role in the superior inhibition of α - over β -glucosidases by deoxynojirimycin **4**^[39, 153] (Table 11). The ring nitrogen atom of protonated **4** is well positioned to form a hydrogen-bonded ion pair with the (anionic) catalytic nucleophile of α -, but not of β -glucosidases (Figure 21 a, b). The reverse is true for isofagomine (**56**),^[154–156] in which the position of the (protonated) nitrogen atom is better suited to interact with the catalytic nucleophile of a β -glucosidase

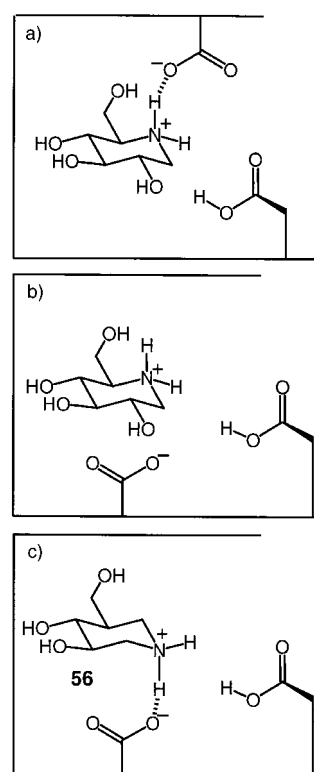
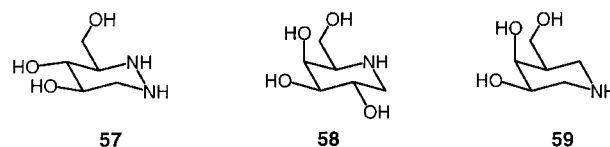


Figure 21. Proposed favored binding interaction of deoxynojirimycin in a α -glucosidase (a), but not in a β -glucosidase (b). c) Favored binding interaction of isofagomine in β -glucosidase.

(Figure 21 c), as reflected in its inhibition constants (Table 11). This is consistent with recently results of Bols et al.,^[157] who have shown that the analogous piperazine **57**, with a nitrogen



atom in both positions, is less α -selective than deoxynojirimycin (it binds both α - and β -glycosidases more strongly), and less β -selective than isofagomine (it binds α -glycosidases more strongly and β -glycosidases less strongly). The analogous relationship between the position of the ammonium center and the inhibition of α - and β -galactosidases is observed for deoxygalactostatin (**58**)^[158] and galacto-isofagomine (**59**).^[156, 159] Conceivably, calystegin B2^[160, 161] can imitate either nojirimycin or isofagomine, depending on the orientation in which it is bound to a glycosidase.

3.5.3.2 Glucosidase or Galactosidase?

The models of the active site of family 1 and 2 glycosidases with bound inhibitors, confirmed by X-ray analyses, also allow a second type of selectivity, or lack of it, to be rationalized. For many years it has been observed that certain β -glucosidases and β -galactosidases only hydrolyze “correctly” configured substrates,^[162–167] whereas others show only a minor preference for a specific configuration at C4.^[65, 119, 167–171] Much work has been invested in purifying these enzymes to examine

Table 11. IC_{50} values [μM] of deoxynojirimycin (**4**), isofagomine (**56**), azaisofagomine (**57**), deoxygalactostatin (**58**), and galacto-isofagomine (**59**).

Enzyme (source)	4	56	57	58	59
α -glucosidase (brewers' yeast)	12.6	86	3.9		> 2000
β -glucosidase (almonds)	47	0.1	0.65		0.19
α -galactosidase (<i>E. coli</i>)	–	–	–	0.0016	200
β -galactosidase (<i>E. coli</i>)	–	–	–	0.16	0.012

whether these apparently unselective glycosidases are mixtures, but often these enzymes remained poorly selective in spite of their homogeneity.^[64, 65] Kinetic results obtained over several decades correlate with the aforementioned classification of glycosidases into families.^[52] Thus, β -glucosidases,^[116] 6-phospho- β -glucosidases and -galactosidases,^[169] and myrosinases (thioglycosidases),^[170] all with low *gluco/galacto* selectivity, belong to family 1. Glucosidases showing high *gluco* selectivity belong to family 3.^[162, 165] Family 2 contains two subfamilies: β -galactosidases, which are strongly *galacto*-selective,^[166] and β -glucuronidases, which show low selectivity.^[171]

Homology in amino acid sequences implies structural similarity, and indeed much of the active site in the enzymes of families 1 and 2 is similar. The residues responsible for catalysis (both Glu), hydrogen-bonding interactions with HO-C2 and HO-C3 (Asn and His), and hydrophobic interaction with the α face of the substrate (Trp) are observed in all crystal structures,^[56, 105, 111, 119] and conserved in all sequences^[172] (see Figures 15–17).

Family 1 enzymes have conserved Trp and Gln residues in positions where they can interact with either an axial or an equatorial C4 hydroxyl group (see Figure 22).^[173, 174]

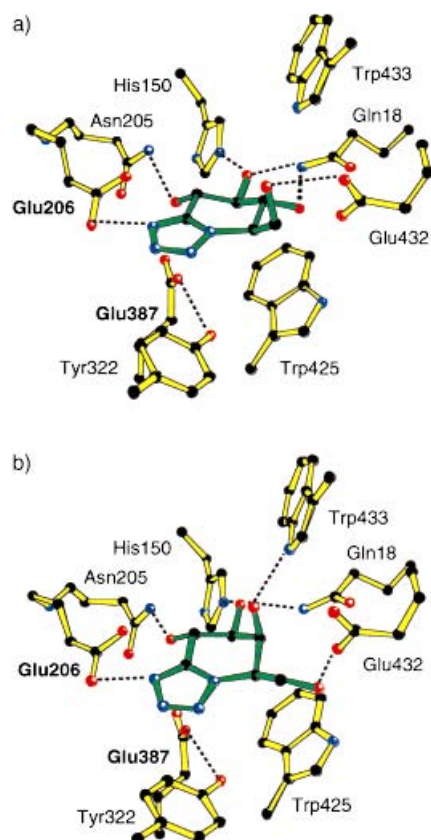


Figure 22. Modeled complexes a) between *S. solfataricus* β -glycosidase (family 1) and *gluco*-tetrazole **20** and b) between *S. solfataricus* β -glycosidase (family 1) and *galacto*-tetrazole **22**.

Both subfamilies of family 2 possess a conserved Asp residue in a position to form a hydrogen bond with an axial C4 hydroxyl group for the representative structures of the *E. coli* β -galactosidase^[104] and human β -glucuronidase,^[120] which both

hydrolyze *galacto*-substrates. These structures are distinguished by the positioning of the conserved Trp residue onto which the pyranose ring stacks. In the human β -glucuronidase, the equatorial C4 hydroxyl group can form a hydrogen bond with the indole nitrogen atom of Trp587 (Figure 23), whereas the equivalent Trp568 in the *E. coli* β -galactosidase (see Figure 15) is shifted upwards, interacts with the hydrophobic face of a galactopyranoside, blocks access to an equatorial hydroxyl group, and prevents glucosides from fully entering the active site.

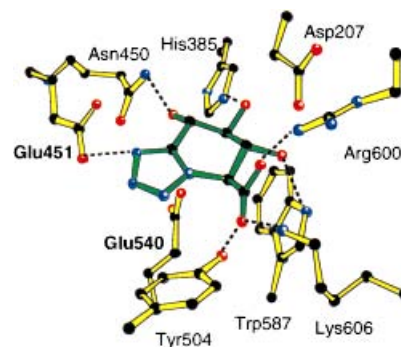


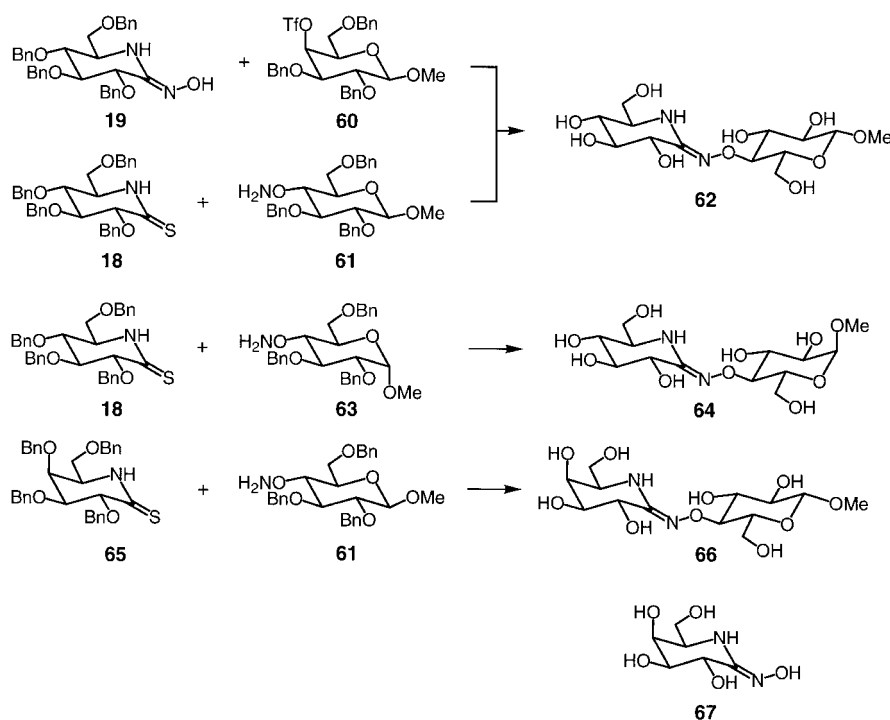
Figure 23. Complex between human β -glucuronidase (family 2) and *gluco*-tetrazole **20**.

So far, no crystal structure has been solved for a family 3 glucosidase, precluding an understanding of the interactions responsible for the high *gluco* selectivity of these enzymes.

4. Aglycon Selectivity—Inhibition by Disaccharide Analogues

Most of the inhibitors discussed in the preceding sections are analogues of the glycon part of the substrate or the transition state. The inclusion of the aglycon part of the natural substrate to the inhibitory glycon portion, for example by the construction of a disaccharide analogue, should ideally lead to increased inhibitory potency and α/β selectivity. A number of research groups have prepared disaccharide analogues of potent inhibitors such as trehazolin,^[175] isofagomine,^[155] valienamine,^[176] 1-deoxynojirimycin,^[177–180] 1-deoxymannojirimycin,^[181, 182] and other azasugars.^[173–190]

The oxime functionality of a hydroximolactam or hydroximolactone is oriented more closely towards the position occupied by the aglycon of a β -glycoside than that of an α -glycoside. A oxime substituent should therefore mimic the aglycon of a β -glycoside more closely than that of an α -glycoside, and a thus substituted hydroximo compound should be a more selective inhibitor of β -glycosidases. The cellobioside analogues **62** and **64** were synthesised by alkylation of the benzyl-protected D-gluconohydroximo-1,5-lactam **19** with the *galacto*-configured triflate **60**, or by condensation of the benzyl-protected thiolactam **18** with the *gluco*-configured hydroxylamines **61** or **63**, followed by debenzilation with lithium in EtNH₂. The lactoside analogue **66** was obtained from the protected D-galactonothiolactam **65**.^[191]



The cellobioside analogues **62** and **64** both inhibit the β -glucosidase from *C. saccharolyticum* as strongly as the unsubstituted hydroximolactam **13**; the second pyranoside ring neither impedes nor enhances the binding to this enzyme (Table 12). However, binding to the yeast α -glucosidase is weakened by a factor of at least 1000, showing that the second pyranoside ring cannot be accommodated in the active site of this glycosidase. The sweet-almond β -glucosidases, with a known preference for a hydrophobic aglycon,^[59] are an interesting case: the inhibition by the β -cellobioside analogue **62** is only four times weaker than that by **13**, whereas that by the α -cellobioside **64** is 100 times weaker. This is in keeping with the interaction with (presumably) a hydrophobic and flat aglycon, incompatible with the axial methoxy group of **64**.

The α/β selectivity for the cellobioside analogues **62** and **64** is mirrored by the lactoside analogue **66**. Thus, the potent inhibition of coffee-bean α -galactosidase by the unsubstituted hydroximolactam **67** ($K_i = 5 \mu\text{M}$) is significantly weakened by the introduction of the glucosyl moiety in **66** ($K_i = 250 \mu\text{M}$), whereas both oximolactams **66** and **67** are potent competitive inhibitors of the *E. coli* β -galactosidase (family 2) with a K_i of 100 nM.

The K_i values of **66** ($3.3 \mu\text{M}$) and **67** ($2.5 \mu\text{M}$) against the β -glucosidase from *C. saccharolyticum* (family 1) are similar to

those for the *gluco*-analogues **13** and **62**, confirming that family 1 glucosidases are not selective for the configuration at C4, and also that the aglycon binding site is adapted for hydrophilic as well as hydrophobic residues. This is in keeping with the role of this enzyme as an *exo*-cellulase, and the observation that it also readily hydrolyzes *p*-nitrophenyl glycosides.^[192] For these interpretations, one should not forget that the distance between glycon and aglycon is larger for the oxime ethers than for the disaccharides. Although the glycosidic bond is progressively stretched during cleavage, it is by no means clear how this aspect affects the inhibition by the disaccharide analogues.

The bovine-liver β -galactosidase is inhibited about 100 times more strongly by **67** ($K_i = 10 \mu\text{M}$) than by **66** ($\text{IC}_{50} \approx 2.5 \text{ mM}$), in keeping with the strong preference of the bovine-liver glycosidase for aryl pyranosides. This contrasts

somewhat with the results for the inhibition of the sweet-almond β -glucosidases, which also have a preference for aryl pyranoside substrates,^[59] but for which the cellobioside analogue **62** binds only four times more weakly than the unsubstituted **13**. It would seem that the aromatic aglycon binding site in the bovine-liver β -glycosidase is more selective than that in the sweet-almond β -glucosidases. Unfortunately the amino acid sequence of neither glycosidase has been reported, which makes it impossible to draw any specific conclusions from these results regarding the structure of their active site, and thus the reasons behind their differing preferences for aromatic aglycons.

5. Concluding Remarks

The synthesis of suitable glycosidase inhibitors, kinetic data, structural information about glycosidases or glycosidase–inhibitor complexes, and the interpretation of these results in the light of the reactivity of organic compounds are ideal prerequisites to elucidate details of enzymic reaction mechanisms. Exact information about these mechanisms, in its turn, allows the rational design of stronger and more selective inhibitors. The consideration of the protonation in

Table 12. K_i and IC_{50} values of the lactam oximes **13**, **62**, **63**, **66**, and **67**.

Enzyme	$K_M^{[a]}$	pH	K_i [μM] or in italics IC_{50} values [μM]				
			13	62	63	66	67
β -glucosidases (almonds)	3.0 (1.2)	6.8	16	<i>60</i>	<i>1000</i>	–	–
β -glucosidase (<i>C. saccharolyticum</i>)	1.2 (1.2)	6.8	3.3	3.6	2.0	3.3	2.5
α -glucosidase (brewers' yeast)	1.2 (1.2)	6.8	2.9	2500	> 5000	–	–
β -galactosidase (bovine liver)	0.24 (0.24)	7.0	–	–	–	2500	10 ^[58]
β -galactosidase (<i>E. coli</i>)	0.04	6.8	–	–	–	0.1	0.1
α -galactosidase (coffee beans)	0.19	6.0	–	–	–	250	5

[a] K_M values [mM] for corresponding 4-nitrophenyl hexopyranosides. In parenthesis, starting concentration of the substrate [mM].

the ring plane, of the direction from which the protonation occurs (*syn* versus *anti* protonators), and of the conformational changes during glycoside cleavage will prove valid in the design of inhibitors. It should be mentioned in this context that recent results from K. Piens in the laboratory of M. Claeyssens (University of Gent) have shown that inhibitors of the hydroxymolactam- and imidazole-type inhibit cellobiohydrolase II (CBH II) of *Trichoderma reesei*, but not cellobiohydrolase I (CBH I). This is consistent with the assignment of CBH I as a *syn* protonator, and suggests that CBH II may be an *anti* protonator. The question of whether similar selectivities exist for the still poorly known glycosyl transferases promises further thrilling studies in the interplay of organic chemistry, enzymology, protein crystallography, and biotechnology.

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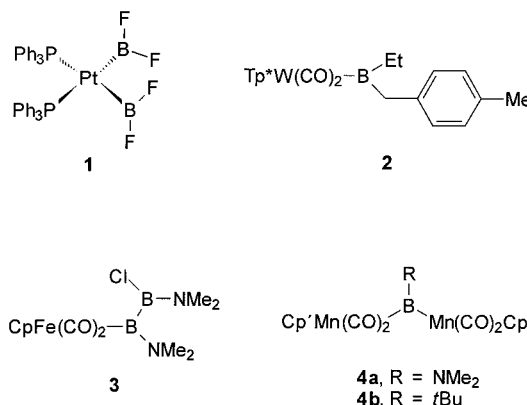
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Metal Complexes Bearing Terminal Borylene Ligands

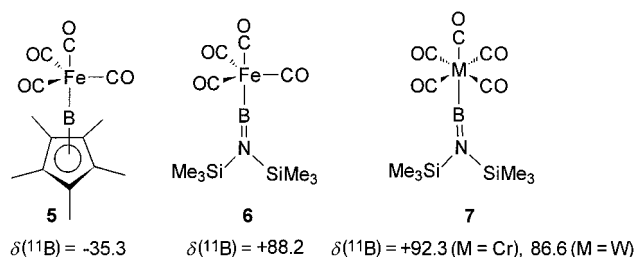
Bernd Wrackmeyer*

The understanding of metal–boron interactions and the challenge to build metal–boron (M–B) bonds provide extremely intriguing research areas in theoretical chemistry as well as in solid-state and molecular chemistry. The development of metallapolyboranes^[1] and metallocarboranes,^[2] compounds in which M–B multicenter bonds play a decisive role, began already in the 1960s. In addition, metal hydridoborates have been characterized more precisely,^[3] simple neutral boranes or anionic borates^[4] have served as π -donor ligands in transition metal complexes, and more recently fascinating complexes with interstitial boron atoms were described.^[5] There have been early attempts at the synthesis of molecules with M–B two-electron, two-center (2e/2c) bonds,^[6] and at that time complexes with borylene ligands (boranediyl, RB) were already considered.^[7] However, the products obtained in these early experiments most probably do not possess metal–boron bonds, at least the physical data are by no means conclusive. There are also some doubts regarding the M–B σ bond for the majority of complexes for which it is suggested that a basic metal center interacts with a borane. Results pertinent to M–B 2e/2c bonds supported by direct structural evidence were only reported very recently,^[8] and this work is primarily related to the investigation of metal-catalyzed hydroboration^[9] and diboration of alkenes and alkynes.^[10] A recent example representative for numerous similar complexes (in particular with catecholboryl groups^[8]) is complex **1**,^[11] an intermediate of the diboration. There are rather few examples for complexes in which a diorganoboryl group is linked to the metal (e.g., **2**;^[12] $\text{Tp}^* = \text{tris}(3,5\text{-dimethylpyrazol-1-yl})\text{hydroborate}$), whereas it appears that the access to complexes bearing aminoboryl groups at the metal^[8] is more facile (e.g., **3**^[13]). Since it proved possible to prepare μ_2 -borylene derivatives (e.g., **4**;^[14] $\text{Cp}' = \text{C}_5\text{H}_4\text{Me}$), the question of complexes with terminal borylene ligands was re-opened.

Complexes with terminal borylene ligands have become a further milestone in the chemistry of metal–boron compounds. The synthesis and structural characterization of such complexes containing one terminal borylene ligand each has been reported recently.^[15, 16] Cowley et al.^[15] have obtained



and fully characterized the tetracarbonyliron complex **5** from the reaction of $\text{C}_5\text{Me}_5\text{BCl}_2$ with $\text{K}_2[\text{Fe}(\text{CO})_4]$. Braunschweig et al.^[16] have mentioned the existence of an analogous complex **6** (not fully characterized), and have described the pentacarbonylchromium- and -tungsten complexes **7**.

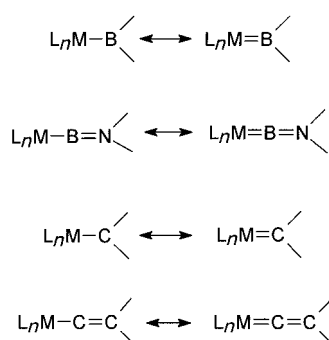


The bonding in **5** is similar to that in the homologous aluminum compound;^[17] the boron atom possesses the formal oxidation number +1 and functions as a weak π acceptor. The chemical shift $\delta(^{11}\text{B}) = -35.3$ lies in the range typical of apical ^{11}B nuclei in *nido*-carboranes. However, to date there is no carbon derivative which can be compared directly with **5**. The question of the relative contributions of canonical structures is more important for **6** and **7** than for **5**. Thus, a carbyne-like canonical structure $[\text{Ar}^*\text{Ga}\equiv\text{Fe}(\text{CO})_4]$ has been discussed for $[\text{Ar}^*\text{GaFe}(\text{CO})_4]$ ^[18] ($\text{Ar}^* = 2,6\text{-bis}(2,4,6\text{-triisopropylphenyl})\text{-phenyl}$). Such a structure may play a role for complexes with terminal RB ligands (R = alkyl, aryl); however, these complexes have not been obtained as yet. The comparison of **6** and **7** with vinylidene complexes is evident, considering the isoelectronic substitution of BN by CC. The short B–N bond (133.8(8) pm) in **7** with M = W supports this argumentation.

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The ^{11}B resonances of **6** and **7** at low field are typical of the low-coordinate ^{11}B nuclei and their integration into the BN π system. An even more reduced magnetic shielding of ^{11}B nuclei can be predicted for the unknown complexes $\text{L}_n\text{M}-\text{BR}$ ($\text{R} = \text{alkyl, aryl}$) (c.f. $\delta(^{11}\text{B})$ values for **4** with $\text{R} = \text{NMe}_2$ (+103) and $\text{R} = t\text{Bu}$ (+170)^[14]). The weak π -acceptor properties of the $\text{BN}(\text{SiMe}_3)_2$ ligand are reflected by the length of the $\text{W}-\text{B}$ bond in **7** (215.1(7) pm; 8 pm longer than in **2**^[12]), and also by the $\tilde{\nu}(\text{CO})$ frequencies which are shifted to lower wavenumbers in **7** with respect to comparable vinylidene complexes.

The comparison of the boryl metal complexes with carbene complexes and of the borylidene complexes **6** and **7** with vinylidene complexes reveal analogous bonding situations. The contribution arising from the canonical structure with the $\text{M}=\text{B}$ bond is always significantly less important than from the one with the $\text{M}=\text{C}$ bond (Scheme 1).



Scheme 1. Comparison of the possible canonical structures of metal complexes with borylene ligands with those of the corresponding carbon analogues.

Future efforts will focus on the synthesis of complexes with terminal borylene ligands RB ($\text{R} = \text{alkyl, aryl}$), the possibility of linking two or even more terminal borylene ligands to one metal atom, and on attempts to combine a variety of other ligands with terminal borylene ligands at the metal center. The enormous synthetic potential of boron chemistry and of transition metal chemistry have to be explored for these new systems, and this will almost certainly guarantee some surprises.

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Keywords: B ligands • boron • transition metal complexes

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Recent Advances in the Conversion of Carbohydrate Furanosides and Pyranosides into Carbocycles

Peter I. Dalko* and Pierre Sinay*

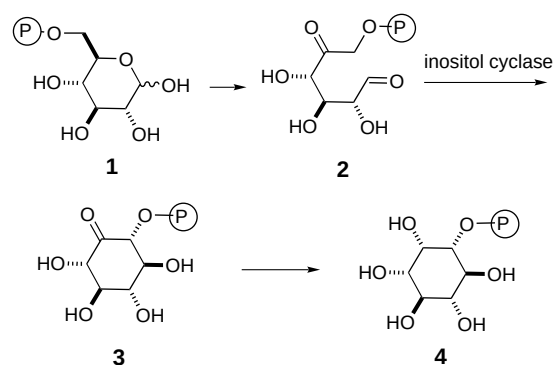
In memory of Stéphan D. Géro

Carbocyclic polyols are important constituents of many biologically active molecules. They exhibit far-reaching biological effects, ranging from cellular regulation to the selective inhibition of enzymes which play key roles in living organisms.^[1, 2] They may be divided into two categories, those containing six- and those containing five-membered carbon rings. Among the most prominent of the six-membered representatives are cyclohexane hexitols, such as inositol derivatives,^[1b] and pseudosugars,^[1f, 2, 3] such as cyclophellitol^[4] and valienamine.^[5] Structural analogues of the latter are constituents of compounds of key pharmacological interest, for example acarbose,^[6] adiposin,^[7] trestatin,^[8] and amylostatin.^[9] Together with a growing number of structurally related alkaloids,^[10] purely synthetic analogues,^[11] and natural products containing five-membered polyols,^[12, 13] these small but synthetically challenging molecules have received growing interest in the last few years.

Owing to their biological significance and the inherent structural challenge they present, a variety of different approaches to obtain optically pure carbocyclic polyols has been developed.^[14] These strategies include 1) cyclization of alicyclic, polyfunctionalized molecules and 2) transformations of conveniently substituted carbocycles. Included in the first strategy are cycloaddition reactions,^[15] Wittig-type olefinations,^[16] radical cyclization approaches^[17] including reductive couplings,^[18] ring-closing methathesis (RCM),^[19] and a variety of anionic^[14] or transition metal mediated^[20] cyclization reactions of advanced polyhydroxylated compounds. Historically, a tandem fragmentation/Henry-type cyclization reaction provided the first examples in which cyclohexane^[21] and cyclopentane derivatives^[22] were obtained from a carbohydrate. An elegant application of the Fujimoto–Belleau reaction by Vasella et al.^[23] and the anionic rearrangement of anhydrosugars^[24] are also examples of this strategy. Some of the above transformations allow the direct conversion of carbohydrate furanosides and pyranosides into carbocyclic polyols. Strategies which include enantiopure carbocycles as starting materials feature transformations of quinic acid,^[25] inositols, or conduritol derivatives;^[26] desymmetrization of

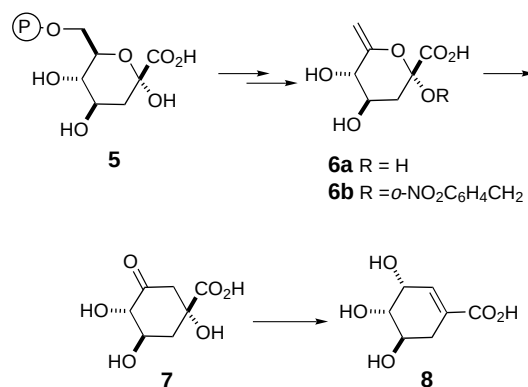
meso cyclohexene derivatives by asymmetric palladium catalyzed hydroxycarboxylation;^[27] enantioselective alkylation;^[28] and transformation of homochiral carbocycles either by microbial^[29, 30] or chemical oxidation.^[28, 31]

Somewhat surprisingly, the biosynthesis of the carbocyclic polyols is not thoroughly understood, probably because their biogenesis does not follow a general, unified path. It has been found that *myo*-inositol derivatives, for example, are formed from D-glucose 6-phosphate (**1**) by stereospecific ring closure under the catalytic influence of inositol cyclase (Scheme 1).^[1b]



Scheme 1. Synthesis of *myo*-inositol 1-phosphate (**4**) from **1** under the catalytic influence of inositol cyclase. P = phosphate.

Other biosyntheses bear strong resemblances to the mechanism by which D-glucose is converted into shikimic acid (**8**; Scheme 2). This is the biogenetic path in which nature produces the benzoid rings of the aromatic amino acids and an extensive range of other metabolites. At an early stage of this sequence the hemiacetal intermediate **6a** undergoes rearrangement and forms the quinic acid derivative 3-dehydroquinic acid (**7**). However, Bartlett and Satake demon-



Scheme 2. Biogenetic synthesis of shikimic acid (**8**).

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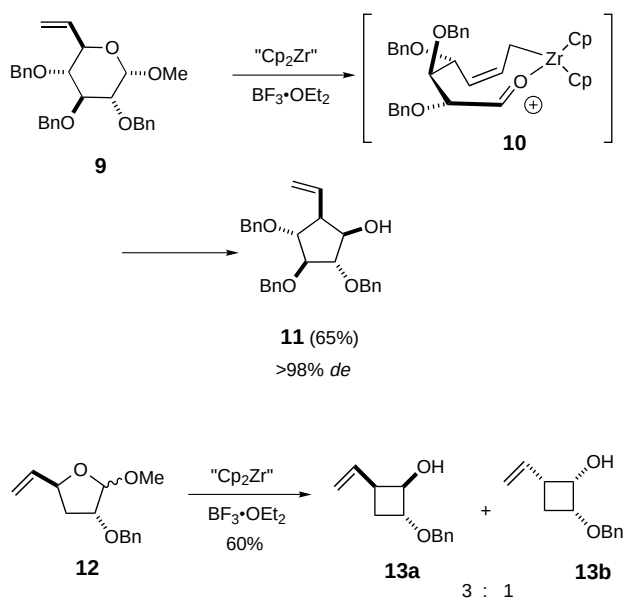
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strated that the unstable **6a**, generated in situ from the *o*-nitrobenzyl-protected α -glycoside **6b**, rearranges spontaneously to **7** in the absence of a specific enzyme.^[32] Moreover, this uncatalyzed reaction gives rise to a single stereoisomer, identical to that of the biosynthetic path. This fact suggests that cyclitol biosynthesis may also be nonenzymatic.

Nature has found a direct way to perform this tandem fragmentation cyclization reaction in a stereospecific manner and under neutral conditions. Human creativity and good luck have uncovered synthetic strategies which can compete efficiently with nature. With the advent of mild, metal-mediated cyclization reactions, the early work of Grosheinz and Fischer,^[21] who converted 6-deoxy-6-nitrohexoses into nitroinositols in a single step, matured to a general strategy of broad interest. The methods that have been used include 1) the ring contraction of carbohydrate derivatives^[33] into functionalized cyclopentanes and cyclobutanes and 2) the conversion of a variety of sugar furanosides and pyranosides into highly functionalized carbocyclic derivatives of five and six carbon atoms, respectively.

Ring Contraction of Carbohydrate Derivatives

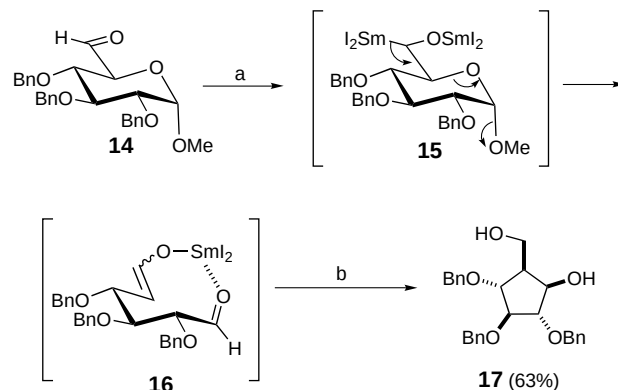
Vinyl carbohydrate derivatives **9** or **12** can be converted into carbocycles **11** and **13** respectively by sequential treatment with "Cp₂Zr" (Cp = C₅H₅) and BF₃·OEt₂ (Scheme 3).^[34] The reaction selectively forms the product in which the vinyl group is *cis* to the newly formed hydroxyl group. Although the



Scheme 3. Synthesis of carbocycles **11** and **13** from vinyl carbohydrate derivatives **9** and **12**. Bn = benzyl.

major stereoisomer was expected to show a *trans* orientation of the vinyl group with respect to the adjacent α substituent, the overall selectivity of the reaction depends intimately on the stereochemistry and nature of the substituents present. Despite the difficulty encountered with different derivatives in controlling stereochemistry, the easy access and the seemingly unhindered choice of the vinyl carbohydrate starting materials renders this methodology appealing.

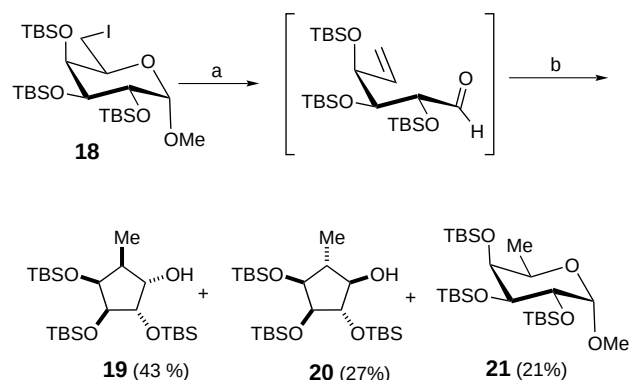
Samarium(II) iodide promotes a comparable transformation of aldehyde **14** to ring-contracted product **17** (Scheme 4).^[35] The presence of HMPA and *tert*-butyl alcohol as a proton source is necessary to obtain a good conversion into the



Scheme 4. Synthesis of **17** from aldehyde **14**. a) SmI₂, THF, hexamethyl phosphoramide (HMPA); b) SmI₂, THF, HMPA, *t*BuOH (2 equiv).

cyclopentane derivative. The reaction is considered to proceed via the samarium ketyl intermediate, which is reduced to the disamarium species **15** under the reaction conditions. After fragmentation, the system is ideally set up for a subsequent aldol cyclization, involving intramolecular nucleophilic attack of the samarium enolate onto the aldehyde through a 5-enol *exo-exo-trig* process. As expected from a metal-linked chelate, the major stereoisomer is that in which the two newly created stereocenters are *cis* with respect to one another and *trans* with respect to the adjacent substituents.

A similar reductive ketyl–olefine coupling is hypothesized as the key cyclization step in the SmI₂-promoted ring contraction of 6-deoxy-6-iodohexopyranosides^[36a] such as **18** (Scheme 5). This sequence requires four single electron



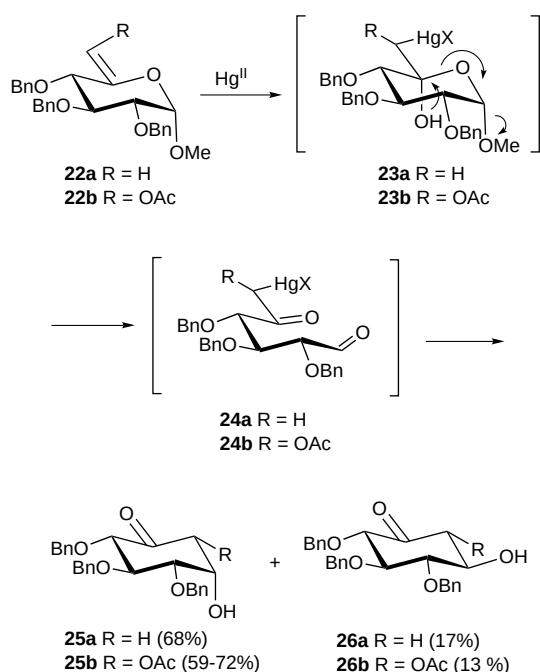
Scheme 5. The SmI₂-promoted ring-contraction reaction of **18** to provide **19**–**21**. a) SmI₂, THF, HMPA; b) SmI₂, THF, HMPA, then H⁺. TBS = *tert*-butyldimethylsilyl.

transfer (SET) steps (i.e., at least 4 molequiv of SmI₂ are necessary to complete the reaction) and consist of 1) reductive dealkoxyhalogenation to give the ring-opened hex-5-enal, 2) an intramolecular ketyl–olefine reductive coupling to afford the ring-contracted organosamarium intermediate,

and 3) the intermolecular trapping of this intermediate to produce branched cyclopentitol derivatives such as **19** and **20**. The cyclopentane products were isolated along with the corresponding 6-deoxypyranoside **21**. In cases where cyclization occurred, the major products usually had a relative *trans* configuration between the newly formed methyl group and the hydroxyl group. In addition, a *trans* orientation between the hydroxyl group and the vicinal alkoxy group was observed.^[36b]

Conversion of Sugar Furanosides and Pyranosides into Highly Functionalized Carbocyclic Derivatives

Sugar enol-ethers which inherently carry both masked nucleophilic and electrophilic functionalities have been converted into carbocycles. The first breakthrough for this class of reaction was the mercury(II)-mediated rearrangement of hex-5-enopyranoside derivatives, generally termed the Ferrier (II) reaction.^[37] For example **22a** gives the highly functionalized cyclohexanones **25a** and **26a** (Scheme 6). This remarkable

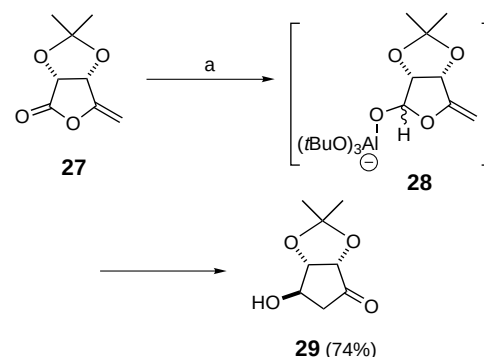


Scheme 6. Synthesis of functionalized cyclohexanones **25** and **26** from **22**.

reaction has provided a practical route to a large variety of bioactive substances such as aminocyclitols,^[38] pseudosugars,^[39] inositols,^[40] and other complex hexitols.^[41] Among the various conditions employed for the Ferrier (II) reaction, both catalytic and stoichiometric methods have been investigated. These afford similar but not identical results.^[14, 42] Also, a “nontoxic alternative” makes use of a catalytic amount of palladium(II) instead of mercury(II).^[43] This modification can slightly alter the selectivity, which is not surprising given the different coordination pattern of the metal center in the transition state. The basic features of this transformation are the loss of the glycosidic functionality, for example as methanol by fragmentation, and the ring-closing aldol reac-

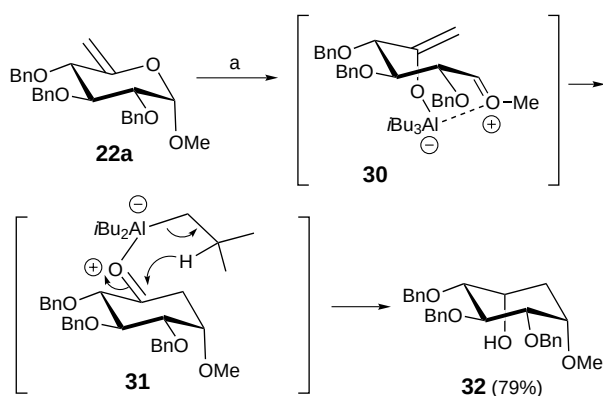
tion. In this cyclization the carbanionic center (C6) attacks the electrophilic carbonyl center (C1). The stereochemistry of the newly formed asymmetric center is influenced by the conformational bias of the molecule, the chemical nature of the functionalities present, and the reaction conditions. Yields of isolated products are also highly dependent upon the experimental conditions. These observations are thought to be due to the preferred conformation of the given sugar derivative in the transition state and to the possibility of formation of a sensitive β -hydroxy ketone, which can undergo elimination and subsequent aromatization. The scope of the reaction was enlarged by the demonstration that functionalized exocyclic olefins may be converted into carbocycles as well. For example, **22b**, made from the corresponding aldehyde, was transformed to inosose derivative **25b** with high stereoselectivity of each newly formed stereogenic center (Scheme 6).

The Ferrier (II) reaction is a quite efficient way to form six-membered carbocycles, but is unsuitable to transform furanosides to cyclopentitols. Subtle changes in the transition state conformation and complexation patterns prevent carbocyclization. The solution for this challenging problem was uncovered with an aluminium hydride based Lewis acid. Five-membered enollactone **27** was converted into the cyclopentanone derivative **29** as a single epimer upon treatment by $\text{LiAlH}(\text{O}t\text{Bu})_3$ (Scheme 7).^[44] Spectroscopic studies have established some mechanistic details. Hydride adds rapidly to the carbonyl, and a stable aluminate complex is formed. The carbocyclization occurs by protonation followed by fragmentation and an aldol-type cyclization process.



Scheme 7. Rearrangement of the enollactone **27** to a cyclopentanone derivative **29**. a) $\text{LiAlH}(\text{O}t\text{Bu})_3$, THF, 0 °C, then NH_4Cl (aq.).

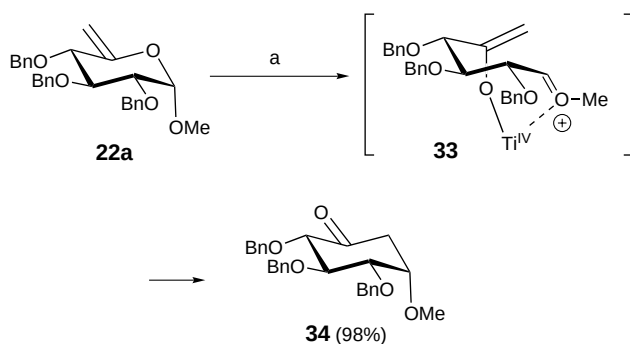
Under the particularly mild conditions of a triisobutylaluminum-mediated rearrangement the fragmentation of the “locked” anomeric substituent in **22a** was avoided. Retention of configuration with high selectivity was observed.^[45] Lewis acid assisted *endo* activation, followed by a ring-opening step, is hypothesized to generate the zwitterionic enolate **30** (Scheme 8). Ring closure is thought to occur by an intramolecular 6-*exo-trig* aldol condensation. The “bonus” of the reaction is the stereoselective formation of a hydroxyl group, resulting from the final reduction of the keto group, probably by intramolecular hydrogen delivery from the less hindered β side. The reaction is fairly general and high-yielding; a wide



Scheme 8. Synthesis of **32** from **22a** via the zwitterionic enolate **30**. a) $i\text{Bu}_3\text{Al}$, toluene, 40°C .

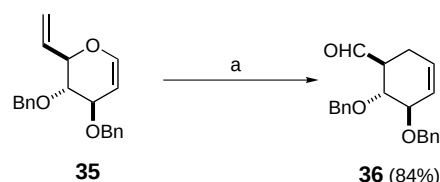
array of sugar starting materials have been used. The good selectivity in conserving the anomeric stereochemical information is worth noting. The reaction proceeds with retention of configuration.

A similar strategy was developed with titanium(IV) derivatives as Lewis acids.^[46] In the presence of $[\text{TiCl}_3(\text{OiPr})]$ the α -D-glycoside **22a** undergoes the expected *endo* cleavage of the glycosidic bond and forms the carbocyclic glycoside **34** with retention of configuration at the former anomeric center (Scheme 9). Although the reaction of the α -glycoside proceeds in nearly quantitative yield, the corresponding β -glycoside gives rise to a mixture of different products. The titanium(IV) reaction is even milder than the triisobutylaluminum-mediated rearrangement and does not result in reduction of the keto functionality.



Scheme 9. Synthesis of carbocyclic glycoside **34** from **22a**. a) $[\text{TiCl}_3(\text{OiPr})]$ (1.5 equiv), CH_2Cl_2 , -78°C , 15 min.

Pseudosugars have been prepared by an elegant thermal [3,3] sigmatropic rearrangement.^[47] This methodology, which is conceptually different from the fragmentation/cyclization strategy discussed above, is based on the work of Büchi and Powell.^[48] Although the generality of this approach using different sugar series remains to be demonstrated, the simplicity is attractive. Vinyl glucal derivative **35** affords the cyclohexene aldehyde **37** on heating (Scheme 10). One of the advantage of this transformation is that the pseudosugar formed retains the original configuration of the starting sugar. D-Glucal derivatives give entry to the pseudo-D-glucal series.



Scheme 10. Synthesis of cyclohexene aldehyde **36** from vinyl glucal derivative **35**. a) 240°C , *o*-dichlorobenzene, sealed tube, 1 h.

Conclusion

The access of enantiomerically pure carbocyclic polyols from carbohydrate furanosides and pyranosides is an attractive proposition. New methods have made it possible to carry out such transformations under mild conditions in good yields and with high, predictable stereoselectivity. Their compatibility with a large variety of substituents gives the discussed rearrangements strategic importance.

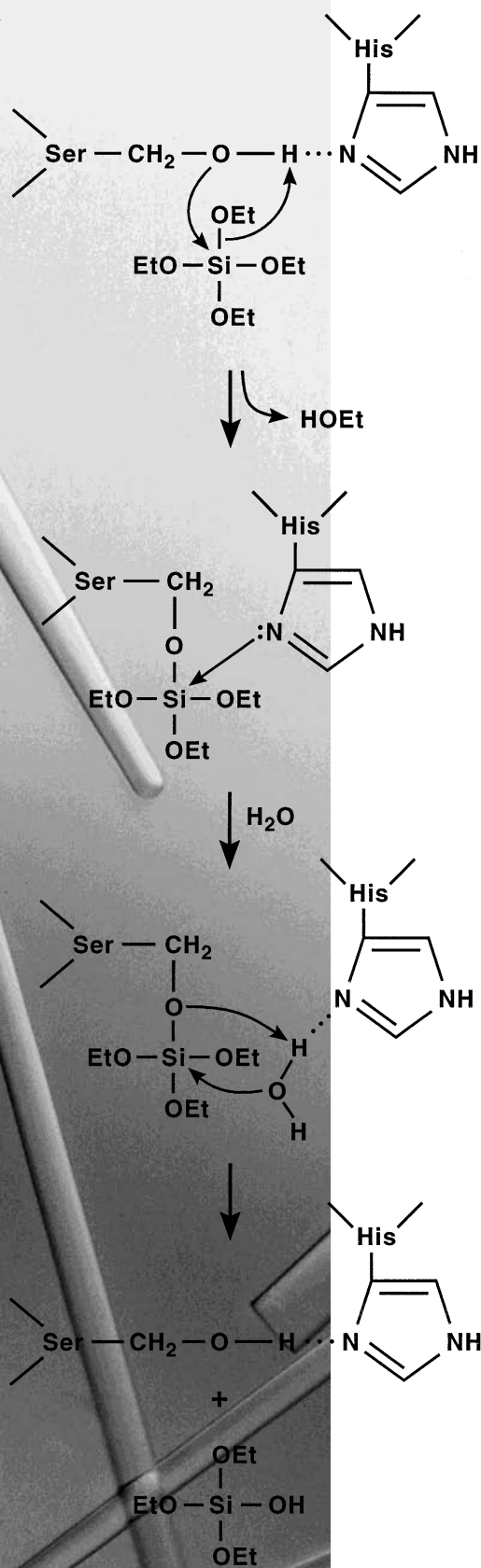
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Keywords: C–C coupling • carbocycles • carbohydrates • cyclitols • cyclizations

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Silica needles (2 mm × 30 μm diameter) make up 75% of the dry weight of a common marine sponge. Protein filaments occluded within these needles (and visible in the photograph) catalyze the synthesis of silica and polysilsesquioxanes from the corresponding silicon alkoxides at neutral pH and 20 °C.
(Photomicrograph by L. J. Friesen)



More information
is given on the
following pages.

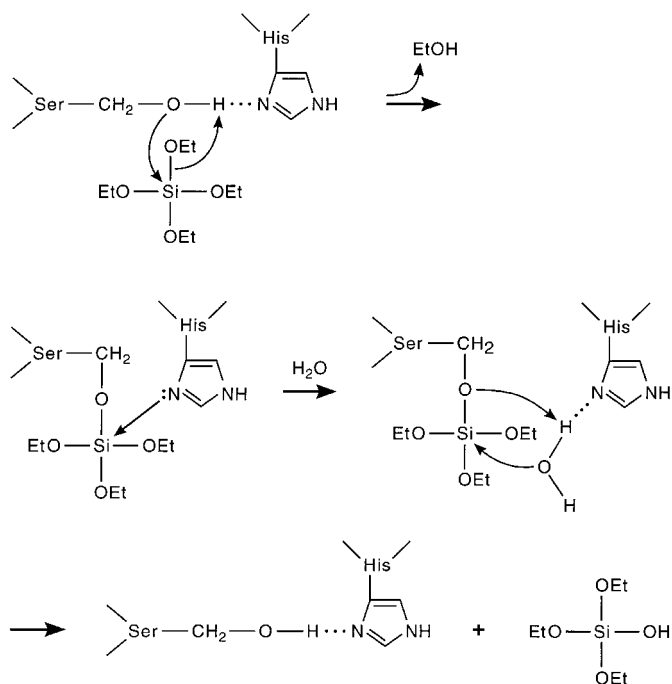
Efficient Catalysis of Polysiloxane Synthesis by Silicatein α Requires Specific Hydroxy and Imidazole Functionalities**

Yan Zhou, Katsuhiko Shimizu, Jennifer N. Cha, Galen D. Stucky, and Daniel E. Morse*

Many living marine organisms synthesize large quantities of shape-controlled silica under mild physiological conditions and at temperatures close to 0 °C.^[1] This is in marked contrast to the conditions of industrial and geochemical synthesis of silica and other polysiloxanes that typically require extremes of pH, elevated temperatures, or both. We recently found that the proteins occluded within the macroscopic silica needles made by a marine sponge can catalyze the synthesis of silica and organically modified silsesquioxanes from the corresponding silicon ethoxides at neutral pH and low temperature.^[2, 3] In addition to this catalytic activity, we observed that when the proteins (that we named “silicateins”, for silica proteins) are assembled into macroscopic filaments (2 mm $\times$ approx. 2 μ m diameter), they serve as scaffolds that spatially direct the synthesis of the polysiloxanes over the surface of the protein filaments. These observations suggested that the biochemical mechanism of catalysis of polysiloxane synthesis mediated by the silicateins might be useful as a model for new routes to synthesis of silicon-based polymers and materials under environmentally benign conditions.

Silicatein α , the principal subunit comprising about 70 % of the mass of the silicatein filaments, exhibits a previously unexpected high structural homology to the well-known hydrolytic enzyme, cathepsin L (a protease), suggesting a possible mechanism for its catalysis of polysiloxane synthesis.^[2, 3] The other two silicatein subunits comprising the protein filaments also are similar.^[2] The close relationship between silicatein α and the hydrolytic enzyme includes high similarities between their amino acid sequences, three-dimensional structures, and membrane-enclosed intracellular localizations. Because the rate of condensation of the silicon

alkoxides is limited by the hydrolysis of the alkoxide (usually catalyzed by acid or base),^[4] we proposed that silicatein α catalyzes this reaction at neutral pH through the activity of the serine and histidine side chains that occupy positions corresponding to the catalytically active, functionally related side chains in the proteolytic enzymes of both the cathepsin L (cysteine–histidine) and trypsin/chymotrypsin (serine–histidine) types.^[3] This suggested mechanism of catalysis (Scheme 1), in which the putative active site serine-26 and



Scheme 1. Key role of the hydroxy group of serine-26 and the imidazole side chain of histidine-165 in the mechanism proposed for silicatein-mediated catalysis. Catalysis of the rate-limiting step in alkoxy silane polycondensation at neutral pH is proposed analogous to the mechanism of peptide bond hydrolysis by the homologous protease.^[3] Hydrogen bonding between the hydroxymethyl and imidazole side chains of serine-26 and histidine-165 increases nucleophilicity of the serine oxygen atom; attack on the silicon atom of the tetraethoxysilane is suggested to form a transitory protein-substrate intermediate that potentially would be stabilized as a pentavalent silicon species through a donor bond from the imidazole nitrogen atom. Hydrolysis of this intermediate releases the silanol product and regenerates the serine–histidine pair.

histidine-165 side chains interact across the substrate binding cleft to form a general acid/base catalyst, is based on the high structural homology between silicatein and the corresponding proteases, the well-known mechanism of hydrolysis of the peptide bond by the corresponding side chains of the proteolytic enzymes (which also act as general acid/base catalysts),^[5] and the requirement for silicon alkoxide hydrolysis to initiate polysiloxane condensation. We present here direct experimental evidence supporting the proposed role of the specific serine and histidine side chains in the mechanism of catalysis of polysiloxane synthesis by silicatein α .

We produced structural variants of the silicatein α protein in which a) the serine residue (with a hydroxymethyl side chain) at position 26, and b) the histidine residue (with an imidazole side chain) at position 165 were specifically

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replaced with an alanine residue (bearing a methyl side chain). These variants were produced by the method known as site-directed mutagenesis, in which the cloned recombinant DNA coding for the silicatein α is modified in a site-specific manner in vitro, and the mutant DNAs then used as templates to direct the synthesis of the corresponding proteins in bacteria. Quantitative comparison of the catalytic activities of the resulting substituted proteins to the activity of the original protein supports the suggestion that both serine-26 and histidine-165 of the silicatein α are required for efficient catalysis of silica synthesis from silicon tetraethoxide at neutral pH (Figure 1). Additional data from these experiments verify the specificity of the catalysis of silica synthesis

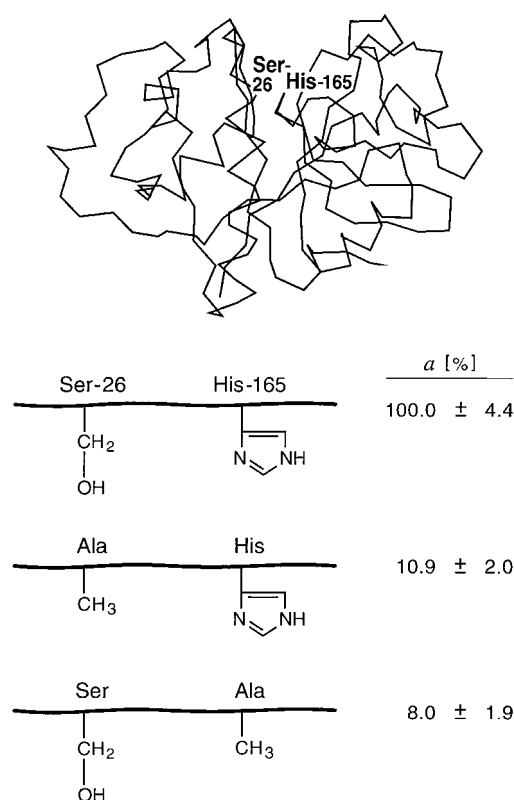


Figure 1. Relative catalytic specific activities (a) of the original and substituted silicatein α proteins. Pictured above is the topology of the protein, based on the three-dimensional structure of the highly homologous cathepsin L, and constrained by three intramolecular disulfide crosslinks at the same positions in the two proteins. Note the close juxtaposition of serine-26 and histidine-165 across the substrate-binding cleft. The three lines below illustrate schematically the protein backbones of the original protein and the two variants produced by site-directed mutagenesis to substitute the methyl side chain of an alanine (Ala) residue in place of the side chains of serine-26 (Ser-26) and histidine-165 (His-165), respectively. Relative catalytic specific activities were determined as described in the Experimental Section.

by silicatein α under the conditions employed. Thus, thermal denaturation, that collapses the three-dimensional structure of the protein, significantly reduces the activity of silicatein α (leaving only $6.3 \pm 1.4\%$ residual catalytic specific activity), proving that the efficient catalytic activity is dependent on the precise three-dimensional conformation of the native protein. Furthermore, the maltose binding protein, whose gene was

used as a "carrier" for the silicatein α gene in the cloning and protein expression (cf. Experimental Section), after purification from the fusion protein, lacks significant activity in the catalysis of silica synthesis from the alkoxide under the conditions employed ($5.4 \pm 1.6\%$ catalytic specific activity relative to that of the native silicatein α). The catalytic activity of the mutant proteins is reduced by an order of magnitude (Figure 1), to a residual value somewhat higher than that of the heat-denatured protein. Similarly, site-directed mutagenesis replacing the active-site serine and histidine side chains of the proteases that have been shown to depend on these moieties for their acid-base catalysis also leaves residual activities higher than exhibited by the corresponding controls.^[6] As shown to be the case with the proteases, this suggests that catalytic efficiency depends on the synergistic interaction between the active-site side chains (and an asparagine side chain that we have not altered),^[6, 7] and that neither substitution alone is sufficient to remove all traces of activity.

The results reported here demonstrate that the functional hydroxy and imidazole side chains of serine-26 and histidine-165, which were identified from the structure of silicatein α as its putative active site, are required for the protein's efficient and specific catalysis of alkoxysilane polycondensation at neutral pH. These observations, and further definition of the structural determinants of the control of the polymerized product by the silicatein molecule, may prove useful in the design of synthetic catalysts for environmentally benign routes to the synthesis of silica and organically modified polysilsesquioxanes.

Experimental Section

All molecular genetic methods were standard.^[8, 9] DNA coding for the silicatein α mature protein was obtained by selective enzymatic replication from its original cloning vector^[2] and integrated into a recombinant bacterial plasmid DNA to produce a fusion protein in which the silicatein α protein is coupled to MBP (maltose binding protein). After verification of the sequence of the fusion protein, and of the ability of the recombinant DNA to direct the synthesis of high quantities of the fusion protein in cells of the bacterium, *Escherichia coli*, variants of the protein were produced by site-directed mutagenesis^[10] to yield altered silicateins in which the serine at position 26 and the histidine at position 165 were specifically replaced with alanine residues (Figure 1). These specific replacements were accomplished by: a) synthesis of oligonucleotides which correspond exactly to the sequences flanking the sites to be replaced, and contain the oligonucleotides coding for alanine in place of the sequence coding for either the serine or histidine; b) molecular hybridization between the replacement oligonucleotides and the complementary single-strand DNA of the fusion-protein plasmid; c) enzymatic replication of the resulting double-stranded intermediate in vitro to produce both the mutant and "wild-type" (native, original) fusion-protein gene; and d) isolation of the sequence-replaced mutant DNA, and confirmation of its identity and structure by direct DNA sequence analysis. The two mutant DNAs and the original native DNA then were introduced separately into cultures of bacteria that were grown in parallel to overexpress the native or mutant silicatein proteins encoded by the recombinant DNAs. Controls verified that no silicatein (or silica-polymerizing activity) is produced from bacteria without the recombinant DNA. After lysis of the bacteria, the silicatein-MBP fusion proteins were purified by maltose-affinity chromatography.^[11] The silicatein proteins were released from the carrier MBP by site-specific cleavage with a highly purified proteolytic enzyme, and the released silicateins and MBP individually purified by conventional means. The

purified silicateins then were reconstituted to their native form by dialysis (at 2 °C) from buffered guanidinium hydrochloride and mercaptoethanol to permit proper refolding and formation of intramolecular disulfide bonds. The reconstituted proteins then were immediately adjusted to equivalent protein concentrations and assayed in triplicate for their catalysis of silica synthesis from tetraethoxysilane at neutral pH (1 h, 20 °C) under conditions described previously.^[3] The silica product was washed by centrifugation, dried by evaporation and quantified colorimetrically with the molybdate reagent after hydrolysis with alkali. Results are expressed as relative specific activities of alkoxy silane polycondensation after correction for the yield of the uncatalyzed reaction. Under these conditions, the average specific activity of the native reconstituted silicatein α protein was 140.0 ± 6.2 nmol silica synthesized per hour per 60 μ g protein; the control value obtained in the absence of any protein was 6.7 ± 2.1 nmol silica synthesized per hour.

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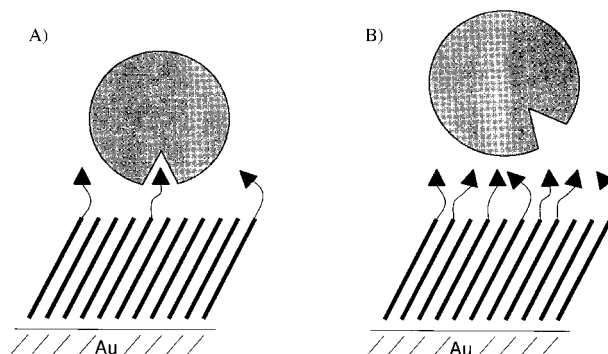
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The Role of Ligand Density in the Enzymatic Glycosylation of Carbohydrates Presented on Self-Assembled Monolayers of Alkanethiolates on Gold**

Benjamin T. Houseman and Milan Mrksich*

The enzymatic modification of immobilized carbohydrates is important in protein trafficking,^[1] viral and bacterial pathogenesis,^[2] and cell migration.^[3] Many model systems, including those that use polymers,^[4] dendrimers,^[5] and liposomes^[6] to present carbohydrates, have provided information on the interactions between proteins and immobilized sugars. These systems, however, present carbohydrates in a heterogeneous environment and offer little flexibility in tailoring the structure of groups surrounding the ligands. These limitations make mechanistic studies of enzymatic processes at interfaces difficult.

Self-assembled monolayers (SAMs) of alkanethiolates on gold are structurally well defined substrates that represent an excellent model system for studies in bio-interfacial science.^[7] Several reports have utilized SAMs to examine the interactions between immobilized ligands and proteins in solution. It is clear from much of this work that the accessibility of a ligand will influence the ability of a protein to bind it.^[8a–d] Ligands immobilized at higher densities,^[8e–f] for example, may have biological properties that are substantially different from those of the same ligand presented at a low density (Scheme 1). Here we report the use of mixed SAMs that present *N*-acetylglucosamine (GlcNAc) and tri(ethylene



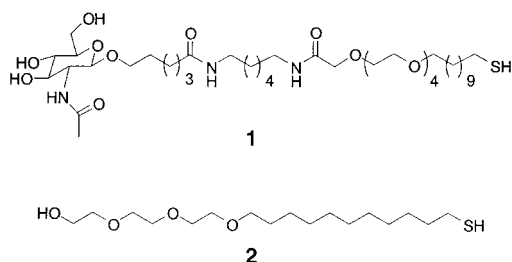
Scheme 1. Ligands immobilized at low density (A) should be more accessible for binding to protein than are ligands immobilized at high density (B).

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glycol) groups to demonstrate that the enzymatic activity of bovine β -1,4-galactosyltransferase (GalTase) depends on the density of immobilized carbohydrate.

We prepared substrates from alkanethiol **1**, terminated in a GlcNAc group, and a second alkanethiol **2**, terminated in the tri(ethylene glycol) group.^[9, 10] The carbohydrate is a substrate



for GalTase, while the tri(ethylene glycol) groups resist the nonspecific adsorption of protein to the model substrate.^[11] The density of GlcNAc at the interface is easily controlled by adjusting the ratio of alkanethiols in the solution from which the monolayer forms. In the presence of divalent manganese ions, GalTase transfers galactose from the donor substrate, uridine diphosphogalactose (UDP-Gal), to the 4-hydroxyl group of GlcNAc (Figure 1).^[12] To quantitate the transfer of galactose to the immobilized GlcNAc groups, buffer containing enzyme, [¹⁴C]UDP-Gal, UDP-Gal, and MnCl₂ was placed onto monolayers and kept for eight hours at 37°C. The substrates were then placed in aqueous solution, and the alkanethiolates were removed from the gold surface by UV

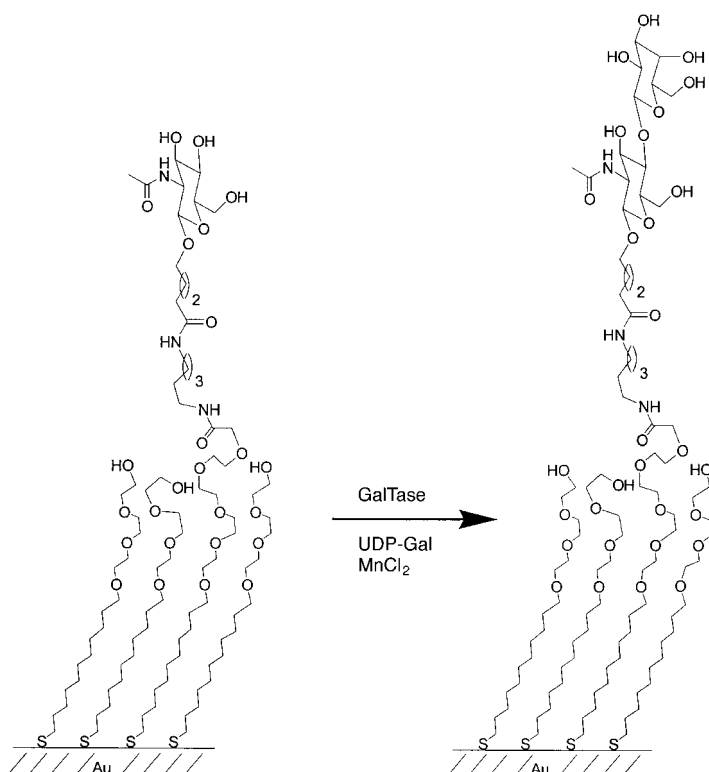


Figure 1. This work used monolayers presenting the carbohydrate *N*-acetylglucosamine mixed with tri(ethylene glycol) groups as a model substrate to determine the relationship between GalTase-mediated glycosylation of the sugar and the density of carbohydrate on the monolayer.

irradiation. The supernatant was analyzed with liquid scintillation counting. This protocol was repeated for monolayers presenting different densities of GlcNAc.

Figure 2 shows the relationship between the density $\chi_{\text{GlcNAc}}^{[13]}$ of GlcNAc on the monolayer and the incorporation of ¹⁴C-labeled galactose. Incorporation of radionuclide increased linearly with density of GlcNAc until $\chi_{\text{GlcNAc}} = 0.7$,

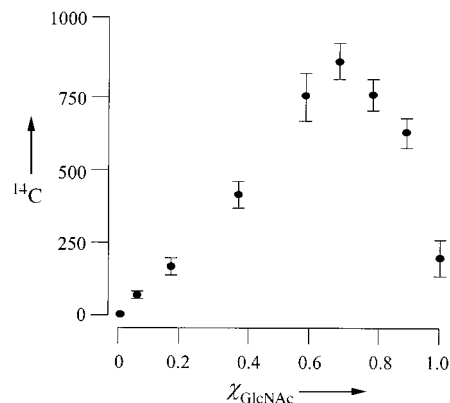
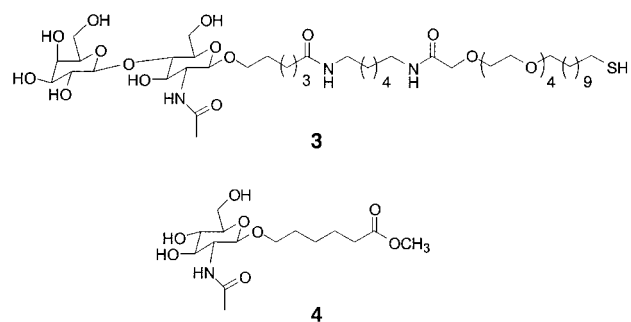


Figure 2. Relationship between χ_{GlcNAc} and the enzymatic incorporation of ¹⁴C from radiolabeled UDP-Gal. A solution (40 μ L) containing GalTase (25 mU), MnCl₂ (10 mM), UDP-Gal (0.4 nmol), and [¹⁴C]UDP-Gal (0.2 nmol) in HEPES buffer (50 mM, pH 7.5) was applied to monolayers having values of χ_{GlcNAc} ranging from 0 to 1.0. The reactions were allowed to proceed for eight hours at 37°C before the coverslips were rinsed with distilled water, dried under a stream of nitrogen, and placed in scintillation vials containing 750 μ L of distilled water. The monolayers were desorbed by exposure to UV light for 4 $\times$ 15 min, and the amount of ¹⁴C incorporation was measured with a liquid scintillation counter. Incorporation of radionuclide was normalized to the area of monolayer (values are reported in units of counts per cm²). Each data point represents the average of four to eight samples.

where it reached a maximum, and then decreased with larger values of χ_{GlcNAc} . Monolayers that presented carbohydrate alone ($\chi_{\text{GlcNAc}} = 1.0$) showed an extreme reduction in glycosylation; the amount of [¹⁴C]galactose on these monolayers was equal to that observed on monolayers with $\chi_{\text{GlcNAc}} = 0.2$. The low yield was not due to a slower rate of reaction since no further glycosylation was observed after the incubation of eight hours. These substrates did not suffer from nonspecific incorporation, since only background levels of [¹⁴C]galactose were detected on monolayers presenting tri(ethylene glycol) groups alone. To illustrate the specificity of the reaction, we subjected monolayers prepared from **2** and the alkanethiol **3**, terminated in a *N*-acetylglucosamine (LacNAc) group, to the reaction conditions and observed only background levels of



^{14}C incorporation. These data show clearly that steric crowding of carbohydrate ligands at the interface inhibits enzyme–substrate interactions in the model system described here; the effect became evident when χ_{GlcNAc} was greater than 0.7. It was not possible to determine whether the decrease in glycosylation resulted from crowding of unmodified ligands or from the influence of LacNAc groups present on a partially modified substrate.

We used surface plasmon resonance (SPR) spectroscopy to measure the association of two lectins having specificity for either GlcNAc or LacNAc with the monolayers. The *BS-II* lectin from *Bandeiraea simplicifolia* binds terminal, non-reducing GlcNAc groups ($K_d \approx 80\text{ nM}$),^[14] while the lectin from *Erythrina cristagalli* binds terminal LacNAc groups ($K_d \approx 400\text{ }\mu\text{M}$).^[15] SPR spectroscopy is an optical technique that measures the angle θ at which polarized light, reflected from the back of a gold-coated slide supporting a SAM, shows a minimum in intensity. The magnitude of this angle depends linearly on the change in refractive index—and therefore the mass of the protein—in the interfacial region.^[16, 17] The specific interaction of the lectins with the monolayers determines the amount of each carbohydrate present on the substrate.^[18] The high molecular weight of the lectins^[19] is beneficial as it provides a larger SPR response, and hence a more sensitive measurement, for the binding event.

Figure 3 A shows the SPR response curves for the binding of each lectin to a monolayer presenting GlcNAc ($\chi = 0.01$). The *B. simplicifolia* lectin ($1\text{ }\mu\text{M}$ in PBS) bound carbohydrate groups at the surface. When the protein solution was replaced with buffer, the amount of immobilized protein fell slowly. Since the lectin can bind cooperatively,^[19] much of the protein remained associated with the substrate. The addition of GlcNAc derivative **4** (1 mM in PBS) and subsequent treatment with buffer removed nearly all of the lectin from the monolayer.^[20] The lectin from *E. cristagalli* ($5\text{ }\mu\text{M}$ in PBS), by contrast, showed no association with GlcNAc groups on the monolayer.^[21]

The response curves in Figure 3 B show the association of each lectin with a monolayer presenting LacNAc groups ($\chi = 0.01$). As expected, the *E. cristagalli* lectin, but not the *BS-II* lectin, bound the disaccharide at the interface. The lectin dissociated upon introduction of soluble LacNAc (2 mM in PBS) and treatment with buffer.^[21] These experiments demonstrate that each lectin exhibits absolute specificity in binding its ligand.

We used SPR spectroscopy to characterize the yield of galactosylation for the enzymatic reaction. A monolayer presenting GlcNAc ($\chi = 0.01$) was treated for 15 min with buffer containing GalTase, MnCl_2 , and UDP-Gal. The solution was replaced with running buffer, and a solution of each lectin was introduced for 20 min to bind GlcNAc or LacNAc groups on the monolayer. Figure 4 A shows that the *E. cristagalli* lectin, but not the *BS-II* lectin, associated with the monolayer and provides clear evidence that the yield of this transformation was essentially quantitative. To characterize the reaction further and to demonstrate that this approach could provide kinetic information, we used the *BS-II* lectin to examine glycosylation after exposure to the enzyme for different amounts of time. A lower concentration of enzyme

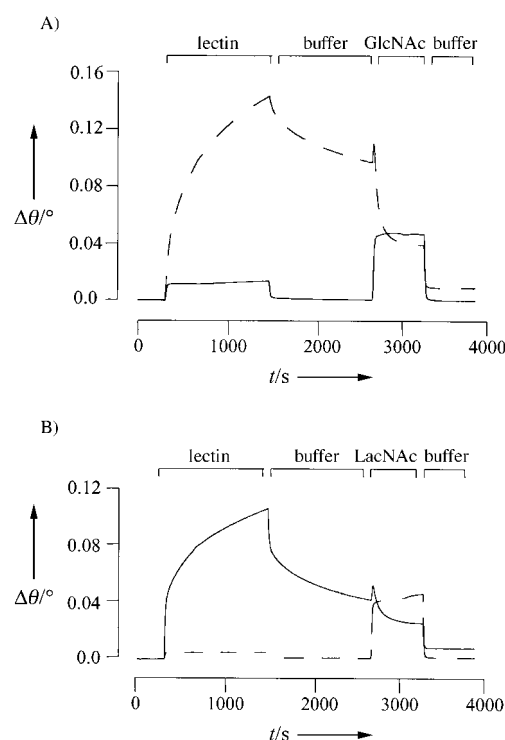


Figure 3. Data from SPR investigations for the binding of *Bandeiraea simplicifolia* *BS-II* lectin ($1\text{ }\mu\text{M}$ in PBS, dashed curve) and *Erythrina cristagalli* lectin ($5\text{ }\mu\text{M}$ in PBS, solid curve) to monolayers presenting A) 1% GlcNAc or B) 1% LacNAc among tri(ethylene glycol) groups. A solution of lectin in PBS was allowed to flow over the monolayers for 20 min, replaced with buffer for 20 min, and followed by a solution of soluble carbohydrate ligand (1 mM **4** or 2 mM LacNAc in PBS) for 10 min. The *BS-II* lectin bound to monolayers presenting GlcNAc groups, while the *E. cristagalli* lectin bound only to monolayers presenting LacNAc moieties. Binding of both lectins could be competitively inhibited by the introduction of soluble carbohydrate ligand.

was used in these experiments to slow the rate of reaction and permit observation by SPR spectroscopy. Figure 4 B shows that a time-dependent decrease in the binding of the *B. simplicifolia* lectin occurs. After 30 min the binding of lectin decreased to background levels because all GlcNAc groups had been modified by the enzyme. No decrease in lectin binding was observed when either MnCl_2 or UDP-Gal was omitted from the reaction mixture. These data show how the combination of SPR spectroscopy and carbohydrate-specific lectins can be used to provide kinetic information for enzymatic modification of immobilized substrates.

This work provides a clear demonstration of the extent to which the density of an immobilized ligand can affect its biological properties. This effect must be considered in model systems that present biologically active ligands at high density. This report also highlights several characteristics of SAMs that make them the best available class of model substrates for studies in bio-interfacial science: They are inert to the nonspecific adsorption of protein; the density of ligand at the interface can be controlled; SPR spectroscopy can be used to measure the association of proteins with immobilized ligands; and the structure and environment of ligands at the interface can be tailored through synthetic organic chemistry. The recognition and glycosylation of immobilized GlcNAc by GalTase has a special relevance to bio-interfacial phenomena,

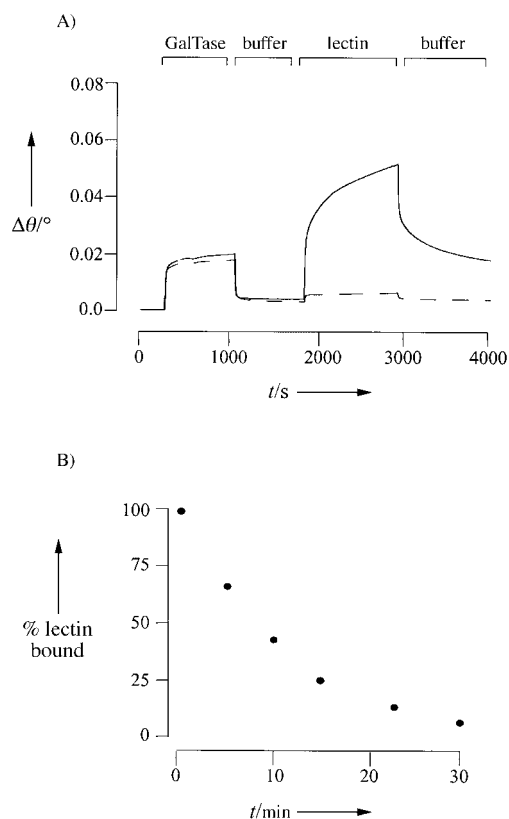


Figure 4. A) The *E. cristagalli* lectin, but not the *BS-II* lectin, binds LacNAc groups resulting from glycosylation of immobilized GlcNAc by GalTase. Monolayers were mounted in the SPR flow cell and treated with buffer containing GalTase (15 μ M in PBS), UDP-Gal (0.4 nmol), and MnCl₂ (10 mM) for 15 min. The reaction mixture was replaced with running buffer for 15 min, and a solution of each lectin (*E. cristagalli* = solid curve; *BS-II* = dashed curve) was introduced to determine the presence of GlcNAc or LacNAc on the monolayer. Quantitative conversion of GlcNAc groups into LacNAc groups was observed. B) Plot of *BS-II* lectin binding versus time. Separate monolayers were treated with the enzyme cocktail (1.75 μ M GalTase) in the SPR flow cell for different amounts of time, and binding of the lectin to the modified surfaces was recorded. All data are reported as a percentage of the binding response observed between the lectin and an untreated monolayer presenting GlcNAc ($\chi = 0.01$).

as it is a key determinant of the migration of cells on laminin matrices.^[3a] We believe that the model system described here will be important for elucidating mechanistic details of this migration process.

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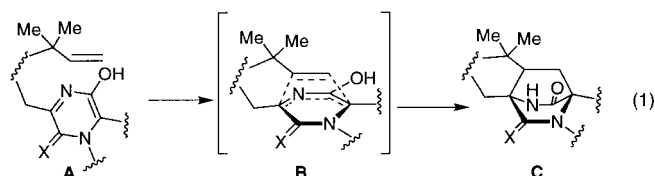
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- [20] The small degree of irreversible binding present after the replacement of soluble carbohydrate with running buffer probably resulted from the nonspecific adsorption of protein to defect sites in the monolayer.
- [21] The changes in $\Delta\theta$ shown in the figure result from an increase in the refractive index of the lectin solution and the carbohydrate solution relative to the buffer; the same changes in $\Delta\theta$ were observed on surfaces presenting tri(ethylene glycol) groups alone.

Reverse versus Normal Prenyl Transferases in Paraherquamide Biosynthesis Exhibit Distinct Facial Selectivities**

Emily M. Stocking, Juan F. Sanz-Cervera,* and Robert M. Williams*

The paraherquamides (**1–10**)^[1] are a group of fungal metabolites that, together with sclerotamide (**11**),^[2] marcfortine (**12**),^[3] asperparaline A (**14**, also aspergillimides, **15**),^[4] and the brevianamides (**16**, brevianamide A; **17**, brevianamide B),^[5] have recently attracted much attention due to the range of interesting biological activities that this family displays, including anthelmintic, paralytic, and insecticidal activities.^[1–5] These substances are the consequence of mixed biogenetic origins, being derived from the oxidative polycyclization of amino acids and isoprene units. Most interesting in this regard

is the emerging body of evidence that supports the notion that the common bicyclo[2.2.2] core structural motif is formed by a biosynthetic intramolecular [4+2] cycloaddition of the isoprene-derived olefin across an azadiene moiety derived from a pre-formed, oxidized piperazinedione [**A** → **B** → **C**, Eq. (1)].^[6, 7]



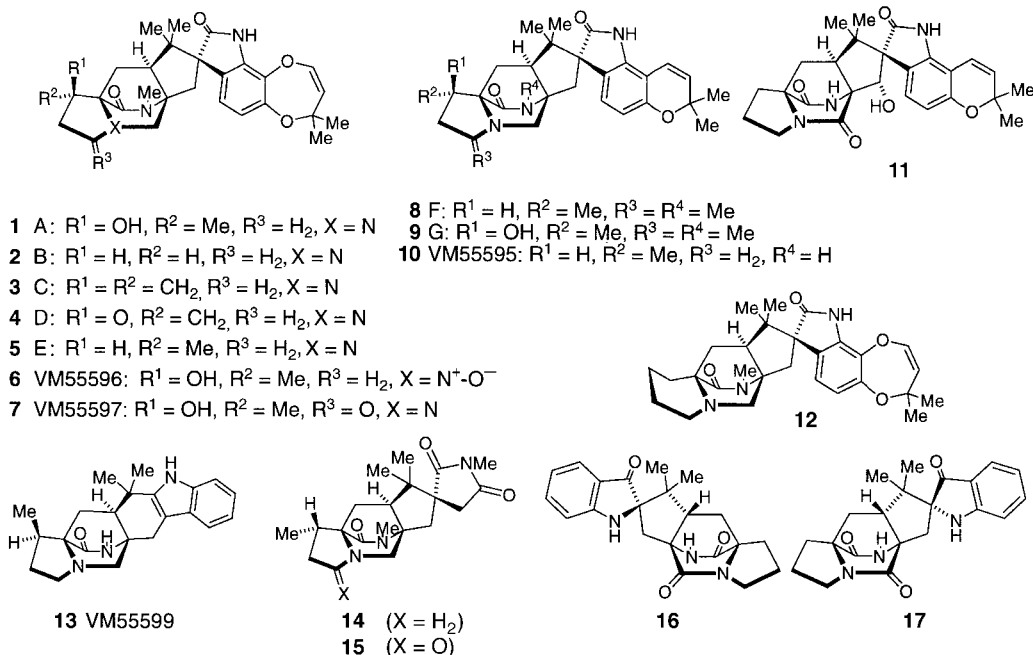
Although [4+2] cycloadditions are used extensively in synthetic organic chemistry, such pericyclic reactions are quite rare in Nature, and in only a few cases has experimental evidence been obtained to support the intermediacy of a

Diels–Alder type of cycloaddition.^[7, 8] Our research efforts have thus focused on the biogenesis of these compounds, with particular emphasis on the key cycloaddition step.

In this family of metabolites, the dimethylallyl group involved in forming the bicyclo[2.2.2] nucleus is apparently introduced by reaction of dimethylallyl pyrophosphate (DMAPP) with the tryptophan unit, resulting in a net *reverse* prenylation at the 2-position of the indole ring. In metabolites **1–12**, there is a second isoprene fragment that is oxidatively added to a phenolic hydroxy group of the tryptophan core. Until recently, it would have

been safe to assume that the isoprene moieties are biosynthesized by the well-known mevalonic acid pathway. However, in the last few years several reports have clearly shown that in some organisms isoprenoids are not formed through this pathway, but rather through the 1-deoxy-D-xylulose pathway. This has been shown not only in bacteria,^[9] but also in the green algae *Scenedesmus obliquus*^[10] and, somewhat surprisingly, in higher plants like *Taxus chinensis*,^[11] barley (*Hordeum vulgare* L.), duckweed (*Lemna gibba* L.), carrot (*Daucus carota* L.),^[12] and *Ginkgo biloba*.^[13] In these cases, it has been shown that the biosynthesis of several cytoplasmic sterols proceeds by the acetate/mevalonate pathway, while the plastidic isoprenoids are synthesized by the new 1-deoxy-D-xylulose pathway.

In the course of isotopic labeling studies aimed at examining the origin of the isoprene units in the paraherquamide structure, we discovered an unexpected stereochemical distribution of the methyl groups derived from DMAPP. We



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carried out feeding experiments with $[U-^{13}C_6]$ -glucose and $[^{13}C_2]$ -acetate which aid in distinguishing between the two pathways using ^{13}C NMR spectroscopy since all the resonances from the ^{13}C NMR spectrum of paraherquamide A have been unequivocally assigned (with the exception of C27 and C28).^[1d] The different labeling patterns to be expected from these two pathways are shown in Figure 1.

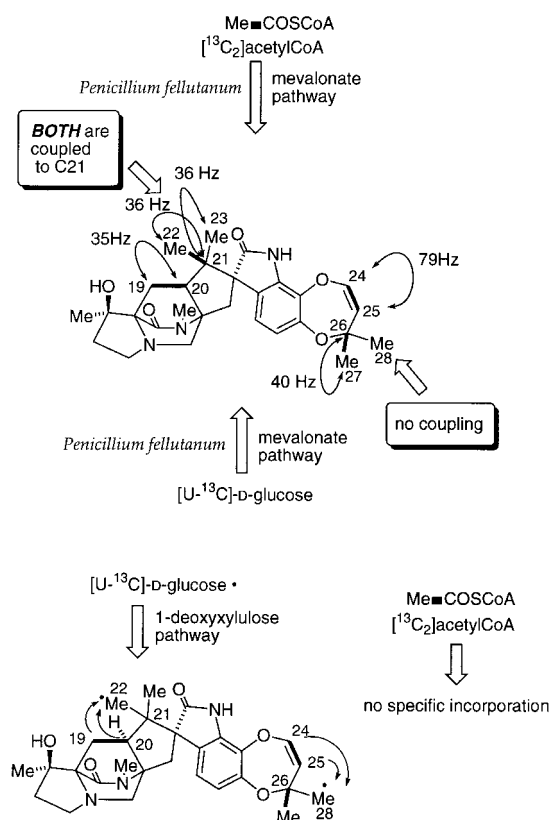


Figure 1. ^{13}C labeling in paraherquamide A corresponding to a feeding experiment with $[U-^{13}C_6]$ -D-glucose and $[^{13}C_2]$ -acetate by the mevalonate (top) and 1-deoxy-D-xylulose (bottom) pathways. Thick lines represent intact acetate units and arrows represent couplings expected and observed in the ^{13}C NMR spectrum.

An initial feeding experiment was carried out on *Penicillium fellutanum* with $[U-^{13}C_6]$ -glucose; harvesting the resulting paraherquamide A provided a labeling pattern in which most signals appeared split around the singlet corresponding to the uncoupled carbon atom, as one would expect from the catabolism of glucose. In this case we focused our attention on the carbon atoms that form the two C_5 units: C19 to C23, and C24 to C28. The ^{13}C NMR spectrum of paraherquamide A^[1d] isolated from a control experiment was used for comparison purposes. Although the specific incorporation was low, the couplings are clearly visible in the ^{13}C NMR spectrum of the resulting paraherquamide A (Table 1).

In order to confirm that acetate, and not 1-deoxy-D-xylulose-5-phosphate, is the key intermediate in the biosynthesis of the two C_5 units, a second feeding experiment was carried out, this time using $[^{13}C_2]$ -acetate. Specific incorporation of intact C_2 units was observed, in agreement with the mevalonic acid pathway (Figure 1). Regarding the carbon

Table 1. Specific incorporations, chemical shifts, and coupling constants for the C_5 carbon atoms of paraherquamide A in the feeding experiment with $[U-^{13}C_6]$ -glucose.

C	δ	$J_{C,C}$ [Hz]	% ^{13}C in each position	% ^{13}C specifically incorpo- rated as intact C_2 units in each position	% ^{13}C in the $[U-^{13}C_6]$ glu- cose used, specifical- ly incorporated in each position
19	22.17	34	1.4	41	0.0075
20	51.42	34	1.9	32	0.0052
21	46.40	36	1.5	36	0.0061
22	20.47	36	1.8	14	0.0018
23	23.71	36	1.9	21	0.0029
24	138.94	81	2.3	35	0.0059
25	115.05	79	2.0	37	0.0065
26	79.81	40	2.0	37	0.0064
27	29.93	40	2.2	40	0.0075
28	29.80	–	1.7	0	0.0037

atoms that form the two C_5 units, C19 to C23, and C24 to C28, the results of the feeding experiment with $[^{13}C_2]$ -acetate were essentially the same as those with $[U-^{13}C]$ -glucose. In both cases, the signal for C-28 at $\delta = 29.80$ showed enhancement with respect to the control spectrum, but no splitting. In the first C_5 fragment, the observed couplings mean that C19 is coupled to C20, while C21 is coupled to C22 or C23, but not to both simultaneously. For the second C_5 unit, the coupling constants show that C24 and C25 are coupled, while C26 is coupled to C27. In this case, C28 shows no coupling.

The non-mevalonic pathway would cause incorporation of $[U-^{13}C_6]$ -glucose in such a way that a long-range coupling would be observed between C19 and one of the methyl groups (C22 or C23) as well as C20 and the same methyl group (C22 or C23), due to the fact that in this case three carbon atoms would come from one glucose molecule, while the other two would come from a different one. However, in our feeding experiment with $[U-^{13}C_6]$ -glucose no long-range coupling between C22 and C19/C20 or C28 and C24/C25 was observed. This result is consistent with the mevalonic acid pathway, in which two pairs of carbon atoms come from two glucose molecules, while the third carbon atom comes from a third glucose molecule, thus precluding long-range couplings. The lack of observed long-range couplings, together with the observed incorporation of $[1,2-^{13}C_2]$ -acetate thus indicate that the operative pathway for the formation of both C_5 units is the mevalonic pathway, as the labeling pattern observed is consistent with that expected for such a mechanism (Figure 1). The electrospray ionization mass spectra of the isolated **1** from both feeding experiments agree with the incorporation of intact C_2 units from acetate.^[14]

It is significant that in the C_5 fragment formed by C24 to C28, C28 shows no coupling with C26, while C27 does. This means that the methyl groups in DMAPP are not equivalent in the biosynthesis of this metabolite. In contrast, in the other C_5 fragment, formed by C19 to C23, both methyl groups show coupling with C20, although not simultaneously. This unexpected result must be interpreted to mean that the *reverse* prenyl transferase presents the olefinic π system of DMAPP in a manner in which both faces of the π system are susceptible to attack by the 2-position of the indole moiety.

The simplest explanation is to invoke binding of the DMAPP in an "upside down" orientation relative to "normal" prenyl transferases which permits a facially nonselective S_N' attack on the π system (Figure 2). In this situation, the pyrophosphate group is likely anchored in the enzyme active site with the hydrophobic isopropenyl moiety being presented in a conformationally flexible ($A \rightleftharpoons B$) disposition with respect to the tryptophan-derived substrate. This is in contrast to the normal mode of prenyl transfer where, the nucleophilic displacement at the pyrophosphate-bearing methylene carbon occurs with inversion of configuration at carbon with the hydrophobic tail of DMAPP buried in the enzyme active site.^[15]

In contrast, the methyl groups in the other C_5 unit (dioxepin moiety) are clearly differentiated; therefore, it is quite likely that this C_5 group (C24 to C28) is introduced in the molecule by direct alkylation with DMAPP by a normal prenyl transferase followed by a stereospecific net oxidative addition to the olefinic system. A plausible mechanism, of several possibilities, for the formation of this ring system, is by face-selective epoxidation followed by ring-opening and dehydration. Significantly, since this isoprene unit is introduced without loss of stereochemical integrity, Ockham's razor can be invoked for there not being a mechanism for scrambling the DMAPP via a dimethyl vinyl carbinol-type intermediate which would necessarily provide stereochemically scrambled isoprene equivalents to the cell's cytosolic pool of DMAPP.

The results of these experiments clearly indicate that the C_5 units in **1** are introduced in stereofacially distinct manners. Since it has been established that prenylation of the indole moiety in the biosynthesis of the related brevianamides occurs in a fashion analogous to that postulated for intermediates **19**^[7d] (Figure 2) and that this is also a reasonable expectation in the paraherquamide biosynthesis, the prenyl transferase that installs this C_5 unit must display DMAPP to the 2-position of the indole in a π facially indiscriminate manner. The work described herein demonstrates the first case where

both a non face-selective and a face-selective addition to the trisubstituted olefinic portion of a DMAPP-derived moiety has occurred within the same molecule.^[16]

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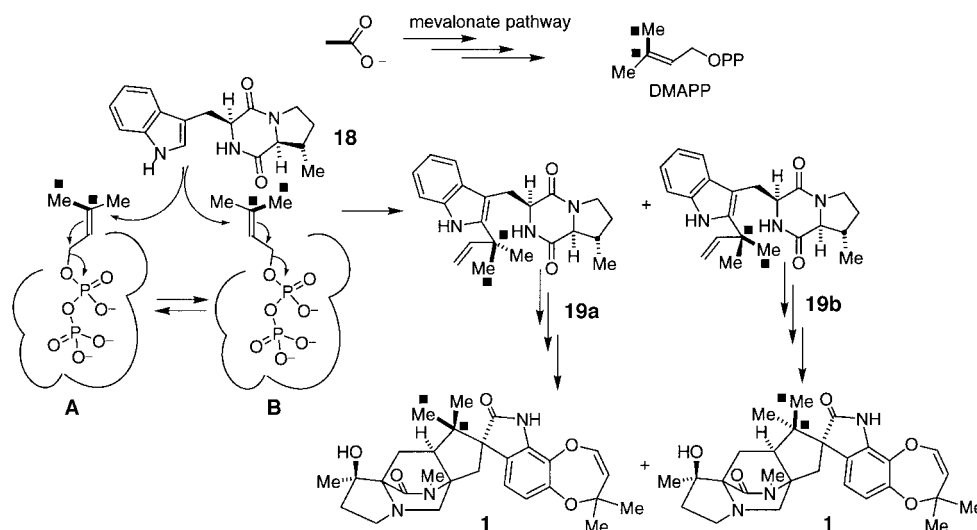


Figure 2. A possible biosynthetic sequence that may explain why C22 and C23 are rendered equivalent in the biosynthesis of paraherquamide A. Thick bonds labeled with ■ represent one intact C2 unit from acetate, incorporated in C3/C5 of individual DMAPP molecules, and in C21, C22, and C23 of **1**. For the sake of clarity and simplicity, the labels that would appear in other positions are not represented (see Figure 1).

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A New Samarium Diiodide Induced Reaction: Intramolecular Attack of Ketyl Radical Anions on Aryl Substituents with Formation of 1,4-Cyclohexadiene Derivatives**

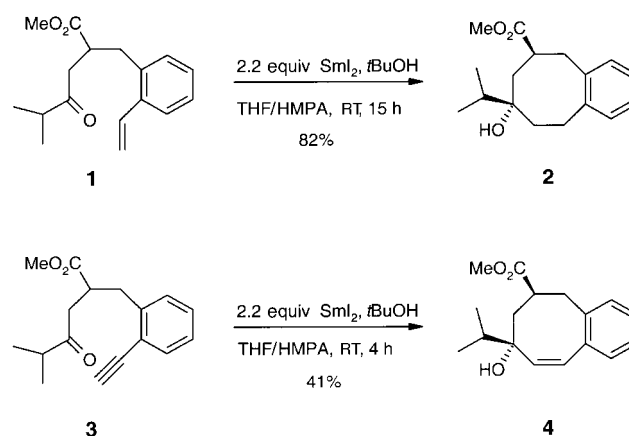
Chimmanamada U. Dinesh and Hans-Ulrich Reissig*

Dedicated to Professor Edward Piers on the occasion of his 60th birthday

Samarium diiodide was introduced by Kagan et al.^[1] as a selective one electron transfer reagent in organic chemistry. Several variants of SmI_2 -induced cyclization reactions^[2] have attained synthetic importance owing to their high stereoselectivity and ability to undergo sequential reactions.^[3] Frequently, ketyl radical anions generated by the electron transfer are the reactive species which add to a multiple bond offered at an appropriate distance. We now report that these ketyl radical anions can attack an aryl moiety in an intramolecular fashion and, after a second electron transfer, lead

to 1,4-cyclohexadiene derivatives. This reaction has not yet been observed in samarium chemistry to the best of our knowledge, and it should gain considerable synthetic importance owing to its high diastereoselectivity.

Motivated by the examples reported by Molander and McKie,^[4] we recently developed a synthesis of benzannulated cyclooctane derivatives.^[5] Our previously unpublished example **1**→**2** (HMPA = hexamethyl phosphoramide) demonstrates that this samarium diiodide promoted transformation proceeds with high diastereoselectivity and in very good yield in spite of the sterically demanding isopropyl group. To explore further mechanistic details of the 8-*endo* cyclization and to obtain new options for functionalizations of the newly generated eight-membered ring, alkyne derivatives such as **3** were subjected to the general reaction conditions. The expected benzannulated cyclooctene **4** was isolated in 41 % yield as a single diastereomer. To our knowledge this is the first successful 8-*endo-dig* cyclization of a samarium ketyl.^[6]

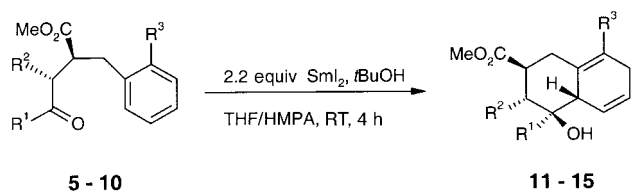


Since they were more readily accessible,^[7] the disubstituted alkynes **5**–**7** were first subjected to samarium diiodide cyclization conditions. However, we obtained products derived from neither an 8-*endo-dig* cyclization nor from the more likely 7-*exo-dig*-reaction, but surprisingly cyclohexadienes **11**–**13** which were formed by attack on aryl substituents. Examples **8** and **9** demonstrate that the alkyne units are not required for the cyclization, as samarium diiodide now affords cyclization products **14** and **15** or the lactone **16**. The cyclohexanone moiety in **6**–**8** allows smooth cyclization;^[8] however, the steric hindrance in isopropyl derivative **10** seems to be too high. Under the conditions applied mainly starting material was recovered and no product was formed. The fact that **1** and **3** were transformed into bicyclic **2** and **4**, and that the precursor **10** was essentially inert, supports the reversibility of the first electron transfer to the carbonyl group. The subsequent steps are responsible for the productivity of the sequence.

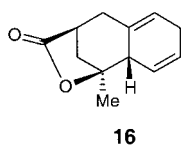
Particularly remarkable is the high diastereoselectivity of the reaction, since in all examples only one diastereomer could be detected. The relative configuration at the two newly generated asymmetric centers of **14** was proven by X-ray analysis.^[9] This showed not only the *cis* arrangement of the bridgehead hydrogen atom with respect to the hydroxyl group, but also the location of R^2 and of the methoxycarbonyl

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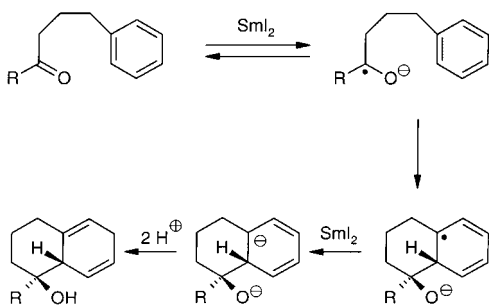


5 , R ¹ = Me, R ² = H, R ³ = $\equiv$ —SiMe ₃	11 , 50%
6 , R ¹ , R ² = -(CH ₂) ₄ -, R ³ = $\equiv$ —SiMe ₃	12 , 88%
7 , R ¹ , R ² = -(CH ₂) ₄ -, R ³ = $\equiv$ —Ph	13 , 91%
8 , R ¹ , R ² = -(CH ₂) ₄ -, R ³ = CN	14 , 60%
9 , R ¹ = Me, R ² = R ³ = H	15 , 24%, + 16 , 36%
10 , R ¹ = <i>i</i> Pr, R ² = H, R ³ = $\equiv$ —SiMe ₃	---



substituent. Of course, it also proved the constitution of compound **14** and the suggested connectivity pattern. We assume that the products **12** and **13** have the same configuration and extend this also to **11**, **15**, and the γ -lactone **16** derived from **15**.

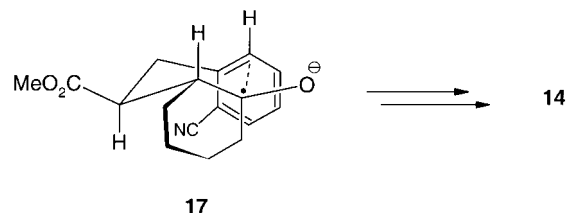
The following mechanism seems likely to us (Scheme 1). As expected for samarium diiodide promoted cyclizations a first (reversible) electron transfer generates a ketyl radical anion,^[2, 3] which now attacks the aryl group in the *ortho* position.



Scheme 1. Proposed mechanism for the SmI₂-promoted cyclization of aryl ketones.

The resulting cyclohexadienyl radical is reduced to a cyclohexadienyl anion by a second electron transfer, and the anion is finally protonated in analogy to the Birch reduction.^[10] It is not clear at the moment whether other steps of this sequence are reversible and whether a direct 6-cyclization (as formulated) occurs or a 5-cyclization to a spiro compound that is followed by a 1,2-shift. This should lead to two regioisomeric products since a priori two C—C bonds are able to undergo the 1,2-migration. Since only one product was obtained, this advocates against this (more complex) mechanism though it does not disprove it.

The configuration of the products is determined in the cyclization step. Thus, for the formation of tricyclic compound **14** we discuss the reactive conformation **17** for the ketyl radical anion. A chairlike folding, as suggested for most of the

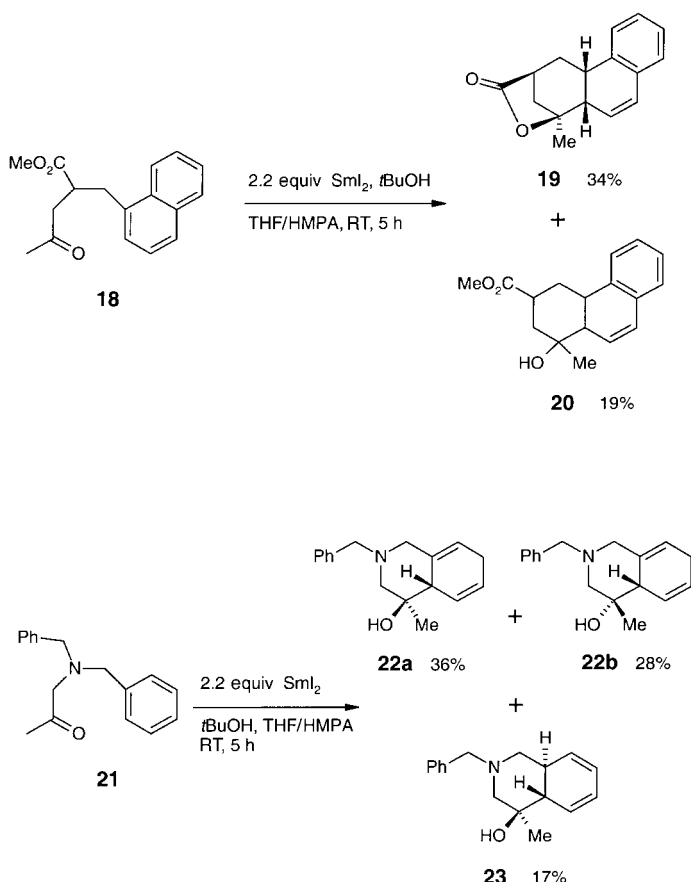


intramolecular additions to C—C double bonds,^[2] with substituents and the ketyl oxygen atom in equatorial positions is considerably favored over conceivable alternatives (e.g., the oxygen atom in an axial position). This model plausibly explains why cyclization of **10** is unsuccessful: The isopropyl group is too bulky to allow the aryl group to occupy a position at a proper distance. This picture illustrates why the second diastereomer of **8** did not react. The carbonyl group should be located equatorial-“exocyclic” in a corresponding conformation, and therefore it could not approach the aryl group.

It must be mentioned here that a related electrochemical reaction of γ -aryl ketones leads to the same type of products with identical relative configuration.^[11] Similar conformational effects have been suggested for the observed stereochemistry. However, for aryl groups with electron-withdrawing substituents and for condensed aromatic compounds the reaction does not stop at the 1,4-cyclohexadiene stage, but further reduction provides cyclohexene and cyclohexane derivatives. The cathodic cyclization of pyridinium salts to quinolizidine and indolizidine derivatives, as reported by Schäfer et al.,^[12] also involves subsequent reduction. In contrast, our samarium diiodide promoted cyclization seems to be redox selective. The examples investigated showed no sign of this type of subsequent reduction, which was most likely to occur during the transformation of **8** to **14**. Related to our experiments is the cyclization of samarium ketyls with arenetricarbonylchromium complexes, as described by Schmalz et al.^[13] Here either a demetalated 1,3-cyclohexadiene derivative is formed or substituted arene(CO)₃ complexes are generated under formal displacement of a methoxy group.

The new reaction mode of samarium ketyls reported here should be of importance for various aspects: First, precursors incorporating aryl substituents, which should serve for other types of cyclization reactions, may react with the involvement of the aromatic group. Second, this reaction should have a high synthetic potential since 1,4-cyclohexadiene derivatives formed by this reductive alkylation may be interesting for many subsequent reactions. Thus, we are currently investigating the scope and limitations of this transformation and relevant mechanistic details. Of particular importance will be the substitution of HMPA by less dangerous additives.^[14] The potential of this new reaction for the synthesis of carbocycles and heterocycles is demonstrated for example **18** with a naphthyl group as ketyl acceptor to give the tetracyclic compound **19** (characterized by X-ray analysis^[9]) and a

second compound **20** (configuration not yet defined), and for the reaction of *N,N*-dibenzyl-substituted amino ketone **21**, which produces isoquinoline derivatives **22** and **23**.



Experimental Section

In a typical experiment, a solution of 1,2-diiodoethane (0.270 g, 0.96 mmol) in anhydrous THF (20 mL) was added to samarium (0.160 g, 1.05 mmol) under an argon atmosphere. The reaction mixture was stirred for 1.5 h at room temperature, and the resulting deep blue solution was treated with HMPA (1.410 g, 7.88 mmol). Stirring was continued for 10 min, and argon was bubbled through the reaction mixture for a further 10 min. To the resulting solution was added a mixture of **8** (0.130 g, 0.44 mmol) and tBuOH (0.065 g, 0.87 mmol) in anhydrous THF (10 mL) over a period of 30 min. The mixture was then stirred for 4.5 h at room temperature, and a saturated solution (10 mL) of sodium bicarbonate was added. The organic layer was separated, the aqueous phase was extracted with diethyl ether (3 $\times$ 10 mL), and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4 . Evaporation of the solvent and column chromatography of the crude product on silica gel (hexane/ethyl acetate 3/1) afforded **14** as colorless crystals (0.075 g, 60%). M.p. 151–153 °C; ^1H NMR (CDCl_3): δ = 5.83 (s, 2H; HC=CH), 3.72 (s, 3H; OCH_3), 3.99 (dd, J = 4.1, 13.5 Hz, 1H; CH_2), 2.89 (s, 3H; CH, CH_2), 2.79 (td, J = 4.0, 12.4 Hz, 1H; CH), 2.34 (t, J = 13.5 Hz, 1H; CH_2), 1.97–1.88 (m, 1H; CH), 1.56–1.43 (m, 7H; 3 CH_2 , OH), 1.38–1.30 (m, 2H; CH_2); ^{13}C NMR (CDCl_3): δ = 174.2 (s, C=O), 150.9 (s, =C), 123.5, 122.4 (2d, HC=CH), 117.8 (s, =C), 104.7 (s, C=N), 75.0 (s, C-OH), 52.0 (q, OCH_3), 50.0, 44.8, 43.3 (3d, 3CH), 36.0, 28.4, 28.0, 23.9, 20.9, 19.6 (6t, 6 CH_2); IR (KBr): $\tilde{\nu}$ = 3515 (br, O-H), 2940–2860 (C-H, =C-H), 2215 (C=N), 1715 (C=O), 1640 cm^{-1} (C=C); elemental analysis calcd for $\text{C}_{17}\text{H}_{21}\text{NO}_3$ (287.4): C 71.05, H 7.36, N 4.87; found: C 71.10, H 7.58, N 4.85.

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Discovery and Optimization of Heterogeneous Catalysts by Using Combinatorial Chemistry**

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Solid catalysts are used in the manufacturing of a vast array of chemicals and fuels, and as such significantly contribute to our economy and high living standards.^[1] In addition, catalysts provide important environmental benefits, such as in catalytic converters for automobiles. However, in spite of their significance and broad utility, the discovery of new catalysts continues to be an arduous and rather unpredictable trial-and-error process. Catalysts traditionally are developed by using a large variety of tedious, time-consuming, and often one at a time methods, characterized and tested for activity, modified,

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characterized and retested until no further improvements are justified. In addition, these investigations have often been conducted independently by different groups, rendering the comparison of activity information difficult. This approach, although time consuming, has been employed successfully for the discovery of a significant number of solid-state catalysts over many decades.^[2] However, there remains a large unexplored universe of binary, ternary, quaternary, and higher order solid-state materials and other complex metal compounds that can have superior catalytic properties. Conventional approaches clearly are inadequate to rapidly synthesize and then screen this vast universe of catalytic materials; therefore, the development of new approaches that will accelerate the process of discovery, optimization, and understanding of heterogeneous catalysts will have a significant impact in catalysis science and engineering.

Combinatorial chemistry, in which a large diversity of chemical compounds are rapidly produced and processed, and the resulting libraries of compounds are then screened for desirable properties to identify lead compounds in a high-throughput fashion, is a particularly attractive approach for the discovery and optimization of heterogeneous catalysts. At present two primary methods exist for the preparation of solid-state libraries: 1) synthesis based on thin film deposition^[3–6] and 2) synthesis using solution-based technologies.^[7, 8] These methods were then used for the discovery of superconducting,^[3] magnetoresistive,^[4] and luminescent^[5–7, 9] materials, and electrochemical catalysts.^[8]

The discovery of heterogeneous catalysts is a great challenge because unlike superconductivity, magnetoresistivity, and electrochemical reactions, which can be tested by contact probes, or non-specific luminescence/fluorescence, the screening of heterogeneous catalysts requires the unambiguous detection of a specific molecule (i.e., a product) in the vicinity of small catalyst sites. To date three methods have been proposed to screen catalyst libraries: 1) in situ optical screening using infrared (IR) thermography^[10, 11] or fluorescence acid–base indicator,^[8] 2) microprobe sampling and scanning mass spectrometry (MS),^[12] and 3) in situ resonance-enhanced multiphoton ionization (REMPI) spectroscopy with microelectrode detection.^[13] The optical methods, although they provide high-speed data on activity, generally provide limited information on selectivity or different reaction products. The mass spectrometric investigation using microprobes involves the sampling and transfer of very small quantities of gases containing product species that are also at low concentrations. In addition, the sampling probe must physically be moved from site to site to screen the entire library. The REMPI technique combines the speed of optical methods and the selectivity of mass spectrometry and allows the high-speed screening of large solid-state catalyst libraries for activity and selectivity without the need to remove samples.

We report herein on the application of REMPI together with novel array microreactors for the discovery of an optimal composition in the ternary Pt/Pd/In metal catalyst system for the dehydrogenation of cyclohexane to benzene. Array microreactors allow the screening of a large number of catalysts in parallel and under identical (standard) operating

conditions. Consequently, measurements become primarily sensitive to the method of preparation of the catalysts. This represents a significant advance over the current practice in catalyst research, in which different catalysts are often studied under different operating conditions, rendering comparisons difficult.

A sketch of the array microreactor system developed is shown in Figure 1. The reactor array consisted of 17 rectangular channels that were micromachined on a flat nonporous silica ceramic slab ($7.5 \times 3.75 \times 0.63 \text{ cm}^3$). Each channel was

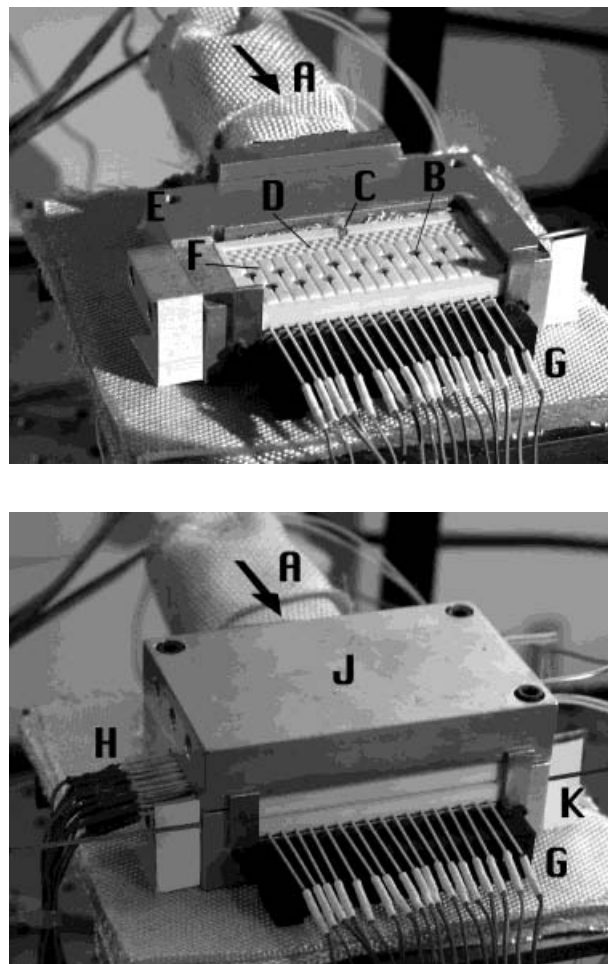


Figure 1. Array microreactor system: Internal details (top); entire reactor system (bottom): A: feed gas preheater; B: catalyst pellets; C: reactant gas inlet; D: flow distribution baffles; E: bottom aluminum heating block; F: reactor channels; G: signal detection microelectrodes; H: power supply; J: top aluminum heating block; K: laser beam.

0.1 cm wide, 0.1 cm deep, and 2 cm long, and possessed a cylindrical well (0.3 cm diameter by 0.2 cm deep) to hold cylindrical catalyst pellets (0.3 cm diameter and 0.1 cm high). In this configuration, the flat surface of the catalyst pellet, when placed into the well, would be flush with the bottom of the reactor channel. A similar flat ceramic slab was used to cover the microreactor array slab and to form isolated channels. This design results in unobstructed gas flow rates through the channels with only wall skin friction providing flow resistance. Consequently, the establishment of nearly identical flow rates in each channel, and therefore identical

contact times between the reactant gases and the catalyst pellets in each microreactor were feasible. The flat and rigid nature of the ceramic slabs also result in the formation of a good gas seal, thereby isolating each channel from one another under a broad range of temperatures.

Ceramic slabs were also machined for optical access and the placement of dedicated microelectrodes as shown in Figure 1. The reactor array was cradled between two aluminum metal heating blocks. In addition, a separate heating block was used to preheat the feed gases to the desired reaction temperature. The temperature of each heating block was independently regulated by using electrical heating elements embedded into the aluminum body and PID controllers (Omega, Stamford, CT). Gas temperatures in the vicinity of the catalyst sites were separately monitored by small thermocouples inserted from the reactor exit to assess their uniformity across the microreactor library, and to establish the time periods for heat up and cool down. These studies revealed the presence of a nearly uniform temperature, within 1 °C, across the entire catalyst library. In addition, the reactor array was shown to heat up and/or cool down to a desired temperature within 30 min of the set point change.

The reactant gases were introduced into the microreactor array through a quartz feed tube and a series of flow distribution baffles that were also micromachined to the bottom ceramic slab (Figure 1). The baffles were used to establish uniform gas flow rates through each channel. The feed stream composition was about 10% cyclohexane in helium carrier gas; this mixture was prepared by bubbling helium gas (1000 cm³ min⁻¹) through liquid cyclohexane in a sparger at about 25 °C. These conditions correspond to a nominal contact time of about 0.04 s to cross the flat surface of the 0.3 cm diameter catalyst pellet in each microreactor. The uniformity of gas flow rates in each channel was checked by passing smoke through the reactor array and by measuring the lengths of the smoke plume jets emanated from each channel. These studies indicated that gas flow rates were approximately the same in each channel.

The supported multimetallic catalyst formulations were prepared by using the conventional co-impregnation method, in which porous alumina pellets were immersed into the aqueous solutions of catalyst precursors at different concentrations. Pellets were prepared by compacting 30 mg of high surface area γ -Al₂O₃ powder (Alfa Aesar, Ward Hill MA, 150 m² g⁻¹) into a cylindrical shape (0.3 cm diameter and 0.1 cm height) using a die. These pellet dimensions allow for easy scale up of newly discovered and/or optimized catalysts for commercial applications. This is in contrast to much smaller scale microreactors proposed by other investigators,^[14] which can possess significant scale up problems. Metal precursor stock solutions of Pt, Pd, and In were prepared from high purity H₂PtCl₆, PdCl₂, InCl₂, and aqueous HCl (Alfa Aesar, Ward Hill MA). The catalyst precursor solution library was prepared automatically by mixing predetermined volumes of different catalyst precursor solutions into individually addressable test tubes in an array using a computer-controlled x,y,z translation table and a high accuracy liquid delivery system. The system utilizes stepper motor controlled syringe pumps and micro solenoid valves to dispense aqueous

solutions within 10 nL accuracy. Following the dispensation of catalyst precursor solutions, deionized water was jetted into each test tube to induce further mixing and to render the final volume to be 50 μ L.

Alumina pellets added to each test tube were allowed to impregnate under the competition of HCl in solution for 24 h in order to attain homogeneous metal distribution within the particles. Subsequently, the solutions were evaporated and the pellets were dried first at 90 °C for 4 h then at 120 °C for 4 h. Finally, the impregnated pellet library was calcined at 500 °C for 2 h and cooled down to ambient temperature in the furnace. The catalyst library consisted of 66 ternary combinations of Pt, Pd, and In in 0.1 % wt increments of each with 1 wt % total metal loading on alumina support. In addition, the final chlorine content of the pellets was also kept at about 1 %. The compositions of the 66 pellets prepared are shown in Figure 2. All the catalyst pellets were prepared and processed in an identical manner without any human intervention.

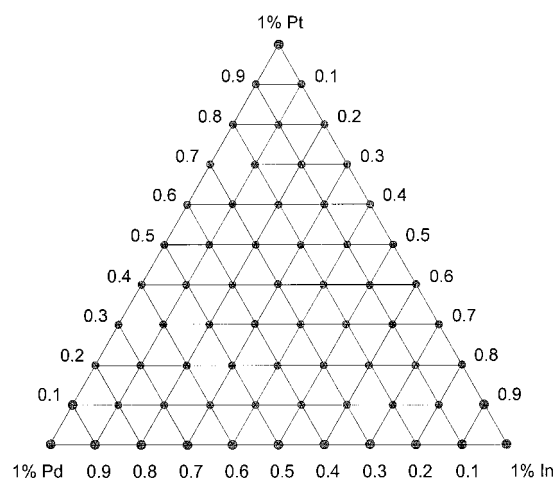


Figure 2. Compositions of the 66 Pt/Pd/In ternary catalyst combinations investigated (1% wt total metal on 30 mg gamma alumina pellet in 0.1 % increments).

The catalysts were tested by using the following procedure: After the placement of new catalyst pellets into the wells of the array microreactor, the system was heated to 350 °C under helium flow. A blank alumina pellet was always placed into the central microreactor (9th well from either side) in all experiments to serve as an internal reference standard. After the temperature had reached 350 °C, the helium flow was switched to pure hydrogen for 2 h to reduce the platinum, palladium, and indium chlorides into metallic form. Following the catalyst reduction step, the reactor temperature was decreased to 300 °C again under helium gas flow. Once the proper operating temperature was reached, cyclohexane feed was introduced. With the existing array microreactor system, this procedure had to be repeated five times to screen the entire library of 66 catalysts. Additional experiments were also undertaken to check the reproducibility of the measurements, including the placement of catalyst pellets at different locations in the array microreactor and duplicate preparation and testing of different combinations. All these studies

resulted in similar results within the limits of accuracy of the measurements (i.e. $\pm 15\%$).

All the catalysts were subjected to an identical pretreatment (i.e. reduction) process before screening in order to standardize the initial conditions. This way the activities of the catalysts can be compared to one another having been on-stream the same time period, thereby minimizing complications related to catalyst deactivation, poisoning etc. In addition, visual inspection of the pellets immediately after the reduction steps also revealed a uniform catalyst metal distribution across the pellet.

The screening protocol demands the unambiguous detection of benzene in a cyclohexane, hydrogen, and helium background, as well as other reaction by-products. In order to accomplish this goal using the REMPI technique one must identify a suitable UV laser wavelength that selectively produces benzene REMPI ions. One such wavelength was identified to be 259.6 nm in our previous studies using time-of-flight mass spectrometry (TOF-MS).^[13] At this wavelength, the first photon energizes the benzene molecule to an excited electronic state, P^* [Eq. (1)], and the second photon ionizes this intermediate state [Eq. (2)].



In the present work, the reactor exhaust gases were also analyzed by TOF-MS using the same 259.6 nm laser light to find out if species other than benzene could have contributed to the signals measured by the microelectrodes.^[15] No photo-ions, other than those with mass 78, were detected by TOF-MS. The laser light in the experiments was obtained by frequency doubling the output of a dye laser (Laser Analytical Systems, LDL 2051) using a second harmonic generator (BBO I). The dye laser was pumped by an excimer laser (Lambda Physik, Compex 102). The energy of the laser beam was maintained at about 300 μ J per pulse during the entire screening process.

The pulsed UV laser light was passed through the optical access windows of the heating block and the ceramic slab, simultaneously crossing the exhaust streams of all the microreactors. The laser beam also passed between the dedicated microelectrodes that were embedded into the top and bottom ceramic slabs (Figure 1). The microelectrodes were made from 0.1 mm diameter steel wires and their tips were flush with the ceramic slab surfaces. This design significantly simplified the process of alignment and calibration of the microelectrodes and the laser beam between catalyst changes and thus improved the speed and accuracy of the catalyst screening process. Multichannel switches were used to individually apply 100 V DC power to each microelectrode sequentially (Stanford Research Systems, PS 350). REMPI signals were detected by using a digital oscilloscope (Hewlett-Packard 54540C). The screening of the catalysts in the array microreactor took two to three minutes.

Figure 3 shows the bar chart of benzene REMPI signals (voltage E in mV) as measured by the oscilloscope for each catalyst (66 total). These measurements correspond to an

average of 10 laser shots. Signal-to-noise ratios were better than 20 throughout the entire screening process. As evident from Figure 3, both Pt and Pd individually exhibited catalytic activities, with Pt being more active than Pd. On the other hand In was not catalytically active for benzene production from cyclohexane. These results are in complete agreement with the previous measurements reported by other investigators.^[16, 17]

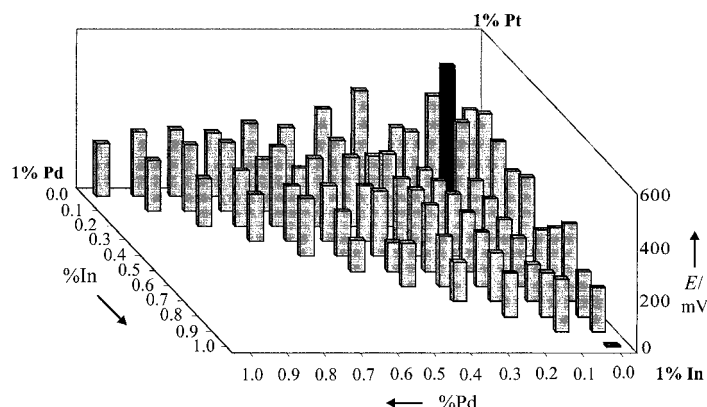


Figure 3. Array microreactor screening results for the ternary Pt/Pd/In catalyst combinations (bar chart for the activities (voltage of measured REMPI signals) plotted against catalyst composition).

The reproduction of the reactivity trends of individual metals is important in generating confidence with regard to the suitability of the system developed for the intended catalyst screening studies. As seen in Figure 3, we also made the significant discovery that a ternary mixture (0.8% Pt, 0.1% Pd, and 0.1% In) exhibited the best benzene productivity of all the metal combinations investigated. These results demonstrate the feasibility of using array microreactors coupled with REMPI screening for the discovery and optimization of heterogeneous catalysts.

In Table 1 the approximate times associated with the execution of each step in the preparation, processing, and screening of the catalyst library are given. As noted earlier, the present array microreactor accommodates a total of 17 catalyst pellets. Consequently, the complete testing of the

Table 1. Time elements associated with the preparation, processing, and screening of catalysts.

Operation	t [h]
Library preparation	
preparation of precursor solutions	0.50
preparation of solution library	0.25
impregnation of pellets	24.00
drying	8.00
calcining (Including heating and cooling)	6.00
total catalyst preparation time	38.00
Library screening (16 pellets per cycle)	
catalyst reduction (Including heating and cooling)	3.50
REMPI screening	0.03
cooling and replacement	1.00
cycle time	4.53
total catalyst screening time (cycle time $\times$ 5)	22.65
Total time to discovery and optimization	60.65 or 2.5 days

entire library of 66 catalysts involved five sequential runs. As shown in Table 1 the preparation and screening of the Pt/Pd/In ternary catalyst library took about 2.5 days to complete. Although this represents a significant advance over conventional methods, which may take months to achieve the same objectives, further acceleration of the overall process is clearly possible. For example, increasing the number of microreactors in each array, that is increasing the level of parallel processing, and decreasing the times for impregnation, drying, calcination, and reduction can significantly speed up the process. However, the latter issues must be carefully implemented as even minor modifications in catalyst preparation methods can dramatically alter the performance of the catalysts. On the other hand, the minimization of catalyst preparation times can be the objective of a combinatorial study such as that described here.

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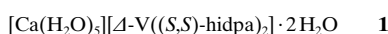
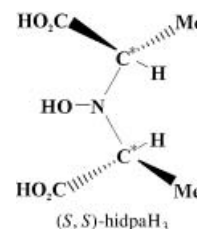
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The Structural Characterization of Amavadin**

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One fascinating aspect of bioinorganic chemistry is that of metal accumulation by living organisms, for example, to provide an appropriate concentration for the use of the metal in one or more specific biochemical functions and/or as a means of protection against toxicity that arises from an excess of metal. A striking example of metal accumulation is provided by three species of *Amanita* mushrooms: *A. muscaria*, the "fly agaric"; *A. regalis*; and *A. velatipes*. Each species concentrates vanadium to levels of up to 400 times those typically found in plants. Studies by Ter Meulen,^[1] Bertrand,^[2, 3] and Meisch et al.^[4] have shown that the accumulation of vanadium by these species of *Amanita* is independent of the age of the mushrooms, unrelated to the vanadium content of the soil, and essentially equally distributed between the stem, skin, and cap.

In 1972 Bayer and Kneifel^[5] isolated a blue, vanadium-containing compound from *A. muscaria* collected in the Black Forest. They named this compound "amavadin" and showed that it is constituted as a 1:2 complex of vanadium and the pro-ligand (*S,S*)-2,2'-(hydroxyimino)dipropionic acid ((*S,S*)-hidpaH₃).^[6] Further studies^[7–10] led to the proposal that the vanadium center of the amavadin exists as an eight-coordinate, non-oxo complex with a novel structure. Herein, we report confirmation of this postulate by X-ray crystallographic determinations on a sample of amavadin crystallized as a phosphoric acid derivative,^[11] and as the salt **1** with Ca²⁺ counteranions.^[12]



The use of Ca²⁺ counterions to crystallize amavadin proved valuable in obtaining good quality crystals for X-ray diffraction analysis, as demonstrated previously for related chemical systems.^[13] The lattice of **1** is comprised of [Ca(H₂O)₅]²⁺ and [Δ-V((*S,S*)-hidpa)₂]²⁻ ions that are linked in the form of infinite chains; a portion of a chain is shown in Figure 1. Each anion is bound by two Ca²⁺ ions through a carboxylate group

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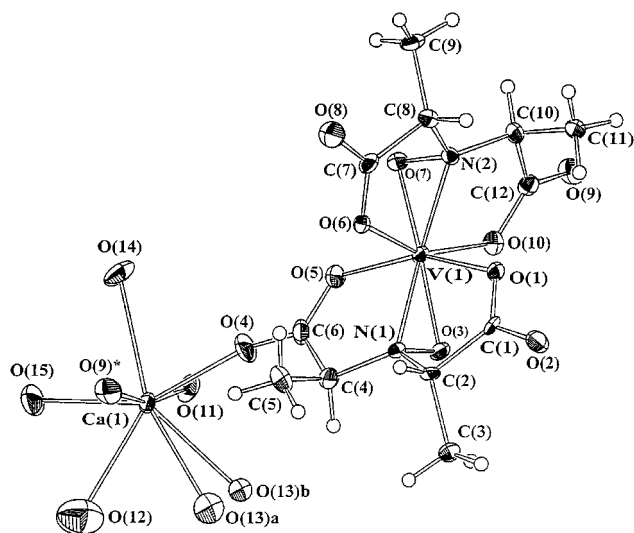


Figure 1. ORTEP view of a portion of the lattice of **1**. (Ellipsoids are shown at the 30% probability level). Selected bond lengths [Å] and angles [°]: V(1)–O(1) 2.028(7), V(1)–O(3) 1.940(7), V(1)–O(5) 2.028(9), V(1)–O(6) 2.042(8), V(1)–O(7) 1.956(7), V(1)–O(10) 2.070(8), V(1)–N(1) 1.999(8), V(1)–N(2) 1.982(8), Ca(1)–O(4) 2.300(9), Ca(1)–O(9) 2.312(9), Ca(1)–O(11) 2.326(9), Ca(1)–O(12) 2.582(17), Ca(1)–O(13a) 2.36(2), Ca(1)–O(13b) 2.48(2), Ca(1)–O(14) 2.571(9), Ca(1)–O(15) 2.389(12), O(1)–C(1) 1.237(12), O(2)–C(1) 1.274(14), O(3)–N(1) 1.395(11), O(4)–C(6) 1.241(15), O(5)–C(6) 1.291(14), O(6)–C(7) 1.242(13), O(7)–N(2) 1.385(12), O(8)–C(7) 1.259(15), O(9)–C(12) 1.243(14), O(10)–C(12) 1.290(13); O(1)–V(1)–O(6) 86.4(3), O(1)–V(1)–O(10) 91.2(3), O(3)–V(1)–N(1) 41.4(3), O(5)–V(1)–O(6) 89.2(3), O(5)–V(1)–O(10) 101.8(3), O(7)–V(1)–N(2) 41.2(3).

on each (*S,S*)-hida^{3–} ligand. Each Ca²⁺ ion is coordinated by an approximately pentagonal bipyramidal arrangement of oxygen atoms comprising five H₂O molecules (O(11), O(12), O(13a,b) O(14), and O(15)) and two unidentate carboxylato groups bound through the O(4) and O(9)* oxygen atoms. The Ca(1)–O(9)* and Ca–O(11) bonds are approximately mutually *trans* (167.3(3)°) and each is orientated approximately perpendicular to an “equatorial plane” formed by the oxygen atoms of two of the Ca²⁺-bound H₂O molecules and the carboxylate O(4) atom.^[14] The Ca²⁺-bound H₂O molecules have close contacts to carboxylate and oxyimino oxygen atoms on four neighboring chains, as illustrated in Figure 2. Thus, the seven water molecules play a significant role in the structure of **1**.^[14]

The structure of the amavadin anion approximates to C₂ symmetry with the twofold axis bisecting the normals to the V(η^2 -NO) planes from each ligand projected onto the least-squares plane of the vanadium atom and four O donor atoms of the unidentate carboxylato groups (the VO₄ plane). The planes of the two V(η^2 -NO) groups are close to being mutually perpendicular (93.6°) and each is essentially perpendicular (89.4° and 91.8° for the V(η^2 -NO) planes involving N(1) and N(2), respectively) to the VO₄ least-squares plane. The unidentate carboxylate oxygen atoms in the same ligand are bonded to the V^{IV} center in a mutually *trans* manner and each of these atoms is significantly displaced from the VO₄ least-squares plane towards the η^2 -NO[–] group of that ligand: the atoms O(1) and O(5) sit below (–0.42 and –0.36 Å, respectively) and O(6) and O(10) sit above (0.43 and 0.36 Å,

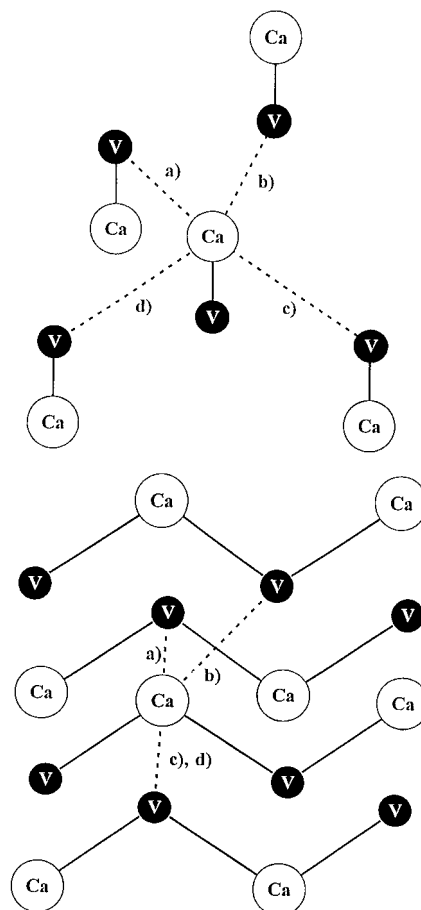
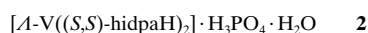


Figure 2. View down the ends (top) and along (bottom) neighboring chains of **1**: only V and Ca atoms are shown (solid lines denote Ca–O–C–O–V linkages, dashed lines denote hydrogen bonding/close contacts between chains (<2.90 Å): a) water O(11)⋯O(7) oxyimino oxygen [2.723(12) Å] and water O(15)⋯O(5) carboxylate oxygen [2.825(14) Å]; b) water O(11)⋯O(2) carboxylate [2.867(12) Å]; c) water O(13a) and O(13b)⋯O(8) carboxylate [2.84(2) and 2.70(2) Å, respectively]; d) water O(14)⋯O(2) carboxylate [2.743(13) Å].

respectively). The results of EPR studies accomplished for *A. muscaria*^[15] and freshly dissolved crystals of **1** are consistent with the V^{IV} center in the former occupying an environment essentially identical to that observed in the latter. This type of coordination geometry was first identified in [NMe₄][NH₄][V(hida)₂] (hidaH₃ = (hydroxyimino)diacetic acid).^[9]

Although the structure of the phosphoric acid derivative **2** of amavadin has been solved to a lower resolution^[11] than that obtained for **1**, it is clear that this system possesses the same type of vanadium center as in **1**. However, the chirality at the metal is of the opposite hand to that in **1**, namely Δ as opposed to Λ . Thus, the geometry of the inner coordination sphere of



the vanadium center in amavadin and related complexes leads to a special chirality at the metal center. The handedness of this chirality is determined by the relative positions of the two η^2 -NO[–] groups, as viewed perpendicular to the VO₄ plane (Figure 3).

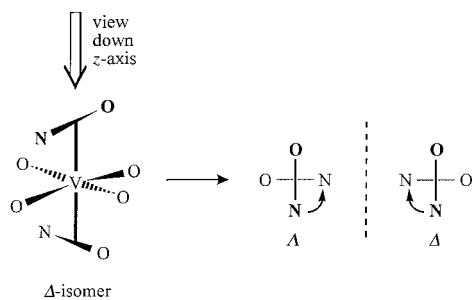


Figure 3. A representation of the chirality at the vanadium center in amavadin.

Thus, amavadin possesses five chiral centers. In the isolated natural product, consistent with earlier conclusions,^[6] the four chiral carbon atoms all have the *S* stereochemistry. We have observed both isomers at the vanadium center in our crystallographic studies: only the Δ form was observed in the crystal selected for X-ray crystallographic analysis of **1**,^[12] whereas the crystal of the H_3PO_4 derivative investigated^[11] contained only the Λ form. Spectroscopic studies of solutions of amavadin^[16] and its V^{V} counterpart^[10] have shown that, as isolated, amavadin exists as an approximate 1:1 mixture of Δ - and Λ -helical forms.

The ability of the carboxylato groups of amavadin-like anions to bind cations, for example, Ca^{2+} , and/or become involved in hydrogen bonding may be relevant to the means whereby amavadin is stored within the *Amanita* mushrooms and possibly to the biological role(s) of this species.

Experimental Section

Isolation of natural amavadin: *Amanita muscaria* was collected from Dunham Forest golf course near Altrincham, Cheshire (UK). An aqueous solution of amavadin was isolated by using the procedure published by Bayer and Kneifel^[5] and crystallized by very slow evaporation. An X-ray crystallographic study^[11] showed these crystals to be constituted as **2**.

Crystallization of natural amavadin with Ca^{2+} counterions: The crystallized amavadin from the procedure detailed above (20 mg, 0.05 mmol) was dissolved in H_2O (0.5 cm^3). CaCl_2 (5 mg, 0.05 mmol) was added and allowed to dissolve before the solution was filtered. A triple layer diffusion with an equal volume of MeOH on top of the amavadin-containing solution, and a top layer of isopropyl alcohol was set up in a sealed Pasteur pipette. After the mixture had stood at room temperature for nine days blue, needle-shaped crystals were collected by filtration.

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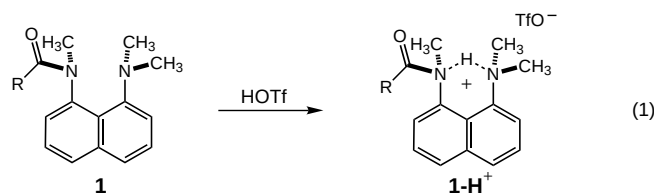
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- [12] Crystal data for **1**: The structure was solved by direct methods^[17] and expanded with Fourier techniques.^[18] The oxygen atoms O(13) and O(17) were disordered over two sites A and B, which were constrained to sum to 1. These atoms were refined isotropically whilst all other non-hydrogen atoms were refined anisotropically. Hydrogen atoms bonded to carbon atoms were placed in calculated positions and refined isotropically; other hydrogen atoms were not included. Data were corrected for Lorentz and polarization effects and an empirical absorption correction, based on azimuthal scans of several reflections, was applied (transmission factors 0.620–1.000). The absolute configuration was determined by refinement of the Flack parameter to give a final value of 0.01(10).^[19] The selection of overall chirality led to the assignment of *S* stereochemistry at all chiral carbon atoms for both **1** and **2**. This stereochemistry had been deduced spectroscopically in the previous independent study.^[6] A refinement was carried out with SHELX97^[20] and other calculations were performed with the TEX-SAN^[21] crystallographic software package. Data: $\text{C}_{12}\text{H}_{22}\text{CaN}_3\text{O}_{15}\text{V}$, $M_r = 525.33$, orthorhombic, space group $P2_12_12_1$, $a = 13.447(7)$, $b = 16.913(7)$, $c = 10.224(4)$ Å, $V = 2325.1(18)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.501$ Mg m⁻³, $T = 293(2)$ K, $\theta_{\text{max}} = 25.06^\circ$. Blue needle crystals ($0.50 \times 0.07 \times 0.05$ mm), 2345 reflections measured, 1856 observed with $[I > 2.0\sigma(I)]$, Rigaku AFC-SR diffractometer, $\text{MoK}\alpha$ radiation $\lambda = 0.7107$ Å, R values ($R(F)$, $wR(F^2)$) 0.0767, 0.2062. 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-102680 and -102668. 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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Strong Hydrogen Bonding to the Amide Nitrogen Atom in an "Amide Proton Sponge": Consequences for Structure and Reactivity**

Christopher Cox, Harald Wack, and Thomas Lectka*

The carbonyl oxygen atom of the amide functional group is universally believed to be the thermodynamically preferred site of protonation in amides;^[1] however, N protonation has been proposed to play a key role in biologically important reactions of peptides and proteins such as acid-catalyzed peptide hydrolysis^[2] and *cis-trans* isomerization about the C–N bond.^[3] Extensive research into the mechanism of acid-catalyzed amide hydrolysis has led to the belief that the O-protonated form is generally the important intermediate on the pathway to hydrolysis.^[4] Still, evidence supporting N protonation in certain cases,^[2] as well as in the related thiol-catalyzed amide hydrolysis reactions important in cysteine proteases,^[5] has also been reported. N-Protonated amides have thus far eluded characterization, probably because they are disfavored thermodynamically relative to the O-protonated form by about 7 p*K*_a units in aqueous solution.^[6] Even species showing strong hydrogen bonding between a donor and the amide N atom are unknown.^[7] Herein we present the first spectroscopic and crystallographic characterization of a strong hydrogen bond between a charged donor (a protonated amine) and an amide nitrogen atom in a model system, and also disclose unusual reactivity of the amide functionality that stems from this interaction.

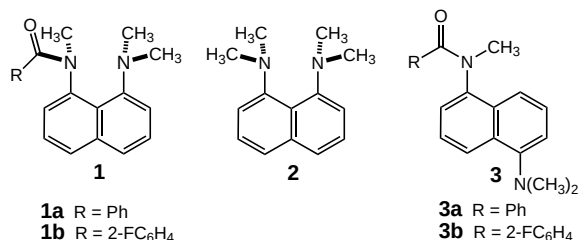
We designed system **1** in which a protonated amine group can act as a donor suitably positioned to engage in a strong intramolecular hydrogen bond with the amide nitrogen atom rather than with the carbonyl oxygen atom [Eq. (1)].^[8] Compound **1** is based on the venerable "proton sponge" **2**



whose exceptional basicity arises from destabilization in the free base due to electron–electron repulsion and also from formation of a strong intramolecular hydrogen bond upon protonation.^[9] "Amide proton sponge" **1** is available in two

steps from 1,8-diaminonaphthalene,^[10] and we propose the isomeric 1,5-naphthalene **3** as a control compound to factor out through-bond inductive effects of the electron-withdrawing protonated amine.

The ¹H NMR spectrum of **1a** in [D₃]acetonitrile in the presence of one equivalent of trifluoromethanesulfonic acid (HOTf) displayed a broad signal due to one proton at δ = 11.0, whereas the analogous signal in control **3a** under the same conditions appeared at δ = 9.4, a difference that hints at the presence of an intramolecular hydrogen bond between the protonated amine group and the amide N atom in **1a-H⁺** [Eq. (1)].^[11] Additional support for N coordination of a proton is provided by ¹³C NMR spectroscopy of **1a-H⁺** in which the carbonyl carbon atom is ¹³C-labeled. Before addition of acid, labeled **1a** shows amide carbonyl peaks at δ = 170.2 and 171.3 corresponding to both the *cis* and *trans* forms of the amide; however, upon protonation the peaks coalesced and shifted downfield to δ = 175.0,^[12] consistent with the formation of a more ketone-like carbonyl group. The fact that both **1a** and **3a** are fully protonated by one equivalent of HOTf in acetonitrile is confirmed by UV/Vis spectroscopy.^[10]



Evidence supporting hydrogen bonding to the amide N atom under these conditions was also obtained by means of IR spectroscopy. Upon protonation of **1a** in [D₃]acetonitrile, the amide C=O stretch shifts +47 cm⁻¹ from 1637 to 1684 cm⁻¹. It has been previously proposed that a shift of this so-called amide I stretch to higher wavenumber is strong evidence for N coordination, if it could be validated that the peaks are correctly assigned.^[13] To support our interpretation, we investigated ¹³C-labeled **1a** under identical conditions and found that the ¹³C=O peak moves from 1597 to 1644 cm⁻¹, again a difference of +47 cm⁻¹, confirming our assignment. Upon protonation of **3a**, the C=O stretch undergoes a small shift of <5 cm⁻¹, indicating that the +47 cm⁻¹ shift observed for **1a-H⁺** is not due to inductive effects.

We also obtained the single-crystal X-ray structure of **1b-H⁺** (Figure 1) that clearly shows the hydrogen atom (H1A) placed between the amine nitrogen atom (N1A) and the amide nitrogen atom (N2A).^[14] The N2A–H1A distance of 2.17 Å and the N2A–H1A–N1A angle of 136° in **1b-H⁺** (as determined by isotropic refinement of H1A's position) classifies the interaction as a moderately strong hydrogen bond by Jeffrey's criteria.^[15] Further evidence supporting the strong hydrogen bond was obtained by examination of the pyramidalization of the amide nitrogen atom (N2A) upon protonation and comparison to that of the free base **1b**, whose X-ray structure was also solved.^[16] As expected, **1b** contains a planar amide unit, as characterized by summation of the three

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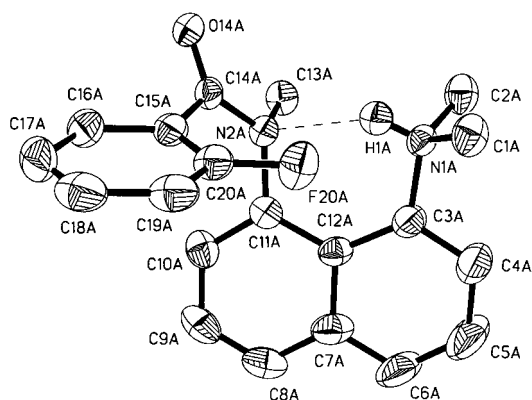
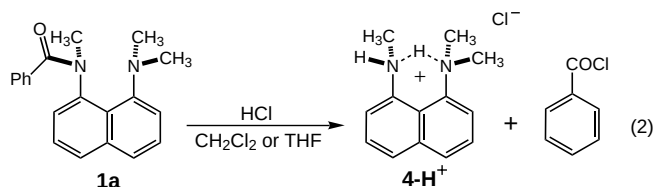


Figure 1. X-ray structure of the triflate salt of **1b-H⁺** (50% ellipsoids).^[14] The unit cell consists of two molecules of **1b-H⁺**, two triflate counterions, and one molecule of water. All hydrogen atoms, except for H1A, have been excluded for clarity. The dashed line indicates the proposed hydrogen bond between H1A and N2A. Selected bond lengths [Å] and angles [°]: N2A–H1A 2.17(4), N1A–N2A 2.869(5), C14A–N2A 1.369(5); N2A–H1A–N1A, 136(3)°.

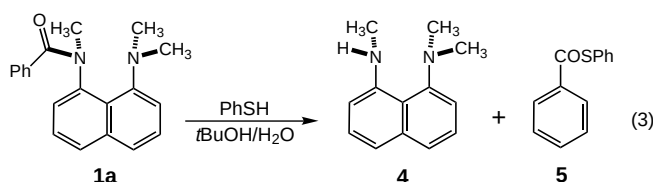
valence angles around N2, $\theta = 359.9^\circ$ (perfectly planar = 360°), and the out-of-plane angle, $\alpha = 7.5^\circ$ (perfectly planar = 0°).^[17] On the other hand, **1b-H⁺** displays $\theta = 349.4^\circ$ and $\alpha = 36.0^\circ$, values that correspond very closely to pyramidalized amides based on strained bicyclic systems.^[17] Thus, **1b-H⁺** is a rare case of a highly distorted amide whose pyramidalization is not the result of a strained backbone.

The first clue to the interesting reactivity of **1-H⁺** was discovered while we were attempting to obtain diffractable crystals of the hydrochloride salt of **1a**. After bubbling anhydrous HCl through a solution of **1a** in CH_2Cl_2 for one minute and storing the test tube in the dark for three days, we did not observe crystallization but instead noted the presence of benzoyl chloride [Eq. (2)]. The conversion in this process



was low, but when we performed the reaction in THF with gentle heating to 50°C for 30 h, the reaction became quantitative based on recovery of 8-methylamino-1-dimethylaminonaphthalene hydrochloride **4-H⁺**.^[18] Under the same conditions control **3a** is inert, underscoring the importance of the strategically placed hydrogen bond in **1a-H⁺**. To our knowledge, this is the only case where HCl reverts an amide to an acid chloride and amine salt, a transformation that can be thought of as the “reverse” of normal peptide bond formation.

A second novel reaction of **1** is the PhSH-mediated conversion to **4** and thioester **5** [Eq. (3)]. Upon heating **1a** with 10 equivalents of PhSH in a thoroughly degassed 50/50 mixture of *t*BuOH/water under a nitrogen atmosphere in a pressure tube at 125°C for 48 h, we observed 55% conversion of **1a** to **4**, as indicated by HPLC analysis of the crude reaction.



The following control reactions were also run under identical conditions: 1) one equivalent of HOTf was added along with **1a** and PhSH; and 2) control **3a** was used instead as the substrate, both with 10 mol % triethylamine and without. In each control there was no indication of any reaction, which suggests a mechanism in which a small amount of PhSH is deprotonated by **1a** and the PhS^- subsequently attacks the amide carbonyl group to yield products. To our knowledge, this is the first example of the thiolysis of an unstrained, nonactivated amide under neutral conditions by a monofunctional thiol, and supports Brown’s mechanistic hypothesis that the thiol-mediated cleavage of amides is subject to rate-limiting proton transfer to a tetrahedral intermediate from a properly aligned general base.^[19] The **1**-PhSH system therefore appears to be a mimetic for the active site of cysteine proteases in which Cys25 and His159 form a highly nucleophilic thiolate/imidazolium ion pair that is proposed to be the active species in these enzymes.^[20]

In contrast to the results with HCl in organic solvents, the triflate salt of protonated amide **1a-H⁺** in aqueous solution showed a surprising lack of reactivity. In the presence of one equivalent of HOTf in a 50/50 mixture of EtOH/ H_2O , **1a** underwent no reaction, even when heated to 150°C for 24 h in a pressure tube. The lack of reactivity in this case supports the hypothesis that the O-protonated form is the intermediate on the pathway to acid-catalyzed amide hydrolysis.^[4] Further studies on the protonation of amide nitrogen atoms and the novel reactivity of amide sponges **1** are currently underway.^[21]

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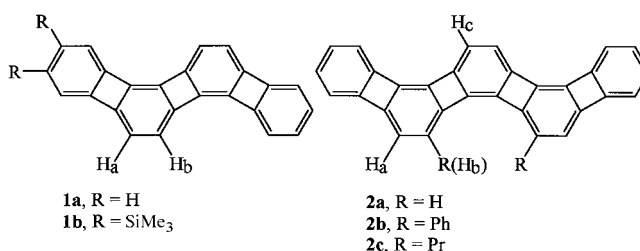
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- [14] a) The unit cell consists of two molecules of **1b-H**⁺, two triflate counterions, and one molecule of water. X-ray crystal data for compound **1b-H**⁺, grown by slow diffusion of Et₂O into a solution of **1b-H**⁺ in acetone: C₂₁H₂₁F₄N₂O_{4.50}S, *M*_r = 481.46, yellow block (0.30 × 0.26 × 0.21 mm), triclinic, *P*1̄, *a* = 12.5179(2), *b* = 12.9868(1), *c* = 14.3698(2) Å, α = 87.722(1), β = 66.638(1), γ = 89.306(1)°, *V* = 2142.84(5) Å³, *Z* = 4, ρ_{calcd} = 1.492 Mg m^{−3}, *F*(000) = 996, Siemens SMART Platform CCD, MoK α radiation, λ = 0.71073 Å, *T* = 173(2) K, 10567 reflections collected, 7152 independent reflections, structure solved by direct methods, difference Fourier synthesis, and full-matrix least-squares on *F*² (SHELXTL-V5.0). *R*1 = 0.0704, *wR*2 = 0.1688 for 4744 reflections; the hydrogen atoms on N1A and N1B were located in the Fourier difference map and refined isotropically, all other hydrogens were placed in ideal positions and refined as riding atoms; one of the triflate anions has significant disorder caused by a rocking motion. b) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-104129 (**1b-H**⁺) and CCDC-104130 (**1b**). 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 Novel Phenylene Topology: Total Syntheses of Zigzag [4]- and [5]Phenylene**

Christian Eickmeier, Daniel Holmes, Heiko Junga, Adam J. Matzger, Frank Scherhag, Moonsub Shim, and K. Peter C. Vollhardt*

Missing from the phenylene topologies hitherto synthesized—linear, angular, and trigonal^[1]—is the zigzag variant, exemplified by the title compounds **1a** and **2a**. These



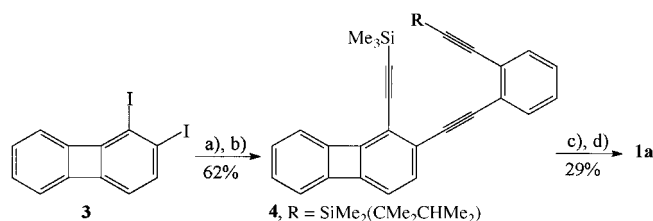
molecules are important as substructure models for the one-dimensional, zigzag phenylene polymer,^[2] the two-dimensional all-carbon net based on the anti-kekulene motif,^[1c, 2a, 3] and the three-dimensional carbon allotropes *O*_h-C₄₈^[4] and *I*_h-C₁₂₀ (archimedeane).^[5] They are also interesting as members of a family of phenylene isomers with different topologies, in particular in comparison to their angular relatives in which

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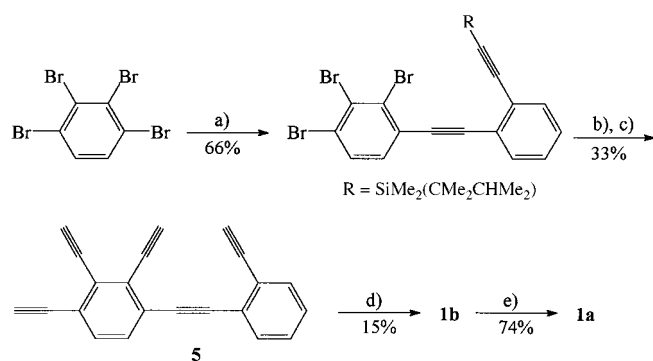
each benzene ring exhibits the same local symmetry and the molecules differ only in the sense of their overall connectivity.^[1a, 6] Finally, they function as additional tests of the accuracy of contemporary calculational techniques, for which the phenylenes have proven to be attractive targets.^[2, 3, 5, 6] We report herein the synthesis of the parent systems **1a** and **2a** and those of their respective derivatives **1b**, **2b**, and **2c**, the latter prepared in order to obtain X-ray structural details.

The initial strategy to the zigzag [4]phenylene frame **1** was patterned after that executed en route to its angular topomer,^[1a] exploiting the regiocontrol exhibited by the Pd-catalyzed alkylation of 1,2-diiodobiphenylene **3**, but inverting the sequence in which the two alkyne substituents are attached (Scheme 1).^[7] The resulting compound **4** was then



Scheme 1. a) *o*-(HC₂)C₆H₄(C₂R) (1.2 equiv; R see Scheme), 10% CuI, 10% [PdCl₂(PPh₃)₂], Et₃N, 23 °C, 15 h; b) Me₃SiC₂H (10 equiv), 7% CuI, 6% [PdCl₂(PPh₃)₂], 50 °C, 2 d; c) Bu₄N⁺F⁻, THF, 23 °C, 40 min; d) [CpCo(CO)₂] (0.9 equiv), *m*-xylene, Δ, *hν*, 18 h (syringe addition of **4**).

deprotected and cyclized in the presence of [CpCo(CO)₂] to furnish **1a**. As the latter did not crystallize in a manner conducive to an X-ray investigation, an improved synthesis was developed which also provided crystalline **1b**. By starting with the selective, stepwise alkylation of 1,2,3,4-tetrabromobenzene,^[1c] the cumbersome construction of **3** was bypassed,^[1a] and the basic carbon frame was assembled by a cobalt-catalyzed cyclization of pentayne **5** in one step to give **1b** (Scheme 2, Figure 1).^[7, 8] The latter is novel, in as much as it constitutes the first such cooligomerization that is both intra- and intermolecular. Protodesilylation of **1b** resulted in **1a**.



Scheme 2. a) *o*-(HC₂)C₆H₄(C₂R) (0.2 equiv; R see Scheme), 6% CuI, 6% [PdCl₂(PPh₃)₂], Et₃N, 50 °C, 24 h; b) Me₃SiC₂H (45 equiv), 30% CuI, 30% [PdCl₂(PPh₃)₂], piperidine, 100 °C, 7 d; c) Bu₄N⁺F⁻, THF, 23 °C, 2 h; d) (CH₃)₃SiC₂Si(CH₃)₃ (solvent), [CpCo(CO)₂] (2.0 equiv), Δ, *hν*, 10 h (syringe addition of **5**); e) CF₃CO₂H/CHCl₃ (1:25), 23 °C, 12 h.

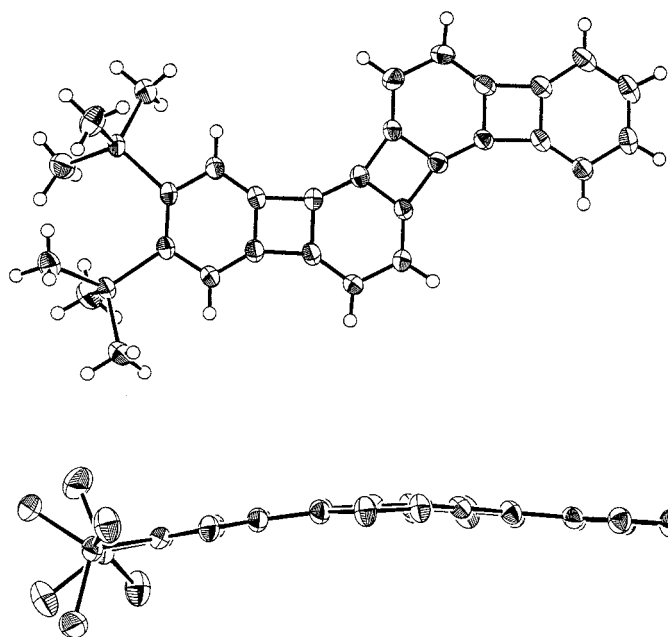
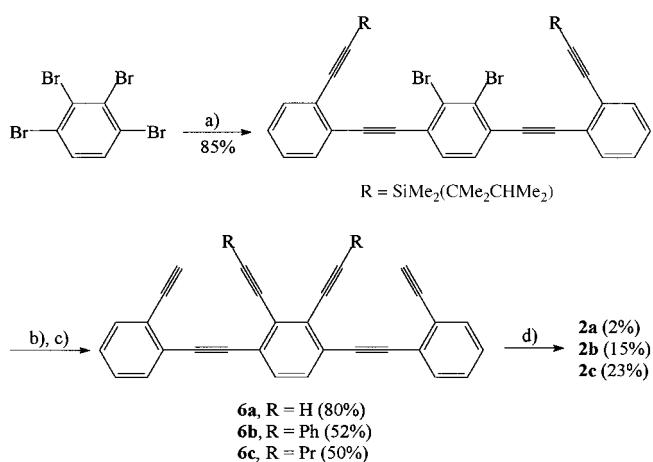


Figure 1. Structure of **1b** in the crystal: views from above (top) and the side (bottom).

Scheme 3 summarizes the construction of **2a–c**^[7] through a variant of an improved route to the angular [5]phenylene nucleus,^[1c] in which the hexaynes **6** are employed as substrates in the final cyclization. X-ray structures were obtainable on crystals of both **2b** and **2c** (Figure 2).^[8]



Scheme 3. a) *o*-(HC₂)C₆H₄(C₂R), (R see Scheme), 3% CuI, 3% [PdCl₂(PPh₃)₂], Et₃N, 23 °C, 5 d; b) RC₂H (20–100 equiv; R = SiMe₃, Ph, Pr), 1–2% CuI, 1–2% [PdCl₂(PPh₃)₂], piperidine, 80 °C, 1–3 d; c) Bu₄N⁺F⁻, THF, 23 °C, 2 h; d) [CpCo(CO)₂] (1 equiv), *m*-xylene, Δ, *hν*, 1–2 h (syringe addition of **6**).

Inspection of the spectral data of these systems (Table 1) is most instructive when the compounds are compared with each other and with the respective isomeric topologies. Thus, the electronic spectra bear the typical features of the phenylenes, with two sets of bands, one at higher energies exhibiting relatively large ε values, the other in the long wavelength range with much diminished intensity.^[1a] As predicted by theory,^[2b–d] the highest wavelength absorptions are much less bathochromically shifted (and hence the HOMO–LUMO

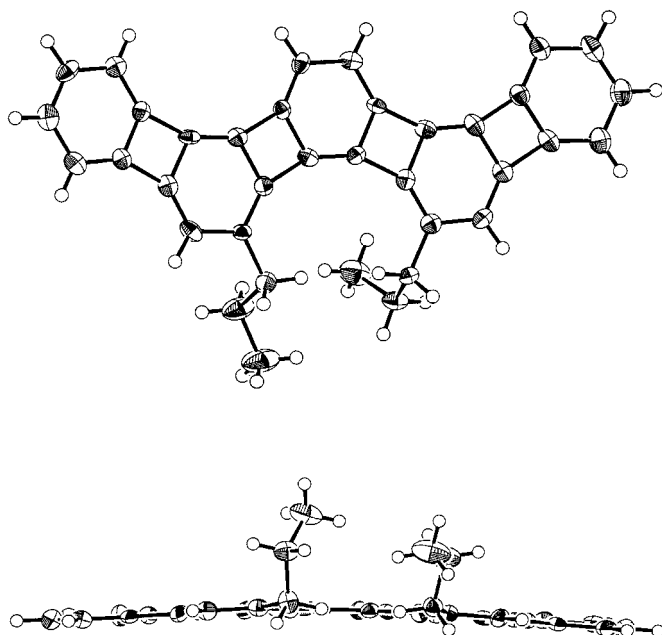


Figure 2. Structure of **2c** in the crystal: views from above (top) and the side (bottom).

gap less rapidly diminished) along the zigzag series ([3] → [4] → [5]phenylene; $\lambda_{\max}$ 428 → 464 → 484 nm) than those of its linear counterpart ($\lambda_{\max}$ 432 → 492 → 530 nm). Interestingly, however, **1a** and **2a** exhibit distinctive (albeit small, $\Delta\lambda_{\max} \sim 15$ nm) shifts of their highest wavelength bands to lower energy, relative to those of their respective angular relatives, indicative of an associated longitudinal transition dipole moment.^[9] In contrast, the ¹H NMR spectra of the zigzag phenylenes are almost identical to those of their angular isomers, the “internal” benzene hydrogen atoms revealing incremental deshielding compared to the linear phenylenes and relative to angular [3]phenylene ($\delta = 6.18$). The corresponding shifts are: **1a**: $\delta_{\text{Ha}} = 6.27$, $\delta_{\text{Hb}} = 6.34$; **2a**: $\delta_{\text{Ha}} = 6.23$, $\delta_{\text{Hb}} = 6.26$, $\delta_{\text{Hc}} = 6.51$. This effect can be attributed to the cumulative influence of bond alternation in the internal benzene nuclei, resulting in relatively lesser paratropicity of the inside compared to the outside cyclobutadienoid rings, a view supported by nucleus-independent chemical shift (NICS) calculations.^[6a] Theoretical estimates^[5a, 6a,c,d,g] also predict zigzag fusion in the [N]phenylenes to be energetically advantaged over linear fusion (e.g. $\Delta\Delta H_f^{\circ}$ (linear → zigzag) $\approx -(4.6-4.7)$ kcal mol⁻¹ for $N=4$, $\approx -(7.1-10.4)$ kcal mol⁻¹ for $N=5$) and, curiously, the zigzag topology to be slightly more stable ($\Delta\Delta H_f^{\circ} \approx 0.1-0.2$ kcal mol⁻¹) than the angular one (HF/6-31G* and B3LYP/6-31G*). The ¹³C NMR data of the respective zigzag (e.g. **1a**, **2c**) and angular topomers^[1a,c] are virtually superimposable ($\Delta\delta < 2$). In short, the spectral data indicate strong similarities between the zigzag and angular topological series (at least up to $N=5$), both pronouncedly deviating in comparison to those of the linear phenylenes.

The X-ray data on **1b**, **2b**, and **2c** corroborate these observations structurally and demonstrate the effect of one- and twofold angular benzocyclobutadienannulation of angular [3]phenylene (Figure 3).

Table 1. Selected physical data for **1a**, **1b**, **2a–c**, and **8**.^[7]

1a: light yellow powder, m.p. > 265 °C (decomp); MS (70 eV): m/z (%): 300 ($[M^+]$, 100), 298 (25), 150 (10); ¹H NMR (400 MHz, CDCl₃): $\delta = 6.90-6.86$ (m, 4H), 6.82–6.77 (m, 4H), 6.34 (d, $J = 6.7$ Hz, 2H), 6.27 (d, $J = 6.7$ Hz, 2H); ¹³C{¹H} NMR (100 MHz, CDCl₃): $\delta = 150.9, 150.2, 149.5, 147.3, 137.2, 135.6, 128.8, 128.4, 119.0, 118.1, 116.5, 114.4$; IR (KBr): $\tilde{\nu} = 3054, 1448, 1414, 1360, 1345, 1252, 1156, 1057, 936, 834, 820, 746, 731$ cm⁻¹; UV/Vis (THF): $\lambda_{\max}$ (lge) = 256 (4.14), 278 (4.44), 284 (4.47), 299 (4.76), 313 (5.03), 338 (4.28), 356 (4.33), 376 (4.29), 399 (3.93), 465 (3.15) nm. High-resolution MS calcd for C₂₄H₁₂: 300.093900; found: 300.093896.

1b: light orange crystals, m.p. 209–210 °C; MS (70 eV): m/z (%): 444 ($[M^+]$, 100), 429 (8), 413 (15), 371 (10), 356 (12), 341 (8), 207 (10), 168 (12), 73 (25); ¹H NMR (400 MHz, CDCl₃): $\delta = 7.12$ (s, 2H), 6.89 (m, 2H), 6.80 (m, 2H), 6.39 (d, $J = 6.7$ Hz, 1H), 6.34 (d, $J = 6.4$ Hz, 1H), 6.31 (d, $J = 6.8$ Hz, 1H), 6.28 (d, $J = 6.4$ Hz, 1H), 0.36 (s, 9H), 0.35 (s, 9H); ¹³C{¹H} NMR (100 MHz, CDCl₃): $\delta = 151.3, 150.8, 150.2, 149.8, 149.5, 148.6, 148.4, 147.9, 147.4, 147.2, 138.2, 137.1, 135.9, 135.7, 128.8, 128.3, 124.4, 123.6, 118.9, 118.1, 116.6, 116.4, 114.7, 114.4, 2.17, 2.15$; IR (KBr): $\tilde{\nu} = 2953, 1543, 1509, 1458, 1411, 1345, 1247, 1055, 855, 828, 754, 730, 669$ cm⁻¹; UV/Vis (THF): $\lambda_{\max}$ (lge) = 259 (4.22), 289 (4.51), 304 (4.77), 318 (4.99), 341 (4.30), 361 (4.34), 381 (4.31), 468 (3.06) nm. High-resolution MS calcd for C₃₀H₂₈Si₂: 444.172958; found: 444.172806.

2a: dark orange powder, M.p. > 260 °C (decomp); MS (70 eV): m/z (%): 374 ($[M^+]$, 100), 372 (18), 187 (10), 78 (34), 69 (46), 57 (48), 55 (50); ¹H NMR (300 MHz, CD₂Cl₂): $\delta = 6.93-6.89$ (m, 4H), 6.86–6.79 (m, 4H), 6.51 (s, 2H), 6.26, 6.23 (AB, $J = 6.6$ Hz, 4H); UV/Vis (CH₂Cl₂): $\lambda_{\max}$ (lge) = 315 (3.97), 326 (4.01), 350 (3.78), 366 (3.79), 484 (2.45) nm. High-resolution MS calcd for C₃₀H₁₄: 374.1096; found: 374.1098.

2b: orange-yellow crystals, M.p. > 300 °C; MS (70 eV): m/z (%): 526 ($[M^+]$, 100), 446 (15), 263 (18), 149 (6); ¹H NMR (500 MHz, CDCl₃): $\delta = 7.31$ (dd, $J = 7.2, 1.0$ Hz, 4H), 6.98–6.93 (m, 4H), 6.89(m, 2H), 6.86(m, 2H), 6.78 (br dd, $J = 7.8, 7.4$ Hz, 4H), 6.68 (br dd, $J = 7.5, 7.4$ Hz, 2H), 6.58 (s, 2H), 6.42 (s, 2H); ¹³C{¹H} NMR and DEPT 90 (100 MHz, CDCl₃): $\delta = 151.0$ (C), 150.2 (C), 148.9 (C), 147.7 (C), 143.3 (C), 138.5 (C), 136.1 (C), 135.5 (C), 134.6 (C), 131.2 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.2 (CH), 125.9 (CH), 119.0 (CH), 118.4 (CH), 116.1 (CH), 115.5 (CH); IR (KBr): $\tilde{\nu} = 3053, 1415, 1336, 1149, 814, 744, 737, 694$ cm⁻¹; UV/Vis (CH₂Cl₂): $\lambda_{\max}$ (lge) = 261 (sh, 4.71), 280 (4.82), 321 (4.93), 339 (4.78), 362 (4.59), 382 (4.64), 446 (4.06), 476 (4.04), 504 (3.60) nm. High-resolution MS calcd for C₄₂ H₂₂: 527.1755; found: 527.1758.

2c: yellow crystals, m.p. 269–270 °C; MS (70 eV): m/z (%): 458 ($[M^+]$, 100), 413 (14), 398 (5), 229 (12), 207 (13), 199 (10), 91 (15), 57 (9); ¹H NMR (400 MHz, CDCl₃): $\delta = 6.96-6.91$ (m, 4H), 6.83–6.79 (m, 4H), 6.49 (s, 2H), 6.13 (s, 2H), 2.44 (t, $J = 7.5$ Hz, 4H), 1.63 (sex, $J = 7.5$ Hz, 4H), 0.97 (t, $J = 7.4$ Hz, 6H); ¹H NMR (500 MHz, CD₂Cl₂): $\delta = 6.96-6.91$ (m, 4H), 6.89–6.82 (m, 4H), 6.54 (s, 2H), 6.18 (s, 2H), 2.46 (t, $J = 7.3$ Hz, 4H), 1.63 (sex, $J = 7.3$ Hz, 4H), 0.99 (t, $J = 7.4$ Hz, 6H); ¹³C{¹H} NMR (100 MHz, CD₂Cl₂): $\delta = 150.9, 150.8, 149.6, 147.8, 145.0, 137.7, 135.3, 134.8, 132.4, 128.9, 128.8, 119.1, 118.5, 116.9, 116.4, 34.4, 23.4, 13.7$; IR (KBr): $\tilde{\nu} = 3059, 2953, 2928, 2869, 1415, 1340, 1158, 867, 829, 742$ cm⁻¹; UV/Vis (CH₂Cl₂): $\lambda_{\max}$ (lge) = 278 (4.37), 318 (4.81), 327 (4.83), 350 (sh, 4.57), 371 (4.65), 381 (sh, 4.61), 400 (sh, 4.30), 444 (3.90), 488 (3.48) nm. High-resolution MS calcd for C₃₆H₂₆: 458.2035; found: 458.2040.

8: pale yellow crystals, m.p. > 200 °C (decomp); MS (70 eV): m/z (%): 490 ($[M^+]$, 100), 461 (9), 368 (12), 289 (10), 256 (18); ¹H NMR (400 MHz, CD₂Cl₂): $\delta = 7.50-7.38$ (m, 4H), 7.08 (d, $J = 7.3$ Hz, 1H), 7.00 (m, 2H), 6.98 (d, $J = 7.4$ Hz, 1H), 6.97 (m, 1H), 6.92 (m, 1H), 6.34 (t, $J = 1.6$ Hz, 1H), 6.17 (s, 1H), 2.34–2.14 (m, 4H), 1.68–1.42 (m, 4H), 1.01 (t, $J = 7.4$ Hz, 3H), 0.89 (t, $J = 7.3$ Hz, 3H); IR (KBr): $\tilde{\nu} = 3070, 2962, 2927, 2855, 1687, 1262, 1097, 1018, 802, 744$ cm⁻¹.

Because the structural features of **2c** are identical within experimental error with those of its angular isomer,^[1c] we can be confident that the following arguments apply equally to both the zigzag and angular series. Thus, in angular [3]phenylene, maximizing the aromatic π delocalization in the terminal benzene rings (in addition to σ strain effects)^[1a] causes

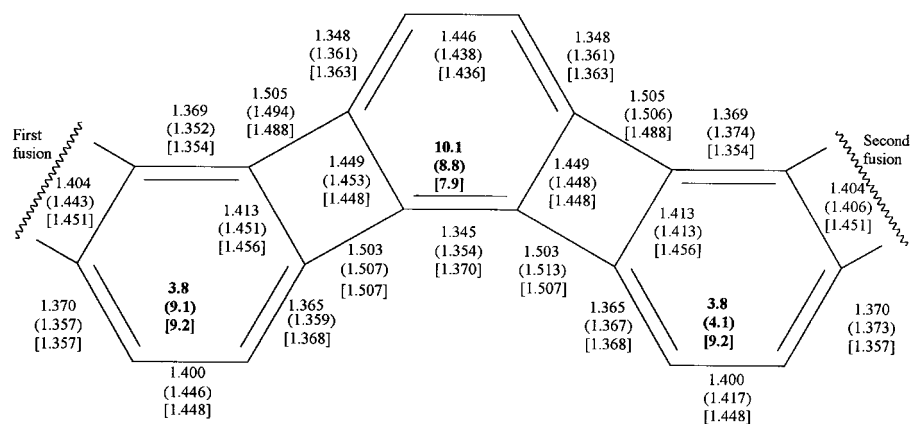


Figure 3. Comparative bond length data [Å] for angular [3]phenylene,^[1a] the unsubstituted portion of **1b** (in parentheses), and the internal fragment of **2c** (in brackets; numbers are averaged for ideal symmetry). Bold numbers indicate the extent of bond alternation (in pm) according to the equation $[\Sigma \text{ single bonds} - \Sigma \text{ double bonds}]/3 (\times 100)$.

pronounced bond alternation in the central cyclohexatriene. Additional single annelation of one terminus, as in **1**, compromises delocalization in the latter, thus attenuating the extent of bond alternation of the "original" cyclohexatriene, that is in **1** the two internal benzenes are less π -bond localized than their counterpart in angular [3]phenylene, while the terminal six-membered rings (exemplified in Figure 3 for **1b** by its unsilylated end) remain essentially unchanged. A second annelation, as in **2**, has more subtle consequences, arguably (considering standard deviations and the presence of substituents in **2b** and **2c**) reinforcing bond alternation in the noncentral internal benzene rings, while somewhat attenuating it in the central ring. The terminal benzene nuclei (not shown in Figure 3) again retain a geometry essentially identical to that of their counterparts in angular [3]- and zigzag [4]phenylene, with minimal bond alternation and, as a consequence, considerably shortened bonds of fusion to the adjacent four-membered rings, signaling relatively stronger cyclobutadienoid, hence paratropic, character in the latter (compared to their "inside" counterparts), in agreement with NICS calculations^[6a] and ¹H NMR data (vide supra). It is noteworthy that the experimental structural data are reproduced closely by calculations (for the parent systems)^[5a, 6d,g] at the HF/6-31G*, and even better at the B3LYP/6-31G* density functional levels. Finally, the previously observed^[1b,c] and calculated^[2a, 5a, 6c,d,g] ready deformability of the phenylenes is evident in Figures 1 and 2 (side views). For **1b** the dihedral angles between the mean planes of adjacent rings range from 0.94 to 5.62°, for **2b** from 0.59 to 2.55°, and for **2c** from 1.15 to 2.53°.

While a thorough investigation of the chemistry for **1** and **2** will be the subject of future efforts, some preliminary experiments on **2c** appear to be consistent with the conclusions presented above. Thus, **2c** is inert to catalytic hydrogenation under conditions (5% Pd/C, 12.2 atm (180 psi)) which lead to hexahydro angular [3]phenylene,^[1a] and, in contrast to angular [3]phenylene,^[10] it does not react with dimethyl butynedioate in the presence of AlCl₃, indicative of lesser cyclohexatrienic reactivity.

Interestingly, however, the compound (in CH₂Cl₂) transforms (albeit in low yield), slowly in air, faster on exposure to O₂, while irradiating with visible light, to give enyne-(Z)-dione **8**,^[7, 8] stereo- and regiospecifically, possibly through perepoxide **7** (Scheme 4), in analogy to the similar oxidation of linear [3]phenylene,^[1a] perhaps signaling increased activation of the affected benzene nuclei relative to the central ring. The spectral data of **8** are as expected for a substituted angular [3]phenylene, and the X-ray determination reveals a highly nonplanar frame (Figure 4) with a distorted ten-

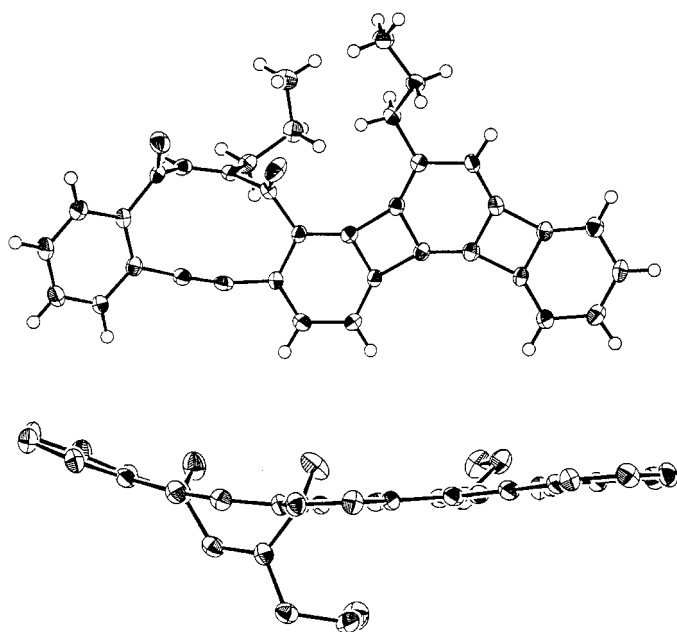
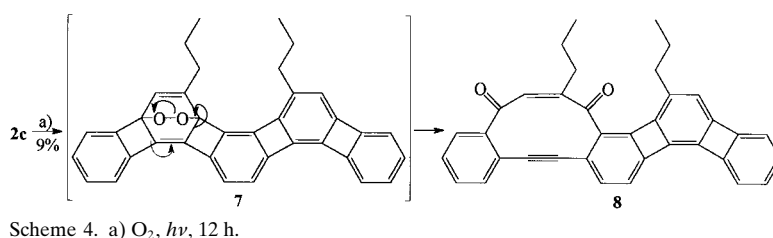


Figure 4. Structure of **8** in the crystal: views from above (top) and the side (bottom).

membered ring in which the double bond, the two oxo functions, and the fused benzene rings are held at relatively large angles to each other.

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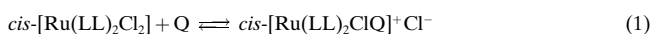
Keywords: antiaromaticity • hydrocarbons • phenylenes • polycycles • strained molecules

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- [7] All new isolated compounds gave satisfactory analytical and/or spectral data (see Table 1).
- [8] X-ray structural analyses: **1b**: Crystal size $0.38 \times 0.34 \times 0.20$ mm³, space group *P2₁/c* (no. 14), scan range $3 < 2\theta < 52.30^\circ$, $a = 16.5909(3)$, $b = 9.1224(1)$, $c = 17.5914(2)$ Å, $\beta = 108.089(1)^\circ$, $V = 2430.84(8)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.167$ g cm⁻³, $\mu = 1.55$ cm⁻¹, 4743 unique reflections at -85°C , of which 3320 were taken as observed [$F_o > 3.00\sigma(F)$], $R = 0.036$, $R_w = 0.046$. **2b**: Crystal size $0.30 \times 0.25 \times 0.20$ mm³, space group *Pbcn* (no. 60), scan range $4.00 < 2\theta < 45.00^\circ$, $a = 22.9475(3)$, $b = 12.8479(2)$, $c = 8.7785(2)$ Å, $V = 2588.14(7)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.351$ g cm⁻³, $\mu = 0.77$ cm⁻¹, 2582 unique reflections at -101°C , of which 1551 were taken as observed [$F_o > 3.00\sigma(F)$], $R = 0.037$, $R_w = 0.042$. **2c**: Crystal size $0.25 \times 0.10 \times 0.10$ mm³, space group *P2₁2₁2₁* (no. 19), scan range $4.00 < 2\theta < 45.00^\circ$, $a = 25.3045(3)$, $b = 5.3273(1)$, $c = 18.5416(4)$ Å, $V = 2499.49(7)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.219$ g cm⁻³, $\mu = 0.69$ cm⁻¹, 4270 unique reflections at -108°C , of which 2444 were taken as observed [$F_o > 3.00\sigma(F)$], $R = 0.054$, $R_w = 0.065$. **8**: Crystal size $0.41 \times 0.24 \times 0.02$ mm³, space group *Pbca* (no. 61), scan range $3.00 < 2\theta < 45.00^\circ$, $a = 20.9160(5)$, $b = 7.5075(2)$, $c = 32.6655(7)$ Å, $V = 5129.35(29)$ Å³, $Z = 8$, $\rho_{\text{calc}} = 1.270$ g cm⁻³, $\mu = 0.77$ cm⁻¹, 4210 unique reflections at -118°C , of which 1873 were taken as observed [$F_o > 3.00\sigma(F)$], $R = 0.029$, $R_w = 0.030$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-103129 (**8**), 103130 (**2c**), 103131 (**2b**), 103132 (**1b**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Coordinative Approach to Mediated Electron Transfer: Ruthenium Complexed to Native Glucose Oxidase**

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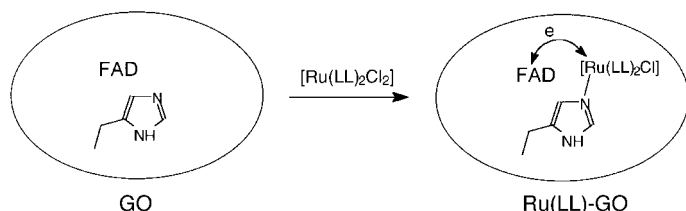
Mediated electron transfer to or from active sites of oxidoreductases^[1] is often accomplished by covalent binding of a redox-active species either to the enzyme surface^[2–5] or to the enzyme active site.^[6] Transition metal complexes such as ferrocene derivatives and osmium compounds are most commonly used. In addition, there are reports on using ruthenium compounds;^[7, 8] however, these did not find widespread application, and this seems rather surprising taking into account the lower cost of Ru compounds and their higher reactivity toward glucose oxidase (GO)^[9] with respect to related Os complexes. Also, an attractive feature of ruthenium complexes of the type *cis*-[Ru(LL)₂Cl₂] (LL = bpy or phen-type ligands) is associated with their ability to coordinate monodentate nitrogen donor ligands like pyridine or imidazole (Q) [Eq. (1)].



If proteins are considered as potential ligands, enzymes appear to be superior, since many of them, including oxidoreductases, contain histidine residues in their active sites. Therefore, it was anticipated that the interaction between an enzyme and *cis*-[Ru(LL)₂Cl₂] would result in binding of the ruthenium center to the imidazole side chain of histidine in such a way that it is delivered close to the active site and thus provides an efficient electron transfer relay (Scheme 1). Glucose oxidase from *A. niger* is a very advantageous enzyme because it contains two imidazole side chains, His 516 and H 559, close to FAD.^[10] Therefore we decided to carry out a “coordinative” loading of the complexes *cis*-[Ru(LL)₂Cl₂] (LL = bpy (**1**) and phen (**2**)) into GO and to investigate electrocatalytic characteristics of ruthenium-modified GO (Ru(LL)-GO). As a result, we succeeded in

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Scheme 1. Schematic illustration of binding of a Ru complex with glucose oxidase and its relay function.

preparing an extremely active modified biocatalyst with superior electrocatalytic properties.

The redox potential of the $\text{Ru}^{\text{III}} + e \rightleftharpoons \text{Ru}^{\text{II}}$ half-reaction is almost ideal for coupling with GO. Since Ru^{II} and Ru^{III} compounds have drastically different abilities to undergo ligand exchange,^[11] the latter are practically inert to ligand substitution,^[9] compounds of Ru^{II} should be used in the complex formation reaction with GO. After the complex formation, Ru^{II} should preferably be oxidized to Ru^{III} in order to keep the redox species more strongly bonded to the protein donor groups. A two-step procedure is utilized, which is reminiscent of development and fixation of black-and-white photographic films: complexation (development) and dialysis (fixation) were performed at different pH values.

Differential pulse voltammetry demonstrated that **2** interacts with imidazole in buffered aqueous solution at pH 5.5. Addition of two equivalents of imidazole to a solution of **2** results in a potential shift from 382 to 330 mV (Figure 1 a,b). Remarkably, a very similar shift, that is from 382 to 340 mV, is

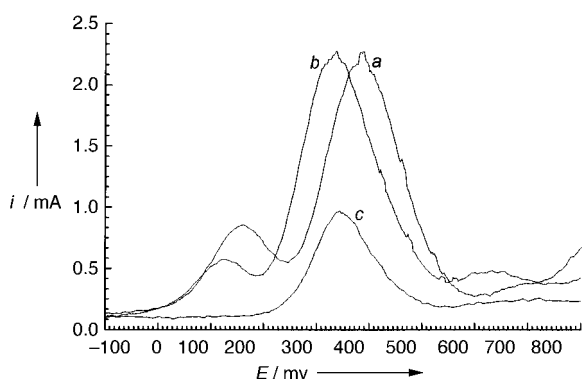


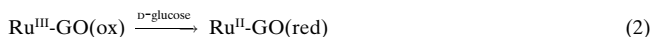
Figure 1. Differential pulse voltammograms of **2** ($9.8 \times 10^{-4} \text{ M}$; 0.01 M phosphate, pH 5.5, 25°C , scan rate 20 mV s^{-1}) a) without addition of imidazole, b) in the presence of two equivalents of imidazole, and c) in the presence of 0.14 equivalents of GO.

observed on addition of GO to the solution of **2** (Figure 1 c). The potential shift is accompanied by a 2.5-fold decrease in current which is in agreement with the estimated value of 2.3 made on assumption that the complex is completely bound to a macromolecule of 150 000 Da.^[12] The presence of one peak in Figure 1 c strongly suggests that Ru^{II} is completely bound to the enzyme and that the binding involves similar donor groups, very likely imidazole side chains of histidine residues. The same conclusions were reached in the case of complex **1**. It is known that Ru^{III} derivatives obtained, for instance, from **2** are readily reduced by D-glucose in the presence of GO, and

that this interaction can be monitored by using UV/Vis spectroscopy.^[9] The same behavior was confirmed by a cyclic voltammetry study. Both **1** and **2** display effective intermolecular coupling with GO, and the corresponding second-order rate constants k for the oxidation of reduced GO by an electrochemically generated Ru^{III} species are equal ($1.5 \pm 0.2 \times 10^5$ ^[9] and $(0.90 \pm 0.06) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively, (25°C , pH 7 (0.01 M phosphate), [glucose] 0.033 M). Interestingly, at pH 7 (adjusted using 0.1 M phosphate) the rate constants are lower; in the case of **1** the rate constant k is $(5.4 \pm 0.2) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. However, under these conditions reaction (1) generates species $\text{cis-}[\text{Ru}(\text{bpy})_2\text{ClQ}]^+$ ($\text{Q} = \text{py}$, imidazole or histidine) with increased formal redox potential which moves anodically by 182, 106, and 104 mV, respectively, relative to E° of **1** (300 mV). This drastically increases k and the corresponding rate constants are $(4.15 \pm 0.01) \times 10^5$, $(3.6 \pm 0.2) \times 10^5$, and $(9.6 \pm 0.4) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Rather surprisingly, the effect practically disappears in 0.01 M phosphate.

The complexation between **1** or **2** and GO was carried out in buffered phosphate solution (0.01 M) at pH 5.5; ratio of GO to Ru^{II} was 1:3.5 and 1:7, respectively. Under these conditions, Ru^{II} is not oxidized by dioxygen into Ru^{III} . Hence, the substitutionally labile species is involved in the “development” step. The dialysis is carried out against the same buffer of pH 7.0. At this pH value, slow oxidation into Ru^{III} is evident, and after 48 h practically all the ruthenium is converted into Ru^{III} . Thus, the ruthenium species becomes fixed to the donor groups of the enzyme. In the case of the above indicated GO to Ru^{II} ratios, all ruthenium binds to the enzyme. It was verified that the properties of the Ru-enriched enzyme do not change after gel filtration, and it is the dialysis coupled with the oxidation that is required for preparation of the active biocatalyst.

The Ru-modified GO (Ru(LL)-GO) was shown to preserve both high catalytic activity and stability. A commonly observed range of the catalytic activity measured spectrophotometrically with respect to 2,6-dichlorophenolindophenol (DCIP)^[13] is 73–82 % as compared to native GO. The stability of Ru(LL)-GO is significant and any loss in activity was not observed after keeping a solution of Ru(LL)-GO for one month at pH 7 and 5°C . Its functional stability is indistinguishable from that of native GO. UV/Vis spectra of Ru(LL)-GO are a superposition of that of GO and Ru^{III} (Figure 2). In particular, Ru(phen)-GO has maxima at 455 and 648 nm arising from FAD and Ru^{III} , respectively (Figure 2 a). Addition of D-glucose induces a color change of Ru(phen)-GO from greenish to purple (Figure 2 b), which is attributed to conversion of the coordinated Ru^{III} into Ru^{II} [Eq. (2); GO(ox) and GO(red) are the oxidized and reduced forms of the enzyme, respectively]. As a result, the spectrum of



reduced Ru(phen)-GO in the range 400–800 nm is similar to that of **2** recorded in the presence of imidazole (Figure 2 c). In particular, the band at 648 nm disappears and the intensity of the 455 nm band drops since FAD is reduced to FADH_2 .^[14] There is a shift in the absorption maximum to 467 nm which

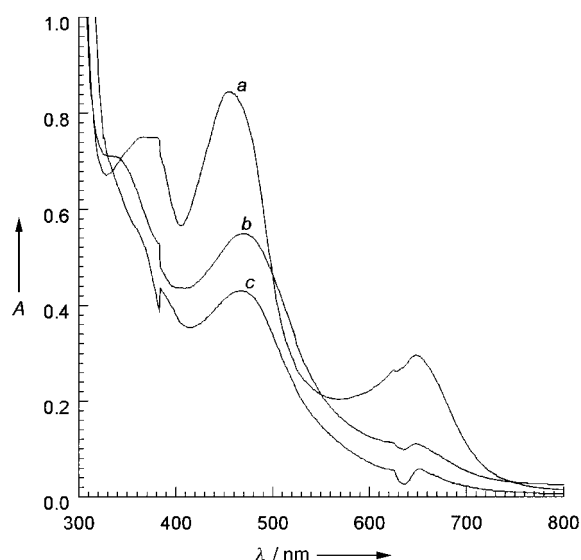


Figure 2. UV/Vis spectra of Ru(phen)-GO (3.4 mg mL⁻¹) a) before and b) after reduction by excess of D-glucose (0.1M). c) UV/Vis spectrum of **2** in the presence of imidazole. Conditions for all spectra: pH 7 (0.01M phosphate), 22 ± 2 °C.

corresponds to Ru^{II}. Since the enzymatic activity is about 75% and assuming that Ru^{II} is the only absorbing species at this wavelength, the amount of the “catalytically active” ruthenium can be estimated by using the extinction coefficient obtained for **2** in the presence of imidazole. Calculations suggest that Ru(phen)-GO and Ru(bpy)-GO have three and one active ruthenium centers, respectively.

The electrocatalytic properties of the Ru(LL)-GO preparations as demonstrated by cyclic voltammetry are presented in Figure 3. There are only weak signals from ruthenium coordinated to glucose oxidase in the absence of D-glucose at 350 and 324 mV for Ru(phen)-GO and Ru(bpy)-GO, respectively, at a scan rate of 10 mV s⁻¹ (traces a). Traces b in

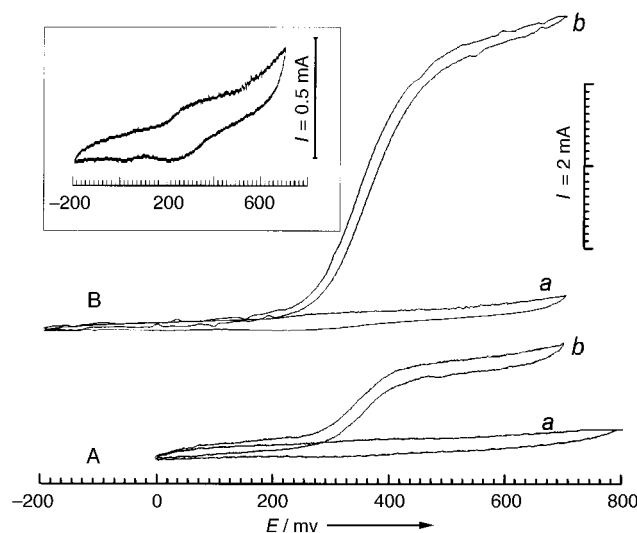


Figure 3. Cyclic voltammograms of Ru(bpy)-GO (A) and Ru(phen)-GO (B) a) in the absence and b) in the presence of D-glucose (0.033M). [Ru(bpy)-GO] 12.7, [Ru(phen)-GO] 17.1 mg mL⁻¹, pH 7 (0.01M phosphate buffer), scan rate 10 mV s⁻¹. The inset shows magnified voltammogram of Ru(phen)-GO in the absence of D-glucose.

Figure 3 recorded in the presence of D-glucose emphasize the impressive electrocatalytic properties of Ru(LL)-GO. In fact, the amplification coefficient I/I_0 is 12.8 for Ru(bpy)-GO and 66.7 for Ru(phen)-GO, (at 500 and 480 mV, respectively, and 10 mV s⁻¹ scan rate). The amplification coefficients obtained for both preparations are summarized in Table 1. Remarkably,

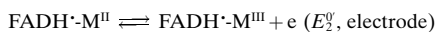
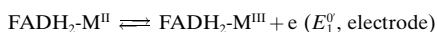
Table 1. Amplification coefficients I/I_0 at 500 (Ru(bpy)-GO) and 480 mV (Ru(phen)-GO) obtained at different scan rates ν ; 25 °C, pH 7 (0.01M phosphate).

	ν [mV s ⁻¹]	I/I_0		ν [mV s ⁻¹]	I/I_0
Ru(bpy)-GO	2	40.9	Ru(phen)-GO	5	93.7
	5	37.8		10	66.7
	10	12.8		20	47.6
				30	20.5
				40	18
				100	7.1

these are significant even at high scan rates and this makes the ruthenium-modified catalysts very promising for biosensor applications.

In order to compare the efficacy of Ru(LL)-GO with the enzyme modified by other redox species, the rate constants for intramolecular electron transfer were estimated by computer simulation of the data in Figure 3 by known methods.^[15]

We applied the model summarized in Scheme 2 and obtained the rate constants 70 s⁻¹ for Ru(phen)-GO and



assumptions: $k_1 = k_2$, $E_1^0 = E_2^0$,^[16] $k_3 = 800 \text{ s}^{-1}$ (saturating glucose concentration)^[14]

Scheme 2. The model applied for the computer simulation.

12 s⁻¹ for Ru(bpy)-GO. For comparison the highest rate constant reported for GO randomly modified with 13 ferrocenecarboxylic acid residues is 3.6 s⁻¹.^[16] We also showed that random modification of GO with 4-pyridylacetic acid followed complexation with **1** resulted in the catalyst with $k_1 = 0.55 \text{ s}^{-1}$. Advantages of the coordinative modification require no further comment.

In conclusion, the extremely simple coordinative modification of GO with Ru^{II}(bpy) or Ru^{II}(phen) complexes affords very catalytically and electrocatalytically active, stable biocatalysts which provide an unprecedentedly high amplification coefficient I/I_0 even at high scan rates and, correspondingly, very high rates of intramolecular electron transfer. This strongly suggests that the ruthenium centers are located close to the enzyme active site and coordinated presumably to 516 and/or 559 histidines.

Experimental Section

Electrochemical measurements were carried out using a potentiostat/galvanostat Model 273A (EG&G Princeton Applied Research). A three-electrode scheme was used with a working glassy carbon electrode, saturated AgCl/Ag reference electrode, and an auxiliary Pt electrode. GO from *Aspergillus niger* (EC1.1.3.4, 285 U mg⁻¹) was a Serva preparation. Ruthenium(II) complexes were prepared as described.^[17]

Modification of GO by Ru^{II}: To a solution of **2** (1 mL, 9.8×10^{-4} M) in 0.01 M phosphate buffer (pH 5.5) a weighed amount of GO (20.5 mg) was added (Ru:GO ratio 7:1) to yield a brownish red solution. The mixture was kept at room temperature for 4 h during which the solution faded, which was attributed to binding to GO and partial oxidation of Ru^{II} to Ru^{III}. The solution was then dialyzed against 0.01 M phosphate buffer (1 L, pH 7.0) for 48 h at 5 °C, and the buffer was changed at least twice. A green solution was finally formed indicative of complete oxidation of Ru^{II} to Ru^{III}. Analysis of the dialyzed solution by differential pulse voltammetry indicated the absence of free ruthenium complex not bound to GO. The amount of protein in the sample as determined according to the Lowry method was 17.1 mg mL⁻¹; the activity determined spectrophotometrically with respect to DCIP was 73.4% relative to the native GO with 100% activity. **Modification of GO by 1** was carried out in a similar way by using a Ru to GO ratio of 3.5:1. The activity was 82%.

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Neutral, Cationic, and Anionic Alkyl Derivatives of Tungsten Bonded to a Calix[4]arene Oxo Surface**

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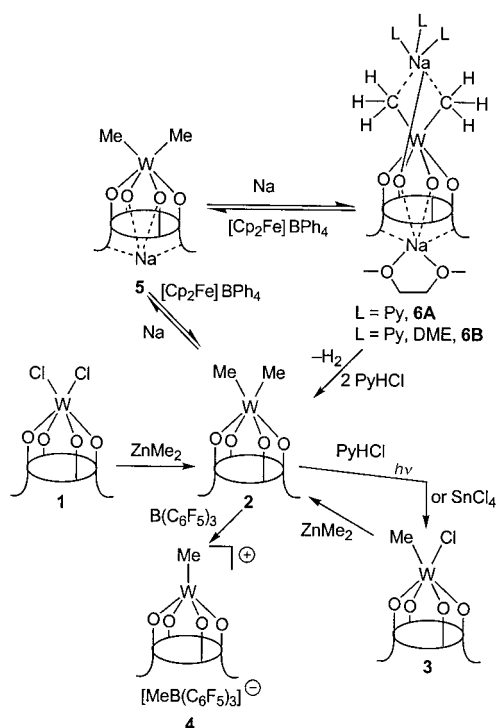
Particular attention is focused on those systems which can mimic at the molecular level the chemical behavior of M–C functionalities bonded to an oxo surface.^[1] The most common approach employs alkoxo and phenoxo groups as ancillary ligands.^[2] However, a quite recent and significant advance in the field was achieved with the preorganized set of oxygen donor atoms from calix[4]arenes,^[3] which bear a number of unique peculiarities^[4] within this context. We report here the generation of a dimethyl tungsten(vi) derivative bonded to an O₄ set of donor atoms, its conversion into an unsaturated monomethyl cation, and its very peculiar redox behavior.

The alkylation of *cis*-(Cl)₂W{*p*-*t*Bu-calix[4]-(O)₄}[^{5]} (**1**) is strongly dependent on the alkylating agent. The use of carbanionic, reducing species, like lithium and magnesium organometallic compounds, led straight to the corresponding anionic alkylidynes,^[4b] while the use of ZnMe₂ in toluene in a 1:1 ratio led cleanly to *cis*-(Me)₂W{*p*-*t*Bu-calix[4]-(O)₄}] (**2**, Scheme 1). The use of smaller amounts of ZnMe₂ produced mixtures of **1** and **2**. The monoalkyl derivative [(Cl)(Me)W{*p*-*t*Bu-calix[4]-(O)₄}] (**3**) could be best prepared by photolysis of **1** in CH₂Cl₂ in the presence of an excess of pyridinium hydrochloride (PyHCl). As **1** does not react with PyHCl in the dark and the photolysis of **1** in C₆D₆ led to evolution of methane and ethylene (¹H NMR), we believe that PyHCl traps an unstable methylene species generated by photolysis.^[6, 7] Compound **3** was also obtained by a ligand exchange reaction of **2** with SnCl₄.

The apparent C_{4v} symmetry of the calix[4]arene fragment, as determined from the ¹H NMR spectra at 298 and 193 K, is a consequence of the equal availability of d_{xz} and d_{yz} as well as d_{z²} orbitals for binding of the two methyl groups. This result emphasizes the very high mobility of the alkyl groups between two equivalent positions over the oxo surface and the absence of a significant energy barrier for movement of the methyl groups between them. In the case of **3**, the C_{4v} symmetry at 298 K is modified to C_s in the ¹H NMR spectrum measured at 188 K. The difference in the metal-to-ligand bonding mode for Me (σ only) and Cl substituents (σ and π) is a factor in the increased barrier to mobility of the ligands Me and Cl over the oxo surface.

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Scheme 1. Synthesis and reactivity of **2**. Py = C₅H₅N.

Complexes **1–3** do not undergo any ligand exchange reaction.^[4d] The methyl group can be transferred to particularly strong Lewis acids—namely, [B(C₆F₅)₃]^[8]—to form the cationic monomethyl derivative [(Me)W(*p*-*t*Bu-calix[4]-(O)₄)]⁺[MeB(C₆F₅)₃][−] (**4**), whose proposed structure is supported by an X-ray analysis (Figure 1).^[9]

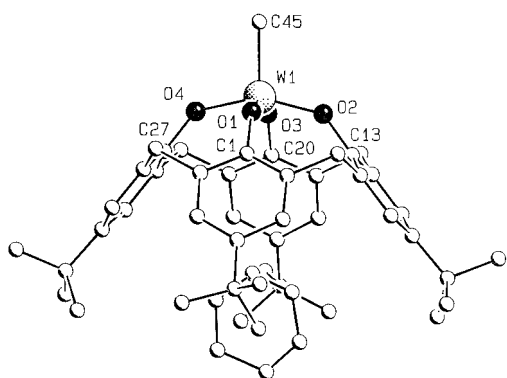


Figure 1. SCHAKAL view of the cation in complex **4**. Selected bond lengths [Å]: W–O 1.837 (av), W1–C45 2.055(8).

The tungsten atom in **4** exhibits a square-pyramidal coordination geometry. The W–C45 bond length (2.055(8) Å) is in agreement with a W–C σ bond.^[10] The W–C45 direction is perpendicular to the plane formed by the O₄ core, from which tungsten is displaced by 0.425(1) Å towards the methyl group. The W–O bond lengths, which are not significantly different from each other (mean value 1.836(3) Å), are in agreement with a significant metal–oxygen π interaction.^[11] The calix[4]arene unit is in a cone conformation, as is usually found in square-pyramidal penta-coordinated metals.^[4, 12]

A particularly interesting reaction is the stepwise reduction of **2** with sodium metal (Scheme 1). Such a reaction is related to what occurs in the solid state rather than in molecular organometallic systems. Complex **2** can be reduced^[13] to *cis*-[(Me)₂W(*p*-*t*Bu-calix[4]-(O)₄)Na] (**5**) and subsequently to *cis*-[(Me)₂W(*p*-*t*Bu-calix[4]-(O)₄)Na₂] (**6**). The reduction of **2** does not produce any change in connectivity for the overall structure. Compounds **5** and **6** can be oxidized back to **2** with stoichiometric amounts of [Cp₂Fe]BPh₄ (Cp = C₅H₅). The diamagnetism of complex **6** is particularly significant in view of the two available d_{xy} and d_{yz} frontier orbitals centered at the metal,^[4d, 4e] and supports a significant energy separation between them (d_{xy} is lower in energy). The NMR spectrum of **6** shows peculiar chemical shifts of the calix[4]arene skeleton which are highly affected by the temperature.

Complex **6** was crystallized from pyridine/1,2-dimethoxyethane (DME), and its structure determined (Figure 2).^[14] In the asymmetric unit there are two crystallographically independent [(Me)₂W(*p*-*t*Bu-calix[4]-(O)₄)Na(dme)][−] ions interacting through the O4 oxygen atom with a sodium cation,

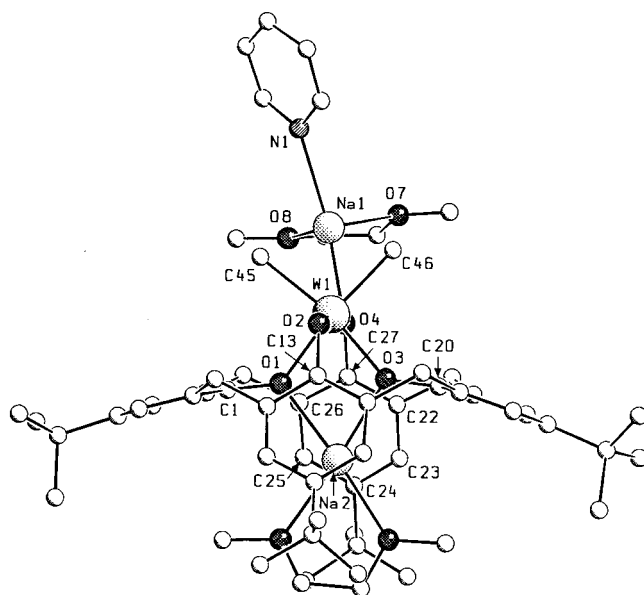


Figure 2. SCHAKAL view of molecule A in complex **6**. Selected bond lengths [Å] and angles [°]: W–O 2.031 (av), W1–C45 2.197(14), W1–C46 2.208(16); C45–W1–C46 96.4(6), O2–W1–O4 167.8(4), O1–W1–O3 77.9(3).

which is bonded to three pyridine molecules in the A anion and to a pyridine and a dme molecule in the B anion.^[12] The geometries of the two anions are very similar. As a consequence of the hexacoordination of tungsten,^[5] the calixarene ligand assumes a flattened cone conformation. The alkali cation is solvated by oxygen atoms from the calix[4]arene and coordinated dme molecule, and interacts in a η⁶ fashion with the arene (C22–C27).^[12]

The tungsten atom of **6** exhibits a pseudo-octahedral coordination involving the O₄ core of the calixarene and two methyl groups (C45, C46). Bonds in the coordination sphere of tungsten are lengthened with respect to the corresponding ones in complex **4**; the lengthening (as calculated with the mean values) are 0.206(9) and

0.126(15) Å for the W–O and W–C bonds, respectively. The methyl groups should be considered as bridging tungsten and sodium ions, the contact distances being rather short (Na1...C45 2.844(15), Na1...C46 3.023(17) Å). The occurrence of **6** in such an ion-pair form with two methyl groups chelating a sodium cation is extremely promising for the chemical reactivity of the alkyl functionality assisted by two metal centers.

Complex **6** might be considered as being formally obtained by the addition of two NaMe units to the d² [W(calix[4]-arene)] fragment. However, it does not readily undergo alkyl transfer reactions, which is typical of metalated species,^[15] but rather can be oxidized back to **2** without affecting the metal–carbon functionalities. The reaction of **6** with proton source like PyHCl, which leads back to **2** and hydrogen, is emblematic of the peculiarities of the system. Such a redox behavior can be explained by protonation of one of the oxygen atoms (the most basic site of the system) followed by an electron transfer from the metal-centered HOMO to the proton by the intermediacy of the oxygen, which can form a π bond with the metal. Such a behavior is quite reminiscent of a metal–oxo surface, more than a molecular system, and sheds light on the very peculiar role of the calix[4]arene ligand, making the comparison with the metal–oxo surface particularly valuable.^[16]

Experimental Section

2: A 2.0 M solution of ZnMe₂ (17.2 mL, 34.4 mmol) in toluene was added to a suspension of **1**·2 C₄H₈ (30.4 g, 28.1 mmol) in toluene (800 mL), and the resulting mixture was stirred at room temperature overnight and then at 50 °C for 5 h to give a red suspension. Bu₄NCl (8.14 g, 29.3 mmol) was added, and the suspension was stirred for 1 h at 100 °C. A solid was filtered off the hot mixture, volatile components were evaporated, and the residue was washed with pentane (300 mL) and dried in vacuo to give red **2** (yield: 18.1 g, 73 %). ¹H NMR (200 MHz, CD₂Cl₂, 298 K): δ = 7.19 (s, 8H; ArH), 4.20 (d, J (H,H) = 12.8 Hz, 4H; *endo*-CH₂), 3.36 (d, J (H,H) = 12.8 Hz, 4H; *exo*-CH₂), 2.62 (s, 6H; Me), 1.24 (s, 36H; *t*Bu); ¹H NMR (400 MHz, CD₂Cl₂, 193 K): δ = 7.15 (s, 8H; ArH), 4.02 (d, J (H,H) = 13.3 Hz, 4H; *endo*-CH₂), 3.34 (d, J (H,H) = 13.3 Hz, 4H; *exo*-CH₂), 2.47 (s, 6H; Me), 1.16 (s, 36H; *t*Bu); elemental analysis calcd for C₄₆H₅₈O₄W (%): C 64.33, H 6.81; found: C 64.48, H 6.98.

3: A mixture of PyHCl (0.96 g, 8.30 mmol) and **2** (4.22 g, 4.92 mmol) in CH₂Cl₂ (200 mL) was irradiated under stirring for 48 h with a Xe lamp (540 W m⁻² at 340 nm). Volatile components were evaporated in vacuo, Et₂O (150 mL) was added, and the mixture was stirred overnight and then filtered. Volatile components were evaporated, and dark brown **3** was washed with pentane (100 mL), collected, and dried in vacuo (yield: 1.90 g, 43 %). ¹H NMR (200 MHz, CDCl₃, 298 K): δ = 7.14 (s, 8H; ArH), 4.70 (d, J (H,H) = 13.2 Hz, 4H; *endo*-CH₂), 3.37 (d, J (H,H) = 13.2 Hz, 4H; *exo*-CH₂), 2.82 (s, 3H; Me), 1.25 (s, 36H; *t*Bu); ¹H NMR (400 MHz, CD₂Cl₂, 188 K): δ = 7.32 (s, 2H; ArH), 7.18 (s, 2H; ArH), 7.10 (s, 2H; ArH), 7.06 (s, 2H; ArH), 4.67 (d, J (H,H) = 12.8 Hz, 2H; *endo*-CH₂), 4.22 (d, J (H,H) = 12.8 Hz, 2H; *exo*-CH₂), 3.46 (d, J (H,H) = 12.8 Hz, 2H; *exo*-CH₂), 3.34 (d, J (H,H) = 12.8 Hz, 2H; *exo*-CH₂), 2.68 (s, 3H; Me), 1.35 (s, 9H; *t*Bu), 1.28 (s, 9H; *t*Bu); elemental analysis calcd for C₄₅H₅₅ClO₄W (%): C 61.47, H 6.31; found: C 61.48, H 6.26.

4: 1.5 C₃H₁₂: B(C₆F₅)₃ (1.47 g, 2.87 mmol) was stirred overnight with **2** (2.47 g, 2.87 mmol) in toluene (200 mL). Volatile components were removed in vacuo. The residue was washed with pentane (100 mL), collected, and dried in vacuo to give brick red **4**·1.5 C₃H₁₂ (yield: 3.38 g, 80 %). ¹H NMR (200 MHz, CD₂Cl₂, 298 K): δ = 7.36 (s, 8H; ArH), 4.77 (d, J (H,H) = 13.2 Hz, 4H; *endo*-CH₂), 3.74 (d, J (H,H) = 13.2 Hz, 4H; *exo*-CH₂) overlapping with 3.67 (s, 3H; Me), 1.23 (s, 36H; *t*Bu), 0.90 (m, 9H; pentane), 0.48 (s, 3H; Me); elemental analysis calcd for C_{71.5}H₇₆BF₁₅O₄W

(%): C 58.06, H 5.17; found: C 58.14, H 5.12. Crystals suitable for X-ray diffraction study were grown from a solution in C₆D₆.

5·3 C₄H₈O: Compound **2** (2.34 g, 2.72 mmol) was stirred with Na (0.0625 g, 2.72 mmol) in THF (120 mL) overnight. The resulting brown solution was filtered, volatile components were evaporated, and pentane (100 mL) was added. Yellow **5**·3 C₄H₈O was then collected and dried in vacuo (yield: 0.89 g, 30 %). Elemental analysis calcd for C₅₈H₈₂NaO₇W (%): C 63.44, H 7.53; found: C 63.54, H 7.57.

6·3 C₄H₈O: Compound **2** (5.74 g, 6.68 mmol) was stirred with Na (0.31 g, 13.6 mmol) in THF (150 mL) overnight. The resulting brown solution was filtered and evaporated to dryness. Yellow **6**·3 C₄H₈O was washed with pentane (100 mL), collected, and dried in vacuo (yield: 5.07 g, 68 %). ¹H NMR (400 MHz, CD₃CN, 298 K): δ = 9.01 (s, 4H; ArH), 8.95 (s, 4H; ArH), 5.91 (d, J (H,H) = 14.2 Hz, 4H; *endo*-CH₂), 3.66 (m, 12H; THF), 2.55 (d, J (H,H) = 14.2 Hz, 4H; *exo*-CH₂), 1.92 (s, 18H; *t*Bu), 1.82 (m, 12H; thf), 1.41 (s, 6H; WMe), –0.28 (s, 18H; *t*Bu); ¹H NMR (400 MHz, CD₃CN, 233 K): δ = 9.82 (s, 4H; ArH), 7.90 (s, 4H; ArH), 5.14 (d, J (H,H) = 14.2 Hz, 4H; *endo*-CH₂), 3.63 (m, 12H; thf), 2.38 (s, 18H; *t*Bu), 2.28 (d, J (H,H) = 14.2 Hz, 4H; *exo*-CH₂), 1.80 (m, 12H; THF), 1.44 (br s, 6H; WMe), –1.26 (s, 18H; *t*Bu); elemental analysis calcd for C₅₈H₈₂Na₂O₇W (%): C 62.14, H 7.37; found: C 61.84, H 7.42. Crystals suitable for X-ray analysis were grown in a solution in pyridine/DME.

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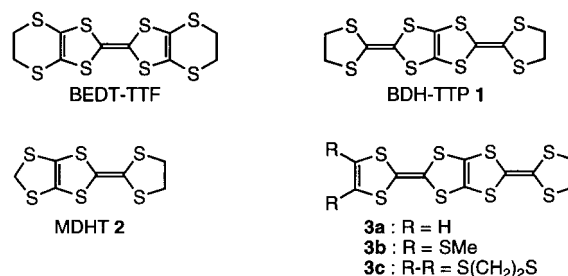
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BDH-TTP as a Structural Isomer of BEDT-TTF, and Its Two-Dimensional Hexafluorophosphate Salt

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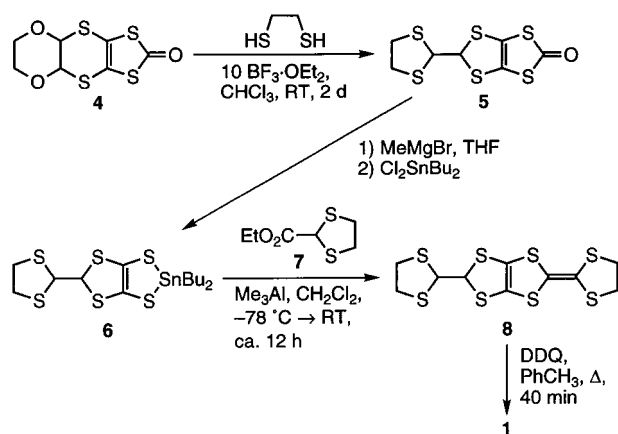
Tetrathiafulvalenes (TTFs) are highly promising π -electron donors for the exploration of new, molecular-based organic metals and superconductors.^[1] Of the TTFs synthesized thus far, bis(ethylenedithio)tetrathiafulvalene (BEDT-TTF) is known to yield the largest number of two-dimensional superconducting salts.^[2] One structural isomer of BEDT-TTF,^[3] the bis-fused dihydrotetrathiafulvalene (DHTTF) compound 2,5-bis(1,3-dithiolan-2-ylidene)-1,3,4,6-tetrathiapentalene (**1**, BDH-TTP), is very attractive because it contains no TTF moiety, yet it would be expected to produce organic metals that retain the metallic state down to low temperatures. This supposition is based on the results of our recent investigations which showed that a) methylenedithio-dihydrotetrathiafulvalene (**2**, MDHT) forms a metallic



(MDHT)₂AuI₂ salt which is stable down to 1.4 K, although its π -electron system is less extended than that of TTF,^[4a] and b) various organic metals are obtained from the fused TTF-DHTTF donors **3a–c** which have more extended π -electron systems than TTF.^[4b] Here we report the synthesis, electrochemical properties, and molecular structure of BDH-TTP as well as the conducting behavior of its charge transfer (CT) complexes and salts.

Our synthetic route to BDH-TTP (**1**) is outlined in Scheme 1. Ketone **5**, a precursor of **6**, was readily available from ketone **4** in 95 % yield.^[5] Reaction of **5** with MeMgBr in THF followed by trapping with Cl₂SnBu₂ gave tin dithiolate **6**, which in a key step underwent Me₃Al-promoted coupling^[6]

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Scheme 1. Synthesis of BDH-TTP (1).

with ethyl 1,3-dithiolane-2-carboxylate (**7**; 2 equiv of Me_3Al in CH_2Cl_2) to afford dithiolane-added derivative **8** of MDHT (52% overall yield from ketone **5**). Oxidation of the C–C bond between two dithioacetal groups of **8** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1.1 equiv) in refluxing toluene furnished BDH-TTP in 89% yield.

The redox behavior of BDH-TTP was investigated by cyclic voltammetry in PhCN/CS_2 (1/1); for comparison, BEDT-TTF was also studied (BDH-TTP: $E_1 = +0.56$, $E_2 = +0.82$, $E_3 = +1.52$, ΔE ($E_2 - E_1$) = 0.26 V (vs. SCE); BEDT-TTF: $E_1 = +0.61$, $E_2 = +0.87$, ΔE ($E_2 - E_1$) = 0.26 V). Two pairs of reversible redox waves and one irreversible oxidation wave (corresponding to E_3) were observed in the cyclic voltammogram of BDH-TTP. The first oxidation potential (E_1) is less positive than that of BEDT-TTF, which suggests that oxidation of BDH-TTP is easier. In contrast, the ΔE value, which reflects the magnitude of the on-site Coulombic repulsion, is equal to that of BEDT-TTF.

The molecular structure of BDH-TTP was determined by X-ray crystallographic analysis of a single crystal obtained by recrystallization from CS_2 . As depicted in Figure 1, the BDH-TTP molecule contains three tetrathioethylene units in an approximately common plane, and the two ethylene end groups are distorted from that plane. In contrast to the

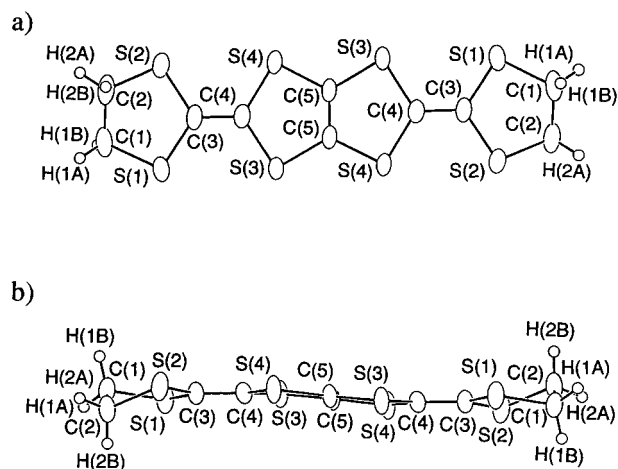


Figure 1. Molecular structure of BDH-TTP (1): a) top view; b) side view.

molecular structure of BEDT-TTF,^[7] this structure is approximately planar.

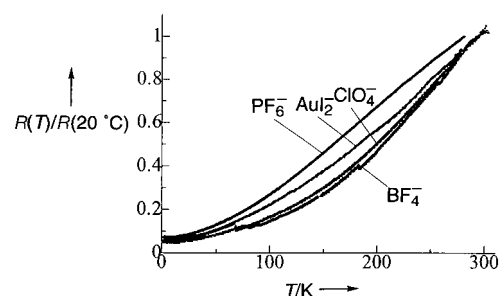
The conducting behavior of the CT complexes and salts of BDH-TTP is summarized in Table 1. As expected from its E_1 value, BDH-TTP reacted with 7,7,8,8-tetracyanoquinodimethane (TCNQ) and the tetrafluoro analogue (TCNQF₄) in

Table 1. Composition and conductive properties of complexes and salts of BDH-TTP (1).

Acceptor	Solvent	1:A ^[a]	σ_{RT} [S cm^{-1}] ^[b]
TCNQ	TCE	1:1	2.0×10^{-7} ($E_a = 0.43$ eV)
TCNQF ₄	TCE	3:2	5.6×10^{-5} ($E_a = 0.42$ eV) ^[c]
I ₃ [−]	PhCl	2:1	230 (metallic at $T \geq 2.0$ K)
AuI ₂ [−]	TCE	2:1	49 (metallic at $T \geq 2.0$ K)
BF ₄ [−]	TCE	2:1	33 (metallic at $T \geq 2.0$ K)
ClO ₄ [−]	TCE	— ^[d]	106 (metallic at $T \geq 2.2$ K)
PF ₆ [−]	TCE	2:1	102 (metallic at $T \geq 2.1$ K)
AsF ₆ [−]	TCE	2:1	49 (metallic at $T \geq 2.0$ K)

[a] A = acceptor. Ratio determined by elemental analysis. [b] Room-temperature conductivity measured by a four-probe technique for a single crystal unless otherwise stated. [c] Data for a compressed pellet. [d] Not determined because this complex may explode during analysis.

1,1,2-trichloroethane (TCE) at room temperature to form CT complexes, but their conductivities at room temperature were fairly low. In contrast, the salts thus far obtained from BDH-TTP and tetrabutylammonium salts with various counter anions in chlorobenzene (PhCl) or TCE by the controlled-current electrocrystallization method^[8] remained essentially metallic down to very low temperatures. Moreover, as the temperature decreased, their similar resistive behavior was independent of the structure and volume of the counter anions (Figure 2).


 Figure 2. Temperature dependence of the relative electrical resistance, $R(T)/R(20^\circ\text{C})$, of the metallic BDH-TTP salts with different counter anions.

The crystal structure of $(\text{BDH-TTP})_2\text{PF}_6$ was also determined by X-ray crystallographic analysis. The structure consists of κ -type sheets of BDH-TTP donor molecules and sheets of PF_6^- anions (Figure 3). The interplanar distance between pairs of donor molecules is 3.53 Å, and the dihedral angle of the molecular planes between pairs is 82°. These values are almost identical to those in κ -(BEDT-TTF)₂Cu(NCS)₂.^[9] In the donor sheet, each molecule is linked by several S...S contacts shorter than the sum of the van der Waals radii (Figure 4). The large intermolecular overlap integrals are also calculated, not only within a pair

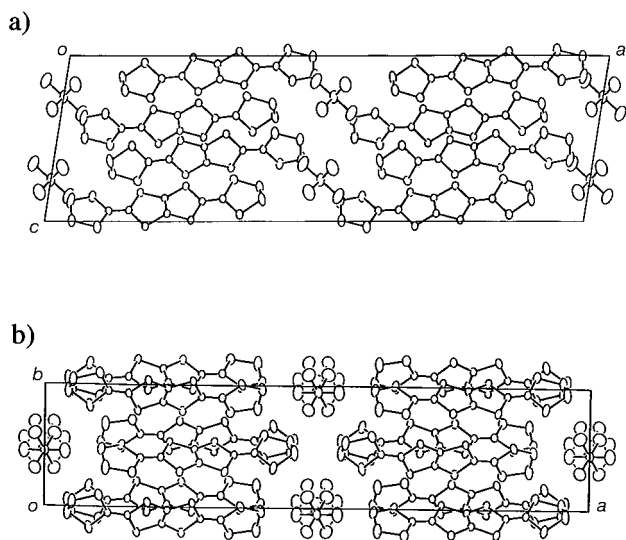


Figure 3. Crystal structure of (BDH-TTP)₂PF₆ viewed along the *b* axis (a) and the *c* axis (b).

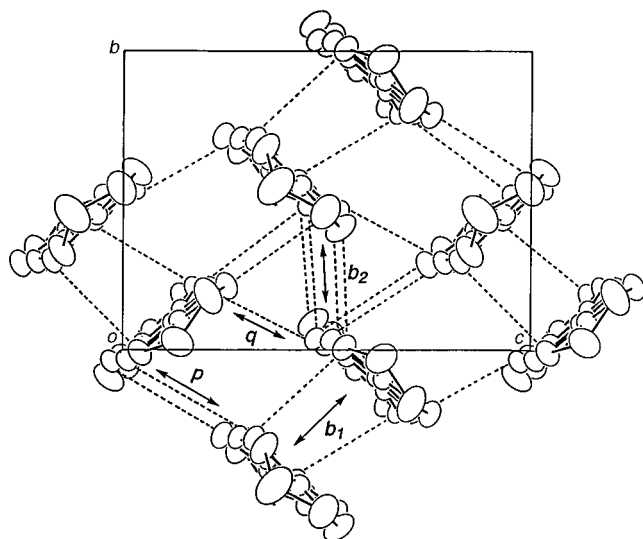


Figure 4. Donor arrangement of (BDH-TTP)₂PF₆. Intermolecular S...S contacts (<3.70 Å) are indicated by the dashed lines. The values of intermolecular overlap integrals are $b_1 = 20.7$, $b_2 = 19.6$, $p = 5.9$, and $q = -7.5 \times 10^{-3}$.

of donor molecules but also between pairs which form the 2D interaction in the *bc* plane. This 2D electronic structure is responsible for the metallic behavior down to low temperatures.

We have also determined the character of the highest occupied molecular orbital of BDH-TTP. Figure 5 shows its

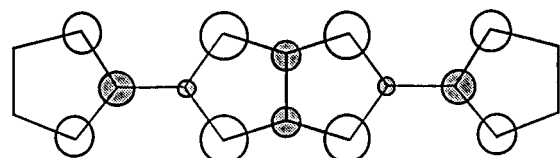


Figure 5. The highest occupied molecular orbital (HOMO) of BDH-TTP (1).

distribution as calculated by the extended Hückel method. It is noteworthy that in contrast to other TTP-type donor molecules, the atomic orbital ($2p\pi$) coefficients of two carbon atoms on the central double bond in the DHTTF backbone differ significantly.^[10]

In conclusion, the BDH-TTP donor is demonstrated to produce a metallic 2D salt. One synthetic target in this area of chemistry is the construction of a donor which is not based on TTF and which tends to stack with 2D interactions.^[11] Our studies on the DHTTF-based donor, described herein, represent a step forward in this direction. Further investigations on X-ray crystallographic analyses of other BDH-TTP salts and crystal growth with complex anions, for example, [Cu(NCS)₂]⁻ and [Cu{N(CN)₂}X]⁻ (X = Cl and Br), are currently in progress.

Experimental Section

1: M.p. 205 °C (decomp.); ¹H NMR (400 MHz, CDCl₃): δ = 3.47 (s, 8H); EI-MS: *m/z* (%): 386 (35, [M⁺+2]), 384 (100, [M⁺]), 280 (34), 268 (24); HR-MS calcd for C₁₀H₈S₈: 383.8392, found: 383.8389; elemental analysis calcd for C₁₀H₈S₈: C 31.22, H 2.10; found: C 30.98, H 1.98.

6: M.p. 163 °C (decomp.); ¹H NMR (400 MHz, CDCl₃): δ = 3.14–3.25 (m, 4H), 3.40–3.51 (m, 4H), 4.86 (dd, *J* = 10.5, 10.5 Hz, 2H); EI-MS: *m/z* (%): 388 (35, [M⁺+2]), 386 (100, [M⁺]), 281 (90); HR-MS calcd for C₁₀H₁₀S₈: 385.8548, found: 385.8552; elemental analysis calcd for C₁₀H₁₀S₈: C 31.05, H 2.61; found: C 31.06, H 2.51.

Data for X-ray crystallographic analysis were collected at *T* = 293 K on a MacScience MXC18 four-circle diffractometer, and all calculations were performed using CRYSTAN (MacScience, Japan). 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-102772 (1) and CCDC-102773 (1)₂PF₆. 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).

Crystal data for **1**: C₁₀S₈H₈, *M_r* = 383.84, monoclinic, space group, *P*2₁/*n*, *a* = 9.583(2), *b* = 9.593(2), *c* = 8.106(2) Å, β = 106.41(2)°, *V* = 714.8(3) Å³, *Z* = 2, ρ_{calcd} = 1.783 g cm⁻³, $\mu(\text{Cu}_{\text{K}\alpha})$ = 112.65 cm⁻¹, crystal dimensions 0.3 × 0.15 × 0.05 mm, min./max. transmission = 0.57. Data were collected with graphite-monochromated Cu_Kα radiation (λ = 1.54178 Å) with the ω –2 θ scan technique to a maximum 2 θ of 120° and corrected for absorption by the difabs method. The structure was solved by direct methods and refined by full-matrix least squares (non-hydrogen atoms with anisotropic displacement parameters; all hydrogen atoms refined in isotropic approximation; 86 variables) against *F*² with 994 reflections [*I* > 2.00σ(*I*)] out of 1175 independent reflection to yield *R* = 0.0434 and *R_w* = 0.0579; max./min. residual electron density 0.29/–0.39 e Å⁻³.

Crystal data for (1)₂PF₆: C₂₀S₁₆H₁₆PF₆, *M_r* = 912.64, monoclinic, space group *C*2/*c*, *a* = 35.800(9), *b* = 7.946(3), *c* = 11.116(4) Å, β = 98.75(2)°, *V* = 3125.5(17) Å³, *Z* = 4, ρ_{calcd} = 1.939 g cm⁻³, $\mu(\text{Mo}_{\text{K}\alpha})$ = 12.029 cm⁻¹, crystal dimensions 0.35 × 0.3 × 0.1 mm, min./max. transmission = 0.77. Data were collected with graphite-monochromated Mo_Kα radiation (λ = 0.71073 Å) with the ω –2 θ scan technique to a maximum 2 θ of 60° and corrected for absorption with the difabs method. The structure was solved by direct method and refined by full-matrix least squares (non-hydrogen atoms with anisotropic displacement parameters; all hydrogen atoms refined in isotropic approximation; 203 variables) against *F*² with 3660 reflections [*I* > 2.00σ(*I*)] out of 4327 independent reflection to yield *R* = 0.0421 and *R_w* = 0.0615; max./min. residual electron density 0.91/–0.67 e Å⁻³.

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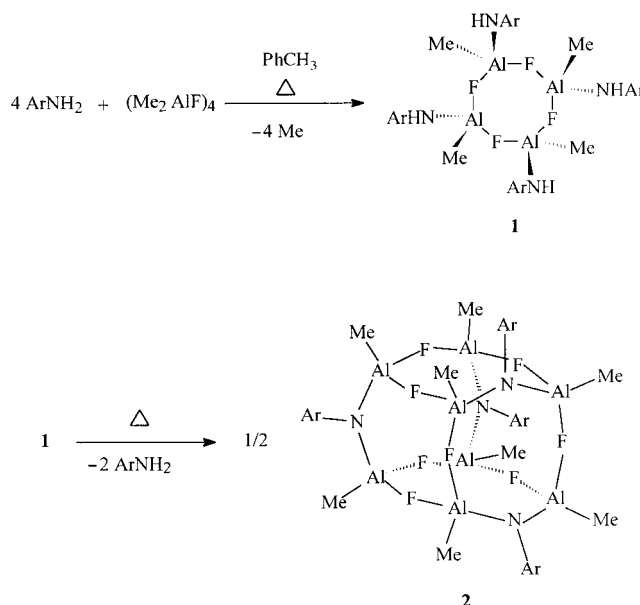
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[[MeAl(μ_2 -F)]₂N(2,6-*i*Pr₂C₆H₃)]₄— A Molecular Al-F-N Cage Compound**

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AlN and AlF₃ are high-melting, temperature-stable solids that are insoluble in organic solvents. In the last years soluble precursors of both compounds have become available which lead to AlN^[1] and AlF₃^[2], respectively, upon elimination under relatively mild conditions. Our goal now was to find out whether it is possible to combine the two systems and to synthesize soluble precursors containing Al, F, and N. Here we

describe the synthesis of [(2,6-*i*Pr₂C₆H₃NH)MeAl(μ_2 -F)]₄ (**1**) and its pyrolysis to the first Al-F-N cage compound **2**, which was characterized by X-ray structure analysis,^[3] NMR and IR spectroscopy as well as mass spectrometry. Compound **2** was obtained as colorless crystals after a two-step elimination reaction from Me₂AlF and (2,6-*i*Pr₂C₆H₃)NH₂ (Scheme 1).



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

In the first step of the reaction, one methyl group at each Al atom of the eight-membered starting material (Me₂AlF)₄^[4] was replaced by a (2,6-*i*Pr₂C₆H₃)NH residue under methane elimination. Compound **1** was isolated and characterized: In the EI mass spectrum the peak for [M – 3Me]⁺ was detected at *m/z* 902, the ¹⁹F NMR signals (δ = –141 and –140) for the Al-bridging fluorine atoms were in accord with those of known Al-F-Al substructures,^[5,6] and the elemental analysis confirmed the composition. The pyrolysis of **1** then surprisingly took place under (2,6-*i*Pr₂C₆H₃)NH₂ elimination. This reaction sequence, which takes place at two very different temperatures, allowed the isolation of **2** in high yields.

Compound **2** crystallizes in the tetragonal space group *P*4₂*c* with one-quarter of a molecule in the asymmetric unit; the remaining three-quarters are generated by the $\bar{4}$ axis. The center of the structure is a cubic cage, the six faces of which form four eight-membered Al₄N(μ_2 -F)₃ rings in a half-chair conformation and two likewise eight-membered Al₄N₂(μ_2 -F)₂ rings in a boat conformation (Figure 1). All rings consist of alternating metal and nonmetal atoms. To complete the coordination sphere each Al atom carries a methyl group, and each N atom is bound to a 2,6-diisopropylphenyl residue.

The mean Al–N bond length in **2** (1.788(3) Å) lies within the typical range for Al–N bonds,^[7] the Al–F bonds (on average 1.785(3) Å) are longer than Al–F single bonds (1.65 Å), but quite typical for μ_2 -bridging F atoms.^[8] The aluminum atoms are all coordinated in a slightly distorted tetrahedron (angular sum 651.5° (ideal tetrahedron: 657.0°)), in which four groups of angles can be distinguished: F–Al–F (1 ×), C–Al–N (1 ×), N–Al–F (2 ×), and C–Al–F angles (2 ×).

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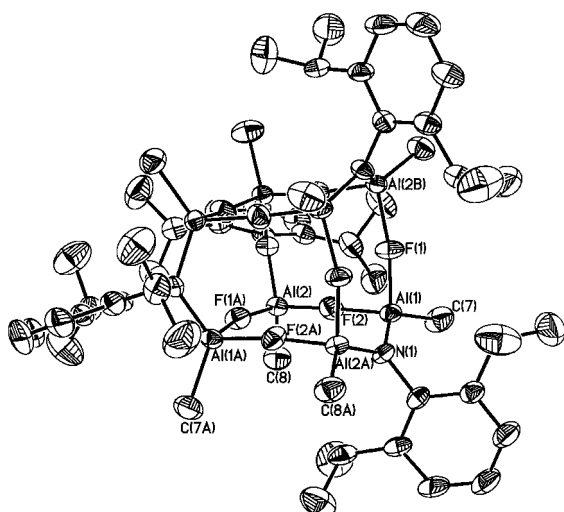


Figure 1. Molecular structure of **2** in the crystal. Selected distances [Å] and angles [°]: Al(1)–F(2) 1.776(3), Al(1)–N(1) 1.793 (3), Al(2)–F(2) 1.792(3), Al(1)–F(1) 1.784(2), Al(2)–C(8) 1.918(4), F(2)–Al(1)–F(1) 92.6(1), F(1)–Al(1)–N(1) 107.5(1), F(1)–Al(1)–C(7) 109.6(2), F(2)–Al(1)–N(1) 107.0(1), Al(1)–F(1)–Al(2B) 164.4(2), Al(1)–F(2)–Al(2) 173.3(2), Al(1)–N(1)–Al(2A) 125.9(2).

The F–Al–F angles are smaller than the ideal tetrahedral angle of 109.5° (on average 91.9(1)°), the C–Al–N angles are larger (on average 126.2(2)°), and the N–Al–F and C–Al–F angles (on average 107.1(1) and 109.6(2)°, respectively) are very close to the ideal tetrahedral angle. The nitrogen atoms in the eight-membered rings have a planar environment within the experimental error: The angular sum around them amounts to 359.8(2)°; the mean deviation from the best plane is 0.003 Å. The two Al–N–Al angles are equal for reasons of symmetry and amount to 125.9(2)°. The two independent Al–F–Al angles differ significantly: If the bridging fluorine atom belongs to an $\text{Al}_4\text{N}_2\text{F}_2$ ring, the angle is 164.4(2)° (Al(1)–F(1)–Al(2B)), if it belongs to an Al_4NF_3 ring, 173.3(2)° (Al(1)–F(2)–Al(2)).

Compound **2** can be considered as an entry into a new class of compounds and can draw a varied chemistry with it due to its reactive substituents. For its use in metal organic chemical vapor deposition (MOCVD) methods the system has to be modified in future work in such a way that good leaving groups are introduced as substituents in order to prevent the deposition of carbon.

Experimental Section

1: Me_2AlF (20 mL of a 2.5 M solution in toluene) was added dropwise to a solution of (2,6-*i*- $\text{Pr}_2\text{C}_6\text{H}_3$) NH_2 (9.41 mL, 50.0 mmol) in toluene (75 mL) at room temperature. Subsequently the reaction mixture was heated for 10 h under reflux, during which time the evolving methane was led off through a mercury pressure relief valve. After all volatile components were removed in vacuo, the residue was washed with *n*-hexane (30 mL), and **1** was obtained as a white solid (40.3 g, 85 %). M.p. 152 °C; ^1H NMR (200 MHz, C_6D_6 , TMS): δ = –0.22 (m, 12 H, AlCH_3), 1.15 (m, 48 H, $\text{CH}(\text{CH}_3)_2$), 3.35 (m, 8 H, $\text{CH}(\text{CH}_3)_2$), 3.85 (s, 2 H, NH), 4.05 (s, 2 H, NH), 6.95 (m, 12 H, arom. H); ^{19}F NMR (235 MHz, C_6D_6 , CFCl_3): δ = –141 (s, 2 F), –140 (s, 2 F); IR (KBr, nujol): $\tilde{\nu}$ = 3369, 3258, 1621, 1592, 1433, 1379, 1343, 1309, 1255, 1211, 1168, 1100, 1043, 976, 887, 866, 820, 762, 705, 670, 570, 545, 427 cm^{-1} ; MS (EI): m/z (%): 902 (2, $M-3\text{Me}$), 162 (100, $\text{C}_{12}\text{H}_{18}$); elemental analysis calcd for $\text{C}_{52}\text{H}_{84}\text{Al}_4\text{F}_4\text{N}_4$: C 65.8, H 8.9, Al 11.4, N 5.9; found: C 65.7, H 9.2, Al 11.1, N 6.2.

2: Compound **1** (4.7 g, 5.0 mmol) was heated to 165 °C for 3 h. The crude product was then washed with *n*-hexane (15 mL), and **2** was obtained as a white microcrystalline solid (3.8 g, 80 %). Single crystals were grown from *n*-hexane at –22 °C within two months. M.p. 180 °C; ^1H NMR (200 MHz, C_6D_6 , TMS): δ = –0.31 (m, 24 H, AlCH_3), 1.51 (m, 48 H, $\text{CH}(\text{CH}_3)_2$), 3.40 (sept., 8 H, $\text{CH}(\text{CH}_3)_2$), 6.95 (m, 12 H, arom. H); ^{19}F NMR (235 MHz, C_6D_6 , CFCl_3): δ = –144 to –140 (4 s, 8 F); IR (KBr, nujol): $\tilde{\nu}$ = 1943, 1608, 1577, 1539, 1509, 1419, 1348, 1260, 1168, 1089, 1077, 1039, 1023, 864, 833, 801, 740, 722, 565 cm^{-1} ; MS (EI): m/z (%): 581 (2, $M/2-\text{Me}$), 162 (100, $\text{C}_{12}\text{H}_{18}$); elemental analysis calcd for $\text{C}_{56}\text{H}_{92}\text{Al}_8\text{F}_8\text{N}_4$: C 56.6, H 7.7, F 12.8, N 4.7; found: C 57.0, H 7.6, F 12.4, N 4.7.

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Energetics of Molecular Complexes in a Supersonic Beam: A Novel Spectroscopic Tool for Enantiomeric Discrimination**

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Enantiomeric discrimination takes place when a chiral selector (C) forms with a pair of enantiomers (M) two diastereomeric molecular complexes (MCs) of different stability (thermodynamic enantioselectivity). This is the basis of chiral chromatography, enzymic resolution, asymmetric synthesis, and NMR spectroscopic discrimination with chiral auxiliaries.^[1, 2] Although the principles of thermodynamic enantioselectivity find eminent applications in many fields, very few experiments have been designed so far to establish at the microscopic level the nature and energetics of the various interactions in diastereomeric MCs.

Thermometric measurements of the energetics of aggregation of chiral ions in solution to make diastereomeric ion pairs were first carried out by Arnett and Zingg.^[3] These authors pointed out that the average difference in thermodynamic stability between diastereomeric combinations of several chiral amines with mandelic acid enantiomers may span from zero to 200–350 cal mol⁻¹, depending upon the structure of the amine and the nature of the solvent.

A way to evaluate the intrinsic nature of the various interactions in diastereomeric MCs and to eliminate the moderating effects of solvent on their energetics is to study their features in the isolated state. Here we report on the first spectroscopic determination of the binding energy in isolated

diastereomeric MCs, with special regard to the dependence of the binding energy on the configuration of the chiral solvent molecule.

Weakly bound MCs, which would be unobservable at room temperature, can be readily generated in the isolated state by supersonic expansion of their components. Under these conditions, they are formed at an average temperature of a few Kelvins^[4] and can be spectroscopically discriminated. The spectral analysis is facilitated by the fact that, at low temperatures, only the lowest rotational and vibrational levels are populated. Lahmani and co-workers recently characterized jet-cooled diastereomeric MCs by different laser-induced fluorescence (LIF) spectra.^[5–7] Discrimination between diastereomeric MCs was obtained by us^[8] with resonance-enhanced multiphoton ionization (REMPI) spectroscopy combined with time-of-flight (TOF) mass spectrometry.^[4, 9] Accordingly, the REMPI-TOF spectra of the diastereomeric molecular complexes **Rr** and **Rs**—formed from the combination of the chiral chromophore (R)-(+)-1-phenyl-1-propanol (**R**) with (R)-(-) (**r**) and (S)-(+)-2-butanol (**s**), respectively—display different features. In particular, their most intense band, assigned to the electronic band origin, is red-shifted relative to the S₁ ← S₀ band origin of the isolated **R** molecule ($\Delta\nu = -79$ cm⁻¹ (**Rr**), -92 cm⁻¹ (**Rs**)), indicating an enhancement of binding energy of both **Rr** and **Rs** adducts in the S₁ state relative to the S₀ state. The difference between the red shifts ($\Delta\nu[\mathbf{Rr}] - \Delta\nu[\mathbf{Rs}] = 13$ cm⁻¹) reflects a S₁ ← S₀ energy gap for **Rs** which is smaller than for **Rr**.

The binding energy of the diastereomeric **Rr** and **Rs** adducts was measured by two color resonance two photon ionization (2cR2PI) experiments. The species under investigation is selectively excited to the S₁ state by absorption of one photon at a fixed frequency ν_1 ($h\nu_1$ in Figure 1), and then ionized by a second photon of variable frequency ν_2 ($h\nu_2$ in Figure 1). The experimental procedure is the following: the species under investigation was first submitted to one color resonance two photon ionization (1cR2PI) experiments,^[8] in which it is excited to a discrete S₁ state by absorption of one photon of frequency ν_1 , and to the ionization continuum by absorption of another photon with the same frequency ν_1 . Once having obtained the 1cR2PI-TOF mass spectrum of the selected species at the resonance frequency ν_1 , the intensity of the laser emitting at ν_1 is lowered so as to reduce the TOF ion pattern to less than 10%. Then, by superimposing a second laser of variable frequency ν_2 , which alone does not produce any significant signal in the spectral region of interest, there is a pronounced increase in the intensity of the TOF signal of a characteristic ion only when the 2cR2PI process takes place. The value of ν_2 corresponding to the signal onset provides a measure of the ionization threshold of the species.

The energetics involved in diastereomeric **Rr** or **Rs** is determined as follows: The dissociation energy D_0'' of ground-state **Rr** is computed from Equation (1), namely, from the difference between its dissociative ionization threshold ($h\nu_1[\mathbf{Rr}^*] + h\nu_2[\mathbf{R}^+ + \mathbf{r}]$; Figure 1) and the ionization threshold of bare **R** ($\text{IP}(\mathbf{R}) = h\nu_1[\mathbf{R}^*] + h\nu_2[\mathbf{R}^+]$). The dissociation energy D_0^+ of ionic cluster **Rr**⁺ is calculated from Equation (2), namely, from the difference between its dissociative ionization threshold ($h\nu_1[\mathbf{Rr}^*] + h\nu_2[\mathbf{R}^+ + \mathbf{r}]$; Figure 1) and its

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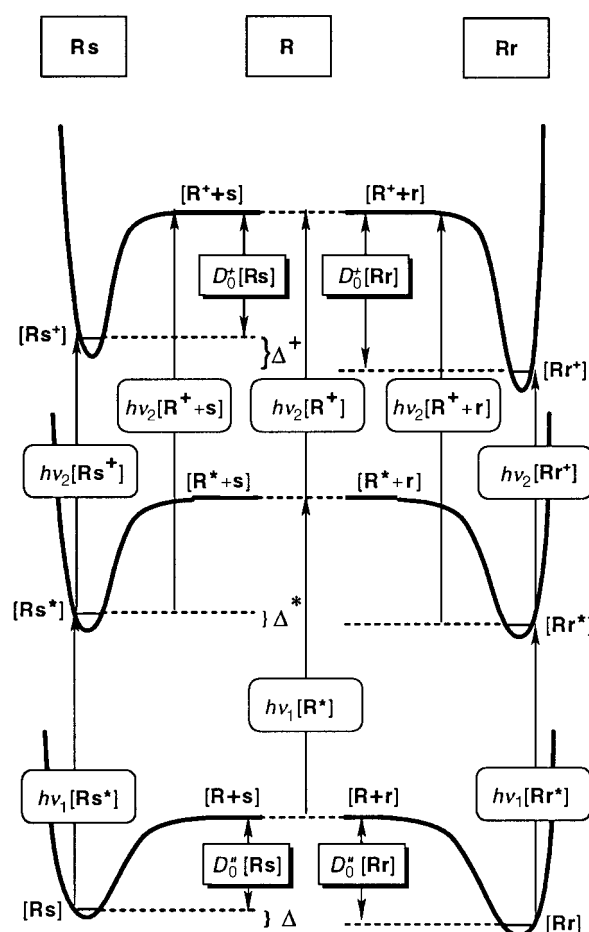


Figure 1. Schematic representation of the potential energy curves for the heterochiral complex **Rs** (left) and the homochiral complex **Rr** (right). The arrows on the center of the diagram refer to the bare chromophore **R**. Excitation (**[Rs*]** and **[Rr*]**), ionization (**[Rs⁺]** and **[Rr⁺]**), and dissociative ionization energies (**[R⁺+s]** and **[R⁺+r]**) are reported in Table 1. The terms Δ , Δ^* , and Δ^+ refer to the experimental energy gap between **Rr** and **Rs** in their ground, first excited, and ionized state, respectively.

ionization threshold ($\text{IP}(\mathbf{Rr}) = h\nu_1[\mathbf{Rr}^*] + h\nu_2[\mathbf{Rr}^+]$). Of course, similar equations can be derived for the heterochiral complex **Rs**.

$$D_0'' = h\nu_1[\mathbf{Rr}^*] + h\nu_2[\mathbf{R}^+ + \mathbf{r}] - \text{IP}(\mathbf{R}) \quad (1)$$

$$D_0^+ = h\nu_1[\mathbf{Rr}^*] + h\nu_2[\mathbf{R}^+ + \mathbf{r}] - \text{IP}(\mathbf{Rr}) = h\nu_2[\mathbf{R}^+ + \mathbf{r}] - h\nu_2[\mathbf{Rr}^+] \quad (2)$$

The band-origin region of the mass-resolved 2cR2PI excitation spectrum of bare **R** is characterized by a peak at 37577 cm^{-1} and two other major peaks at 37618 and 37624 cm^{-1} . By analogy with the spectral features of similar molecules,^[10, 11] this triplet of bands can be interpreted as being due to either different stable conformers or vibronic transitions. We tend to support the first hypothesis since analysis of the excitation spectrum around 530 cm^{-1} above the electronic $S_0 \leftarrow S_1$ origin reveals the presence of a mirror triplet at 38106 , 38148 , and 38155 cm^{-1} . These vibronic bands around 530 cm^{-1} above the electronic $S_0 \leftarrow S_1$ origin ($6b_1$) characterize the spectrum of many substituted benzenes and are independent of the nature of the substituent.^[11]

The 2cR2PI ionization spectrum of bare **R** is taken by setting ν_1 at a value corresponding to any of the bands between 37577 and 37624 cm^{-1} and by varying ν_2 . All these measurements provide the same value for the appearance potential of **R⁺** (8.84 eV) within the limits of experimental uncertainty ($\pm 0.01 \text{ eV}$).

The 2cR2PI ionization spectra and dissociative ionization spectra of the diastereomeric pair **Rr** and **Rs** are taken in the same way, by setting ν_1 at a value corresponding to their band origin. Figure 2 displays the 2cR2PI ionization spectrum and

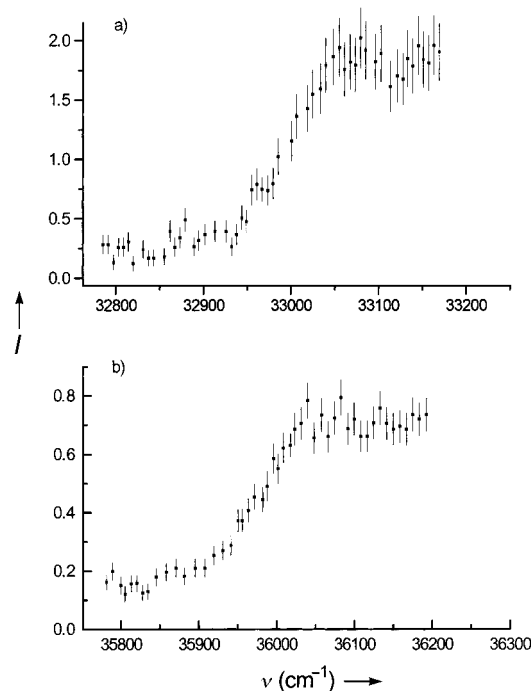


Figure 2. a) The 2cR2PI ionization spectrum and b) the dissociative ionization spectrum of the complex **Rr** at a threshold of m/z 210 and a total stagnation pressure of $4 \times 10^5 \text{ Pa}$. Intensity I is given in arbitrary units.

dissociative ionization spectrum of the **Rr** complex. Similar spectra are obtained for the other diastereomer **Rs**. The appearance potential and dissociative appearance potential of **Rr** and **Rs** are listed in Table 1, together with their derived D_0'' and D_0^+ dissociation energies. The D_0'' values demonstrate that the homochiral **Rr** complex is appreciably more stable toward dissociation than the heterochiral **Rs** adduct. This trend extends to the S_1 excited complexes as well, that is, $\Delta^* = D_0''[\mathbf{Rr}^*] - D_0''[\mathbf{Rs}^*] = \Delta - 13 \text{ cm}^{-1} = 1.1 \pm 0.4 \text{ kcal mol}^{-1}$ (Figure 1).

Table 1. Photon energies for excitation, threshold ionization, and threshold dissociative ionization of the **Ra** (**a = s** or **r**) complexes and their derived binding energies in the neutral (D_0'') and cationic ground state (D_0^+); see also Figure 1.

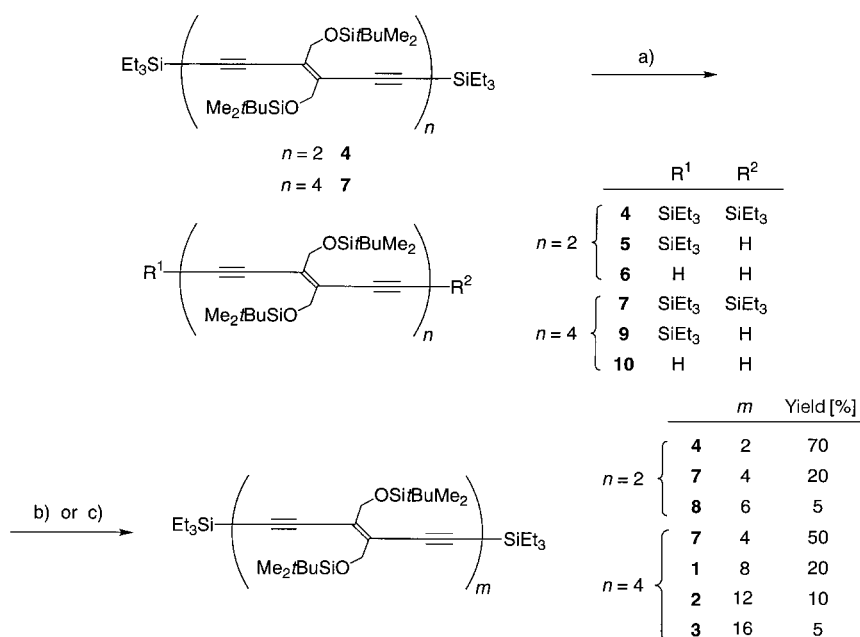
	$h\nu_1[\mathbf{Ra}^*]$ [cm ⁻¹]	$h\nu_2[\mathbf{Ra}^+]$ [cm ⁻¹]	$h\nu_2[\mathbf{R}^+ + \mathbf{a}]$ [cm ⁻¹]	$D_0''^{[a]}$ [kcal mol ⁻¹]	$D_0^+^{[b]}$ [kcal mol ⁻¹]
Rr	37498 ± 1	32950 ± 40	35890 ± 40	5.9 ± 0.2	8.4 ± 0.2
Rs	37485 ± 1	33160 ± 40	35540 ± 40	4.8 ± 0.2	6.8 ± 0.2
				$\Delta = 1.1 \pm 0.4$	$\Delta^+ = 1.6 \pm 0.4$

[a] D_0'' as derived from Equation (1) ($\text{IP}(\mathbf{R}) = 8.84 \pm 0.01 \text{ eV}$). [b] D_0^+ as derived from Equation (2).

polymer that are required to furnish size-independent optical, redox, or other properties, and this concept has been quite useful in the theoretical and experimental understanding of π -conjugated polymers.

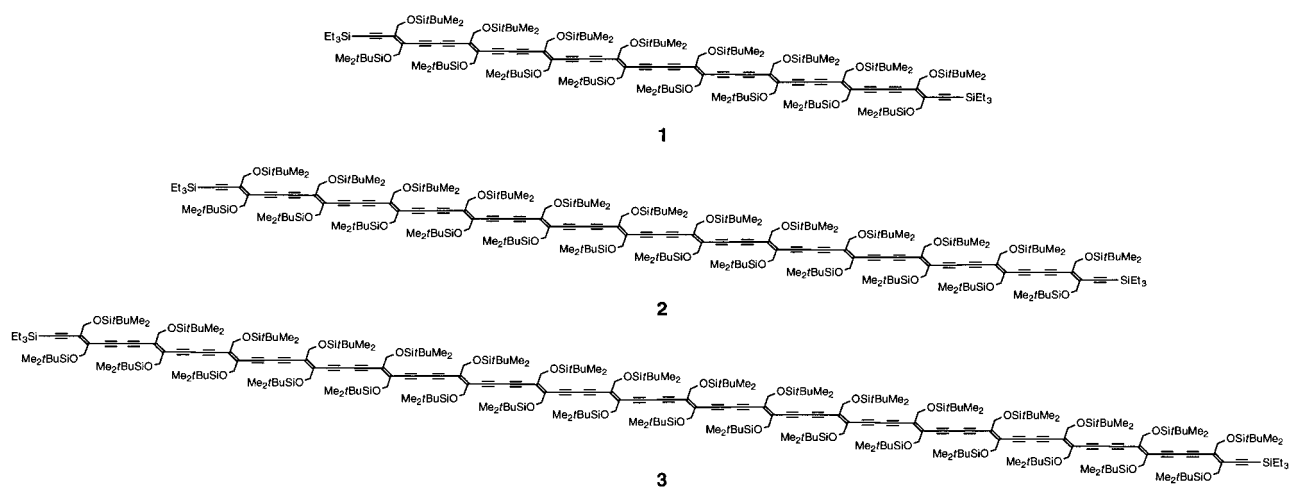
To determine directly the ECL of poly(triacetylenes) experimentally^[5, 7] we have now dramatically extended the oligomeric series by preparing a 6.07 nm long octameric (**1**), a 8.99 nm long dodecameric (**2**), and a 11.9 nm long hexadecameric PTA rod (**3**).^[8] With its linearly conjugated 16 double and 32 triple bonds between the terminal Si atoms hexadecamer **3** is currently the longest linear, fully π -conjugated molecular wire without aromatic repeat units.^[9] We also show that the direct determination of the ECL from the saturation of linear optical properties and the Raman frequency^[10] of the acetylenic C=C stretch with increasing oligomeric length is in good agreement with the initial predictions.

The synthesis of oligomers **1–3** started from dimer **4**, which was prepared in 97 % yield by an oxidative Hay coupling of mono-Et₃Si-protected (*E*)-3,4-bis[[(*tert*-butyl)dimethylsilyloxy]methyl]hex-3-ene-1,5-diyne (Scheme 1).^[4, 11] Statistical



Scheme 1. Synthesis of PTA oligomers by statistical deprotection–oligomerization sequences. a) NaOH, THF/MeOH 1/1, 20 °C, 20 min. b) (for $n = 2$): CuCl, *N,N,N',N'*-tetramethylethylenediamine (TMEDA), CH₂Cl₂, 4-Å molecular sieves, air, RT, 2 h. c) (for $n = 4$): CuCl, TMEDA, toluene, 4-Å molecular sieves, dry O₂ atmosphere, reflux, 1 h.

followed by workup, the oligomers formed were purified by preparative size exclusion chromatography (SEC; 5 × 180-cm



deprotection of **4** under kinetic control with NaOH in THF/MeOH 1/1 at room temperature afforded mono-deprotected **5** and bis-deprotected **6**, besides recovered **4**, and this mixture was oligomerized directly under Hay conditions. This protocol avoided the handling and purification of the rather unstable unprotected alkynes. Reaction times for the cleavage of the Et₃Si groups in **4** were typically and reproducibly around 10 min, and product formation was monitored by thin-layer chromatography. After workup the concentrated solution was diluted with dry CH₂Cl₂ containing 4-Å molecular sieves and then TMEDA and CuCl were added. After vigorously stirring the reaction mixture (2 h) under an atmosphere of air

glass column filled with Bio-Beads SX-1 (Bio-Rad), CH₂Cl₂). This strategy permitted the synthesis of predominantly tetrameric **7** together with minor amounts of hexameric **8** and traces of unseparable polymers (<5 %).^[11] Recovered dimer **4** was then used in the next deprotection–oligomerization cycle, thus making this route very efficient.

The same procedure starting from tetrameric **7** to produce a mixture of **7**, mono-deprotected **9**, and bis-deprotected **10** by statistical deprotection, followed by oligomerization, yielded monodisperse octameric **1**, dodecameric **2**, and hexadecameric **3** as the main products besides recovered **7**. The reaction conditions were only slightly changed, and the oligomeriza-

tion was performed in toluene under reflux in an atmosphere of dry O_2 (see experimental section). Purification of all oligomers up to dodecameric **2** was achieved by preparative SEC as described above, whereas the isolation of pure hexadecameric **3** required preparative SEC on a commercial TosoHaas TSKgel G3000 HR column ($5\mu m$; 2.15×60 cm).^[12] A final purification of the oligomers was achieved in each case by precipitation from MeOH followed by centrifugation. An improved synthesis of hexadecameric **3** (20% yield) used octameric **1** as the starting material in the statistical deprotection–oligomerization sequence. The larger molecular weight difference between the various oligomers obtained in this conversion greatly facilitated the SEC separation and purification of **3**.

The oligomers exhibit an increasingly deep yellow color with growing chain length; they are readily soluble in aprotic solvents such as toluene and CH_2Cl_2 but insoluble in protic solvents. The pure oligomers are highly stable for months under standard laboratory conditions, and their melting points increase in the order $69^\circ C$ (**4**), $151^\circ C$ (**7**), $183^\circ C$ (**8**), $202^\circ C$ (**1**), $219^\circ C$ (**2**), and $>220^\circ C$ (**3**).

The purity of the oligomers was clearly revealed by MALDI-TOF mass spectrometry in the positive ion and linear detection mode. When using 3-(3-indolyl)acrylic acid as a matrix the hexadecameric rod **3** only displayed the molecular ion $[M+Na]^+$ as the parent ion at m/z 6056 (6055.5 is calculated for the most abundant isotope in the molecular ion cluster, $^{12}C_{329}^{13}C_3H_{574}O_{32}^{28}Si_{31}^{29}Si_2^{30}Si_3^{23}Na^+$, Figure 1). No trace of the octameric starting material **1** was detected in the spectrum.

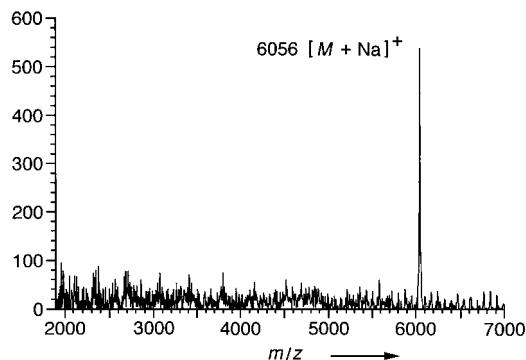


Figure 1. MALDI-TOF mass spectrum of **3** (intensity in arbitrary units, matrix: 3-(3-indolyl)acrylic acid).

The excellent solubilities of the oligomers, largely a consequence of the laterally appended $Me_2tBuSiOCH_2$ groups, allowed full characterization of the molecular rods by ^1H and ^{13}C NMR spectroscopy. Whereas the ^{13}C NMR spectrum of octameric **1** depicted 24 separate resonances for the C atoms of the conjugated backbone (8 $C(sp^2)$ resonances between $\delta = 129$ and 133 , 2 outer alkyne $C(sp)$ resonances at $\delta = 102$ and 107 , and 14 interior alkyne $C(sp)$ resonances between $\delta = 81$ and 88), the $C(sp^2)$ and inner alkyne $C(sp)$ resonances in dodecameric **2** and hexadecameric **3** showed severe overlap in the expected spectral regions. This suggests that individual $C(sp^2)$ and $C(sp)$ atoms, respectively, begin to

resemble each other electronically. The information at which point a single monomer in a conjugated polymer loses its electronic individuality is important for the determination of the chain length at which a polymer can delocalize charge carriers without that carrier experiencing end effects.^[13] It is noticeable that the ^{13}C NMR resonance overlap starts at an oligomeric length (between octamer and dodecamer), which corresponds to the predicted ECL for poly(triacetylenes) by UV/Vis spectroscopy. Both NMR and IR spectra do not provide any indication for photochemical *trans*→*cis* isomerization of the molecular rods **1**–**3**;^[14] we therefore have high confidence that these compounds are present in the all-*trans* configuration shown. Furthermore, X-ray crystal structure analyses on PTA oligomers revealed a preference of the planar backbones for the depicted *s-trans* conformation of the double bonds;^[15] such geometry also accommodates best the bulky, lateral silyl groups.

The UV/Vis spectra of the new oligomers in $CHCl_3$ are shown in Figure 2a. The deconvoluted^[16] longest-wavelength absorption maximum (λ_{max}) shifts bathochromically with increasing chain length for the shorter oligomers up to octameric **1**,^[4] whereas for the higher oligomers this shift vanishes. Thus $\Delta\lambda_{max}$ upon changing from hexameric **8** to

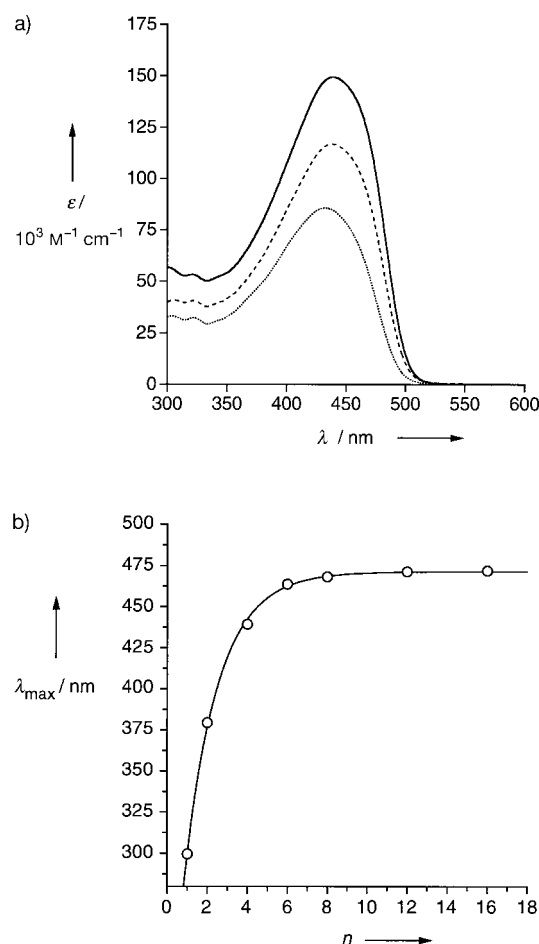


Figure 2. a) Electronic absorption spectra of the PTA oligomers in $CHCl_3$ at $20^\circ C$. b) Convergence of the deconvoluted λ_{max} values (from the monomeric to hexadecameric rod: $\lambda_{max} = 299.6 \pm 0.1$; 379.4 ± 0.1 (**4**); 439.2 ± 0.2 (**7**); 463.7 ± 0.8 (**8**); 468.1 ± 0.8 (**1**); 471.2 ± 0.6 (**2**); 471.8 ± 0.6 (**3**)) with an overlaid exponential fit.

octameric **1** is 4 nm, from octameric **1** to dodecameric **2** is 3 nm, and finally from dodecameric **2** to hexadecameric **3** no additional increase in $\lambda_{\max}$ was observed (Figure 2b). From these data, the ECL in a PTA backbone can now be determined to occur around $n=10$, thus verifying the previously estimated value of $n=7-10$ monomeric units.^[4] A more elaborated determination of the ECL by using an exponential equation^[5] provided the same ECL of $n=10$ and $b_1=0.594$, a parameter that indicates how “fast” the limit of convergence is reached.

Raman scattering studies of the PTA oligomers in CHCl_3 solutions^[17] at 20 °C showed an exponential decrease in the frequencies of the triple and double bond stretches $\nu_{(\text{C}=\text{C})}$ and $\nu_{(\text{C}\equiv\text{C})}$ with lengthening of the π -conjugated backbone. The two resonances also increase significantly in intensity with increasing chain length. From the plotted data for the acetylenic stretch (Figure 3) the ECL can be directly estimated to lie in the range of 10 monomer units, which is in excellent agreement with the UV/Vis data.

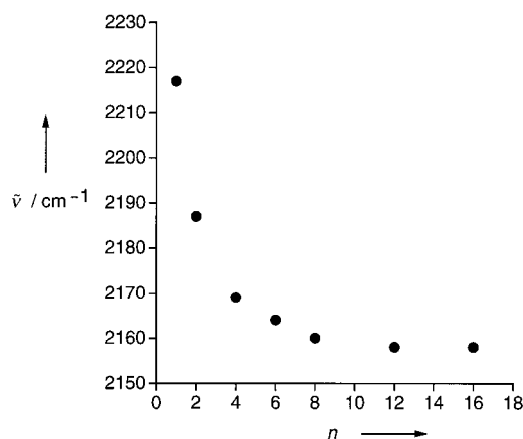


Figure 3. Plot of the Raman-active stretches $\nu_{(\text{C}\equiv\text{C})}$ for the PTA oligomers as a function of the oligomeric length. The wavenumbers are (from the monomeric to the hexadecameric rod): $\tilde{\nu}=2217, 2187$ (**4**), 2169 (**7**), 2164 (**8**), 2160 (**1**), 2158 (**2**), 2158 (**3**) cm^{-1} .

The SEC traces of the product mixture obtained by deprotection–oligomerization of **8** indicated that even higher monodisperse PTA oligomers, such as a 24-mer, should be stable and isolable; attempts to prepare such molecular rods of unprecedented length using the fast and efficient statistical synthetic protocol described in this communication are now under way.

Experimental Section

Synthesis of 1–3 from 7: A solution of 1M aqueous NaOH (1 mL) at 20 °C was added to a solution of **7** (0.10 g, 0.059 mmol) in THF/MeOH 1/1 (30 mL). After TLC (SiO_2 , n -hexane/AcOEt 40/1) indicated the appearance of bis-deprotected **10** (approximately 10 min), the reaction was immediately quenched by addition of saturated aqueous NH_4Cl (100 mL). After extraction with CH_2Cl_2 (4×50 mL), the collected organic phases were dried (MgSO_4), and concentrated at water aspirator pressure to about 10 mL. Dry toluene (20 mL) containing 4-Å molecular sieves was added followed by TMEDA (0.01 g, 0.01 mL, 0.083 mmol) and CuCl (0.002 g, 0.024 mmol). Dry O_2 was passed directly over the solution while it was heated to reflux for 1 h. After addition of a solution of EDTA (EDTA = ethylenediaminetetraacetic acid, pH 8, 100 mL), the mixture was exhaus-

tively extracted with CH_2Cl_2 , the organic phase washed with saturated aqueous NaCl (100 mL), and dried (MgSO_4). Concentration at water aspirator pressure, preparative SEC, and precipitation from MeOH followed by centrifugation yielded **7** (0.050 g, 50 %), **1** (0.018 g, 20 %), **2** (0.009 g, 10 %), and **3** (0.005 g, 5 %).

Analytical data for **3**: Deep yellow solid, m.p. > 220 °C. UV/Vis (CHCl_3): $\lambda_{\max}$ [nm] (ϵ [$\text{cm}^{-1}\text{M}^{-1}$]): 270 (61300), 281 (61200), 301 (sh, 56800), 321 (53300), 439 (149400); FT-IR (CHCl_3) [cm^{-1}]: 2956(s), 2933(s), 2856(s), 2417(w), 2383(w), 2348(w), 1667(m), 1645(m), 1600(m), 1495(m), 1461(m), 1367(m), 1261(s), 1183(m), 1168(m), 1100(s), 1072(m), 1041(m), 1017(m), 922(w), 839(m), 811(m); Raman (CHCl_3): [cm^{-1}]: 3017(m), 2925(w), 2398(w), 2158(w), 1555(w), 1496(w), 1214(m), 761(m), 665(s), 364(s), 259(s); ^1H NMR (500 MHz, CDCl_3): $\delta=0.069$ (s, 12H), 0.075 (s, 12H), 0.083 (s, 168H), 0.62 (q, $J=7.9$ Hz, 12H), 0.89–0.90 (m, 288H), 0.99 (t, $J=7.9$ Hz, 18H), 4.40 (s, 4H), 4.44 (s, 56H), 4.48 (s, 4H); ^{13}C NMR (125.8 MHz, CDCl_3 + 20 mM [$\text{Cr}(\text{acac})_3$): $\delta=-5.42, 4.03, 7.21, 18.09, 25.60, 63.59, 63.81, 81.52, 82.60-83.15$ (large overlap), 84.93, 86.81, 87.10 (large overlap), 87.18, 87.40, 102.35, 107.25, 129.49, 131.69–132.19 (large overlap), 132.35, 133.08; MALDI-TOF-MS: see Figure 1; elemental analysis calcd for $\text{C}_{332}\text{H}_{574}\text{O}_{32}\text{Si}_{34}$ (6033.16): C 66.10, H 9.59; found: C 66.20, H 9.52.

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- All new compounds were fully characterized by their melting points, UV/Vis, IR, Raman, ^1H and ^{13}C NMR spectroscopies, and MALDI-

- TOF-MS (matrices: 3-(3-indolyl)acrylic acid, 2,5-dihydroxybenzoic acid, or 2',4',6'-trihydroxyacetophenone/ammonium hydrogencitrate), as well as elemental analysis.
- [12] The purity of the oligomers was further checked by analytical SEC on two columns (TosoHaas TSKgel G2500 HR (5 μ m) and TosoHaas TSKgel G2000 HR (5 μ m), 0.78 $\times$ 30 cm) connected in series. Vis detection at λ = 400 nm gave peaks at retention time: t_R = 13.92 (4), 12.98 (7), 12.39 (8), 12.03 (1), 11.56 (2), and 11.30 min (3).
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- [16] The real longest-wavelength transitions are hidden under an inhomogeneously broadened absorption band that arises from molecular vibrations. Therefore, the UV/Vis spectra were deconvoluted by assuming a sum of Gaussian line shapes in energy space, by which all spectra could exactly be reproduced. The software *pro Fit*, Ver. 5.0.0 for Power Macintosh, Quantum Soft Zürich, 1990–96, was utilized for fitting by using a self-written plug-in module.
- [17] Raman spectra were measured on a series 2000R NIR FT-Raman spectrometer (Perkin–Elmer). Excitation occurred at 1064 nm (Nd:YAG laser) with a 700–900 mW laser power.

Separation of a Racemic Mixture of Two-Dimensional Molecular Clusters by Scanning Tunneling Microscopy**

Matthias Böhringer, Karina Morgenstern, Wolf-Dieter Schneider, and Richard Berndt*

The separation of a racemic mixture of chiral molecules into the enantiopure compounds is a fundamental and often challenging problem in chemistry that was first solved by Pasteur in his celebrated experiment in 1848.^[1] With a magnifier and tweezers he discriminated and separated small enantiomorphic crystals obtained from a solution of racemic sodium ammonium tartrate. Pasteur took advantage of stereospecific molecular interactions to obtain sufficiently sized crystallites, each composed exclusively of one enantiomer. One hundred and fifty years after Pasteur's pioneer-

ing experiment scanning probe microscopies now permit visualization of structures with molecular resolution, and chiral aggregates of achiral,^[2–6] racemic,^[7, 8] or chiral^[8–11] compounds have been discriminated on the molecular scale. However the second part of Pasteur's experiment—separation of the enantiomorphic aggregates—has not been performed, to date, on the nanoscale. We show that enantiomers of supramolecular clusters that exhibit two-dimensional chirality can be discriminated *and* separated from a racemic mixture with a scanning tunneling microscope (STM). The experiment is a molecular scale analogue of Pasteur's experiment.

Figure 1 shows the structural formula of 1-nitronaphthalene (NN) and a STM topograph of the clean reconstructed Au(111) surface. The Au(111) surface is comprised of alternating domains of face centred cubic (fcc) and hexagonal

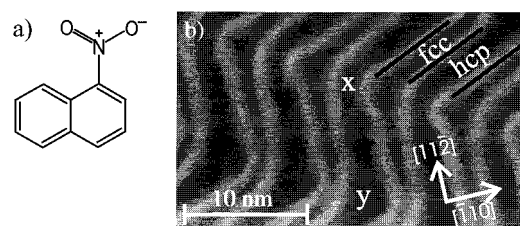


Figure 1. a) Structural formula of 1-nitronaphthalene (NN). b) STM topograph of the reconstructed Au(111) surface. Broad/narrow dark stripes correspond to domains with surface atoms in fcc/hcp positions. Two domain orientations rotated by 120° alternate and form a herringbone pattern. Two inequivalent types of turning points (elbows) of this pattern are observed, which correspond to larger (y elbows) or smaller (x elbows) widths of the fcc domain. Sample voltage $V = -820$ mV, tunneling current $I = 10$ pA.

close packed (hcp) stacking of the surface atoms with respect to the underlying crystal.^[12] The domain walls where the surface atoms are displaced from the hcp or fcc sites appear in STM images 0.1–0.2 Å higher (brighter) than the domains. On large terraces two domain orientations rotated by 120° alternate and form a herringbone pattern.^[13]

STM images of the NN covered surface recorded below about 70 K reveal that molecular aggregates of distinct size and geometry have self-assembled (a monolayer corresponds to a thick layered structure with nine surface atoms per NN molecule). If the isolated molecules are disregarded about 85% of these clusters are composed of ten molecules arranged in a modified pinwheel structure (Figure 2a). The decamers form within the fcc domains near elbows, which is reminiscent of the preferential nucleation of some metals at these sites.^[14] Intriguingly, two related kinds of decamers (L , R) are observed, which behave in a similar manner to an object and its mirror image and cannot be transformed into one another by rotation and translation within the surface plane. We conclude that the confinement of the NN molecules on the Au(111) surface leads to planar aggregates that exhibit two-dimensional chirality. As a consequence of a planar adsorption geometry already on Au(111) single NN molecules are two-dimensionally chiral on the surface. Equal amounts of enantiomeric NN molecules—denoted here as l and r —are expected to be present on the surface. In STM images,

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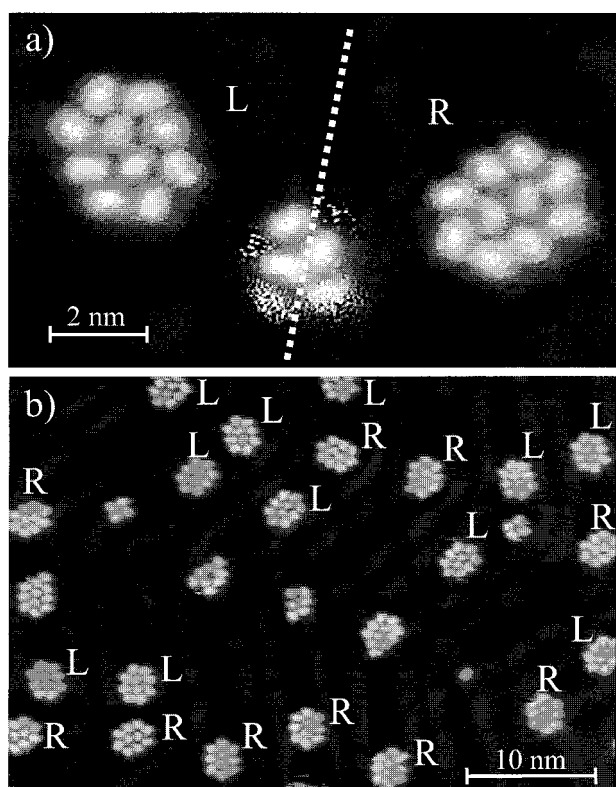


Figure 2. a) Two dimensional chiral decamers (denoted *L* and *R*) formed by NN molecules on the Au(111) surface (imaging conditions: 50 K, –510 mV, 10 pA). b) At 50 K with a coverage of 0.1 monolayers about 85 % of the molecular aggregates are decamers. The remaining clusters are ca. 5 % undecamers, 5 % tetramers, and the rest unordered structures. The decamers nucleate within the fcc domains near γ elbows. Equal amounts of (*L*)- and (*R*)-decamers are observed and hence constitute a racemic mixture (50 K, –330 mV, 10 pA).

however, both enantiomers usually appear as ellipsoids and cannot be distinguished.^[15] The formation of stable NN

decamers may be viewed as the synthesis of two-dimensional chiral supermolecules from an achiral precursor, namely the gas-phase NN. The inversion symmetry of the decamers implies that they are comprised of even numbers of *l* and *r* molecules. Molecular dynamic simulations predict a 8:2 ratio (2:8 for the opposite chirality).^[15] This ratio and the equal numbers of adsorbed *r* and *l* molecules imply that the clusters form a racemic mixture. Accordingly, equal numbers of enantiomorphic (*L*)- and (*R*)-decamers are found on the surface (Figure 2b).

Figure 3 displays a series of experiments at 50 K where the manipulation capabilities of the STM are used to line up decamers within one fcc domain. First, Figure 3a is recorded at imaging conditions, that is with negligible interaction between the STM tip and the molecular aggregate (sample bias –600 mV, tunneling current 10 pA). Then the STM tip is positioned above one of the decamers. At this point the tunneling resistance is decreased by three orders of magnitude (–5 mV, 200 pA) in order to move the chosen decamer through an increased interaction with the tip. Using these parameters the tip is moved slowly, within seconds, to an adjacent fcc domain along the path indicated by an arrow. There the decamer is released by changing back to the imaging conditions. This sequence is repeated for the decamers marked in Figure 3a.

The result of the manipulation was inspected at imaging conditions as shown in Figure 3b. One observes that the decamers have indeed been moved without changing their supramolecular arrangement. In particular, the chirality of the decamers remains unaltered. These experiments support the concept that decamers behave as supermolecules whose stability, structure, and chirality are determined by strong intermolecular interactions.^[15] We note that an enantio-merization of a (*L*)- to a (*R*)-decamer (or vice versa) requires a change from a 8:2 to a 2:8 ratio of (*l*)- and (*r*)-NN molecules.

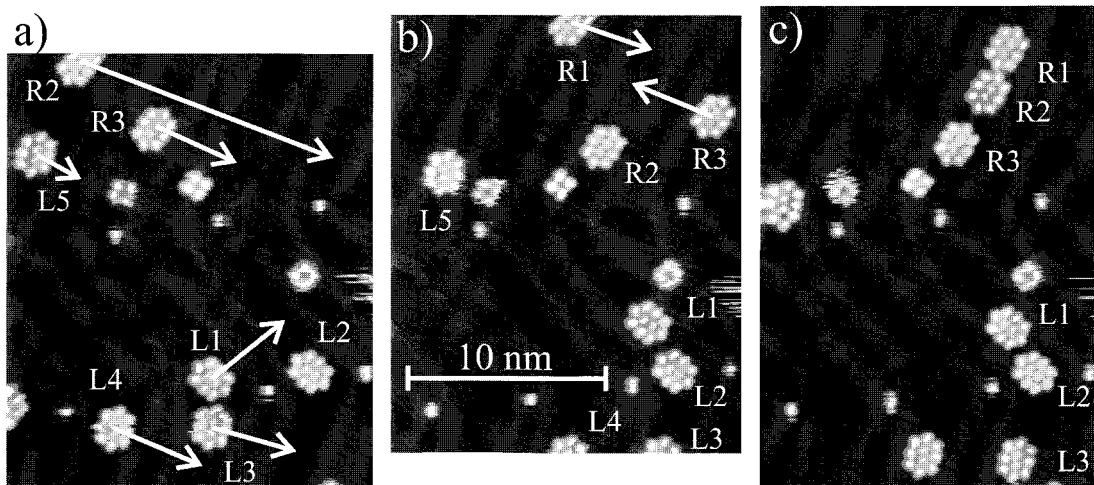


Figure 3. Pasteur experiment in two dimensions on a molecular scale. a) Image (–600 mV, 10 pA) of decamers prior to manipulation. Decamers are subsequently moved by the STM tip along the paths indicated by arrows (tunneling conditions for the manipulation: –5 mV, 200 pA, 50 K). b) Intermediate result of the manipulations. Structure and two-dimensional chirality of the decamers remained unaffected. Decamer *L5* has been moved into the neighboring hcp domain; this decamer will have only a small interaction with the substrate and will be disturbed by the imaging process and so it is disregarded for the rest of the manipulation experiment. c) Arrangement of the decamers at the end of the manipulation sequence. (*L*)-decamers are located in the lower part and (*R*)-decamers in the upper part of a fcc domain.

The manipulation sequence was continued and the decamers were lined up along one fcc domain to separate the racemic mixture into the pure compounds with the (*R*)-decamers in the top right part of the domain and the (*L*)-decamers below (Figure 3c). We note that in terms of the two-dimensional chiral NN molecules this is equivalent to an enantiomeric excess of 60 %.

The present experiment adds the important capability of enantiomer separation to the previous studies on atomic-scale manipulation,^[16] selective dissociation,^[17–19] conformational analysis,^[20] and chiral recognition.^[2–11] Hence scanning probe based techniques are emerging as an important arsenal for nanochemistry. Both chiral recognition and enantioselective manipulation are key capabilities for future stereoselective chemical reactions on a single molecule basis.

Experimental Section

The NN clusters were obtained by dosing sub-monolayer amounts of NN (purified by vacuum sublimation at 300 K prior to use) onto the clean Au(111) substrate at room temperature in ultra-high vacuum and with subsequent cooling to 50 K within 24 h.

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The Carbonyl Chlorine(II) Cation [ClCO]⁺**

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The cation [ClCO]⁺ belongs to a family of linear, three-atom molecules with 30 electrons (such as OCS, ONP, ONS⁺, and ArCN⁺) which are of interest to theoreticians and which exhibit interesting spectroscopic properties. The carbonyl halogen(II) cations [XCO]⁺ (X = F, Cl, Br) are also of practical importance as reactive intermediates in Friedel–Crafts reactions and in plasma etching processes. Whereas the [XCO]⁺ ions (X = F,^[1,2] Cl,^[3] Br^[4]) have been extensively studied in the gas phase (mass spectra, dissociation energies, electron affinities,^[2] rotational^[5] and photoelectron^[6] spectra for [FCO]⁺), and both [FCO]⁺^[5] and [ClCO]⁺^[7,8] have also been the subjects of theoretical calculations, there is only a single report in which the cations [XCO]⁺ (X = Cl, Br, I) have been detected in the condensed phase as transients.^[9] In this study^[9] of the reaction of CO with Cl₂, Br₂, or I₂ in SO₂ClF/SbF₅ by ¹³C NMR spectroscopy at –78 °C, three new singlets at δ = 133.7, 127.0, and 100.4 are observed and attributed to the [XCO]⁺ ions (X = Cl, Br, I) by comparison to the spectra of the corresponding X₂CO molecules. Thus short-lived carbon species are produced in superacidic solutions; however, ¹³C NMR resonances alone are not sufficient evidence to conclusively and unambiguously establish the existence of the carbonyl halogen cations in the condensed phase.

While this manuscript was in preparation, we became aware, from two presentations at conferences,^[10,11] and of a comprehensive, so far unpublished, study of the [ClCO]⁺ ion,^[12] in which vibrational and ¹³C NMR spectra as well as thermodynamic and ab initio calculations are discussed. The results appear to confirm and complement our observations, which are reported here.

We generated the [ClCO]⁺ ion accidentally during attempts to synthesize the homoleptic carbonyl cation [Fe(CO)₆]³⁺. In the reaction of Fe(CO)₅ with CO and Cl₂ as oxidizing agent in SbF₅, the salt [Fe(CO)₆][Sb₂F₁₁]₂ was obtained in high yield and found to be thermally stable to 100 °C.^[13–15] The IR spectrum of this salt contains a new band at 2256 cm^{–1}, which was tentatively attributed to the unknown [Fe(CO)₆]³⁺ ion,^[13,14] because the IR-active fundamental $\tilde{\nu}_{\text{CO}}$ (F_{1u}) of [Ir(CO)₆]³⁺^[16] is observed at 2254 cm^{–1}. We have found in the meantime that the band at 2256 cm^{–1} is also observed when CO and Cl₂ are combined in SbF₅ in the absence of Fe(CO)₅. Hence the band is re-assigned as the $\tilde{\nu}_{\text{CO}}$ band of the [ClCO]⁺

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ion. Optimization of the reaction conditions allows the $[\text{ClCO}]^+$ ion to be generated in such a high yield that its complete vibrational spectrum can be recorded.

If pure SbF_5 is combined with COCl_2 or $^{13}\text{COCl}_2$ or with a 1:1 mixture of CO or ^{13}CO and Cl_2 at a molar ratio of 4:1 in a sealed glass reactor bulb, the reaction mixture solidifies immediately. After ten minutes the mixture liquefies and the characteristic Raman bands of SbF_5 have disappeared. Instead the bands of the $[\text{ClCO}]^+$ or the $[\text{Cl}^{13}\text{CO}]^+$ ion, respectively, are observed (listed in Table 1), together with bands, attributed to oligomeric fluoroantimonate(v) anions of

Table 1. Assignment of the observed vibrational wavenumbers $\tilde{\nu}$ [cm^{-1}] of $[\text{ClCO}]^+$ by comparison with calculated wavenumbers.^[a]

Assignment	Repr.	$[\text{Cl}^{12}\text{CO}]^+$		$[\text{Cl}^{13}\text{CO}]^+$	
		IR _{calcd} ^[b]	IR	Raman	Raman
$2\nu_1$	Σ	4479 (0.07)	4486 (0.6)		4376 (0.4)
$\nu_1 + 2\nu_3$	Σ		3188 (0.6)		3106 (0.5)
$\nu_1 + \nu_2$ (^{35}Cl)	Σ	3052 (1.4)	3056 (2.4)		2995 (1.2)
$\nu_1 + \nu_2$ (^{37}Cl)	Σ	3042 (0.5)	3047 (1.4)		2986 (1.1)
$\nu_1 + \nu_3$	Π		2718 (0.06)		2649 (0.03)
$5\nu_3$	Π		2344 (4.5)		2279 (1.5)
ν_1, ν_{CO} (^{16}O)	Σ	2254 (100)	2256 (100)	2256 (100)	2200 (100)
ν_1, ν_{CO} (^{18}O)	Σ		2212 (0.3)		2155 (1.2)
$2\nu_3$	Σ		945 (3.0)	945 (14)	918 (1.8)
ν_2, ν_{Cl} (^{35}Cl)	Σ	800 (5.9)	803 (7.5)	803 (76)	797 (7.1)
ν_2, ν_{Cl} (^{37}Cl)	Σ	790 (2.0)	794 (4.0)	794 (50)	788 (3.7)
ν_3, ν_{ClCO}	Π	475 ^[c]	468 (27)		454 (27)

[a] The expected band for the overtone $3\nu_3$ is hidden by a group of unassigned bands in the region 1200–1700 cm^{-1} . Values in parentheses are relative intensities. The relative intensities for the Raman bands ν_1 , ν_2 , and ν_3 are calculated by using the basis set RHF/6–311G(3df,3pd)^[19] to be 100:41:0.4 respectively. [b] Ref. [7]. [c] Ref. [8].

the type $[\text{Sb}_n\text{F}_{5n+1}]^-$ with $n > 2$. An unambiguous identification of the counteranion is not possible in the complex reaction mixture. Attempts to record a ^{13}C NMR spectrum of the suspension containing the $[\text{Cl}^{13}\text{CO}]^+$ ion were unsuccessful. The salt is apparently insoluble in SbF_5 and the width of the ^{13}C NMR resonance line is too large to permit a comparison with the results from the earlier study.^[9] Within three days the intensities of the Raman lines of the $[\text{ClCO}]^+$ ion decreased noticeably. The suspension changed to a clear liquid, in which among others bands due to the $[\text{SbCl}_4]^+$ ion are observed at 444, 392, 140, and 124 cm^{-1} .^[17, 18] This implies a Cl/F exchange has taken place, and COFCl and COF_2 as well as small amounts of CF_4 and CO_2 were detected in the gas phase by IR spectroscopy. The IR spectrum of the suspension was recorded on a thin film between silicon discs, prepared inside a drybox.

The observed vibrational band positions of the $[\text{ClCO}]^+$ ion are listed in Table 1 and compared to calculated values of the gas-phase cation.^[7, 8] The calculated band positions were obtained by ab initio calculations with large basis sets,^[7, 8] in which all anharmonicity corrections, with the exception of the Fermi resonances by the ν_3 progression, are taken into account. The agreement between calculated band positions for $[\text{ClCO}]^+(\text{g})$ and the experimentally observed positions for $[\text{ClCO}]^+$ is excellent. Hence the existence of the $[\text{ClCO}]^+$ ion in the condensed phase is clearly and unambiguously established.

The vibrational spectrum of the $[\text{ClCO}]^+$ ion bears some resemblance to those of the isoelectronic species ClCN ^[20] and SCO .^[21] For the Σ representation there are a multitude of anharmonic resonances between various overtones or combinations with ν_1 and ν_2 . These resonances impede a normal coordinate analysis, which requires the use of unperturbed fundamentals. Accordingly the band positions of ν_2 in Table 2 are corrected for Fermi resonance with $2\nu_3$ as described previously.^[21] By using calculated^[8] structural parameters $r_{\text{CO}} = 122.2$ pm, $r_{\text{Cl}} = 156.6$ pm and the vibrational data from Table 2, the following force constants, normalized to 100 pm, are obtained with the program MOLVIB:^[22] $F_{\text{CO}} = 19.2(2)$, $F_{\text{CCl}} = 6.51(5)$, $F_{\text{CO/CCl}} = 0.8(1)$ and $F_{\text{OCCl}} = 0.5034(7) \times 10^2 \text{ N m}^{-1}$. Vibrational data calculated with these force constants are compared in Table 2 to the corresponding input data.

Table 2. Assignment of observed and calculated vibrational wavenumbers $\tilde{\nu}$ [cm^{-1}] of the fundamentals of $[\text{ClCO}]^+$.

Assignment	$[\text{Cl}^{12}\text{CO}]^+$		$[\text{Cl}^{13}\text{CO}]^+$	
	obs.	calcd. ^[a]	obs.	calcd. ^[a]
ν_1 (^{16}O)	2255.6	2256.3	2199.6	2199.4
ν_1 (^{18}O)	2212.2	2212.5	2154.8	2154.0
ν_2 (^{35}Cl)	810.7	811.5	806.1	806.1
ν_2 (^{37}Cl)	801.7	801.8	797.0	796.1
ν_3	467.8	468.0	454.3	454.1

[a] The calculated wavenumbers of the vibrations ν_1 and ν_3 for $^{37}\text{ClCO}]^+$ are 0.3 and 0.5 cm^{-1} smaller respectively, than for $^{35}\text{ClCO}]^+$. [b] Corrected for Fermi resonance with $2\nu_3$ ($X_{33} - X_{11} = 0.25$, $W_{233} = 32.8 \text{ cm}^{-1}$), see text.

The CO stretching force constant F_{CO} of $19.2(2) \times 10^2 \text{ N m}^{-1}$ is significantly larger than in the free CO molecule ($18.6 \times 10^2 \text{ N m}^{-1}$ ^[23]) but clearly smaller than in the $[\text{HCO}]^+$ ion ($21.3 \times 10^2 \text{ N m}^{-1}$ ^[24]). It follows that in the $[\text{ClCO}]^+$ ion, CO is bonded in a very similar manner to that in the homoleptic metal carbonyl cations,^[25] $[\text{M}(\text{CO})_n]^{m+}$, where π bonding is strongly reduced and σ bonding to M causes a polarization of the CO bond.^[25] Hence the agreement in band positions between ν_1 of $[\text{ClCO}]^+$ (Tables 1 and 2) and ν_6 (F_{1u}) of $[\text{Ir}(\text{CO})_6]^{3+}$,^[16] which motivated this work, is not surprising. The force constant F_{CCl} of $6.51 \times 10^2 \text{ N m}^{-1}$ is unusually large but characteristic for Cl–C bonds involving sp-hybridized carbon as in C_2Cl_2 ($5.2 \times 10^2 \text{ N m}^{-1}$,^[26] calculated according to ref. [22]) or in ClCN ($5.3 \times 10^2 \text{ N m}^{-1}$ ^[8]).

We have shown in this study that the superelectrophilic cation $[\text{ClCO}]^+$, generated in the Lewis superacid SbF_5 as reaction medium, can form salts with oligomeric fluoroantimonate(v) anions that are sufficiently stable at ambient temperature to allow a detailed vibrational study. However, the salts cannot be isolated because removal of excess SbF_5 at 25 °C results in slow decomposition, as evidenced by the evolution of COFCl . Interestingly, our earlier attempts^[13–15] at the synthesis of $[\text{Fe}(\text{CO})_6]^{3+}$ ion led to a solid product mixture, in which the $[\text{ClCO}]^+$ ion is one component, that is thermally stable at room temperature for several months.

The reaction of SbF_5 with COF_2 under similar conditions produced the O-bonded adduct $\text{F}_2\text{CO} \cdot \text{SbF}_5$ ^[27] and not the

[FCO]⁺ ion. From a CO/Br₂ mixture and SbF₅ no evidence for [BrCO]⁺ was obtainable. It is concluded that the [FCO]⁺ ion is a stronger electrophile than the [ClCO]⁺ ion, while the [BrCO]⁺ ion is less thermally stable than the [ClCO]⁺ ion.

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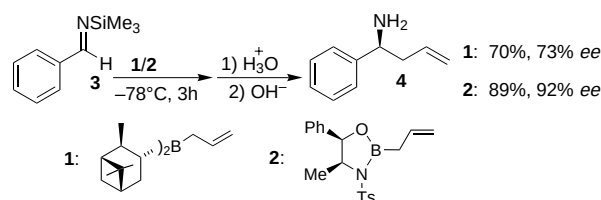
The Critical Importance of Water in the Asymmetric Allylboration of *N*-Trimethylsilylbenzaldehydes with *B*-Allyldiisopinocampheylborane

Guang-Ming Chen, P. Veeraraghavan Ramachandran, and Herbert C. Brown*

Dedicated to Professor Heinrich Nöth on the occasion of his 70th birthday

Among the several α -pinene-based asymmetric synthetic methods^[1] that we have developed over the last ten years, the allylboration of aldehydes with *B*-allyldiisopinocampheylborane (**1**, D-Ipc₂Ball)^[2] has been adopted in numerous syntheses.^[3] Recently, we reported an efficient synthesis of C₂-symmetric diols with very high diastereoselectivity (*de*) and enantioselectivity (*ee*) by the allylboration of various dicarboxaldehydes with **1**.^[4]

On the contrary, there are only a few reports on the allylboration of aldimines, probably owing to the inherent instability of such imines and the lack of reactivity of the more stable *N*-substituted imines.^[5] Recently, Itsuno and co-workers reported the asymmetric allylboration of various *N*-masked benzaldehydes with a variety of allylboration agents, including **1** (Scheme 1).^[6] They concluded that *N*-trimethylsilylaldimine is the most reactive species for such



Scheme 1. Asymmetric allylboration of **3** with **1** or **2**.

allylboration. The reaction can be carried out at -78°C to give the corresponding homoallylamine with up to 92% *ee* upon use of the norephedrine derivative **2** as the chiral auxiliary. Villieras and co-workers adopted Itsuno's methodology for the allylboration of *N*-trimethylsilylaldimines with a 2-ethoxycarbonyl-substituted allylboronate.^[7]

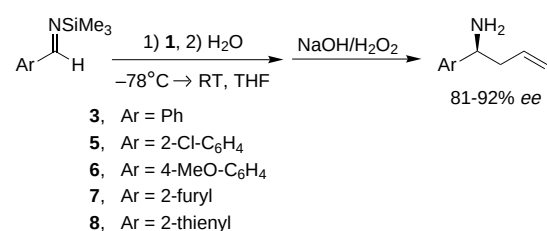
We previously established that the rate of allylboration of aldehydes with **1** is exceptionally fast at -78°C , and fast even at -100°C .^[8] It appeared desirable to obtain comparable information about the allylboration of imines, especially trimethylsilylaldimines, which Itsuno had reported to be very facile. Accordingly, we mixed *N*-trimethylsilylbenzaldehyde (**3**) and **1** in THF at -78°C and followed the rate of reaction by ¹¹B NMR spectroscopy. To our great surprise, we observed no reaction after several hours, not even after a week at room temperature! Clearly there is a discrepancy with the literature

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report of a reaction time of 3 h at -78°C in the case of **1**.^[6] The ^1H NMR spectrum also supported this observation, revealing only the unchanged starting materials. Interestingly, the aqueous workup of this reaction mixture led to a strongly exothermic reaction which provided the product amine in 95 % yield. Apparently, the reaction takes place during the aqueous workup. It might have proceeded by rapid liberation of the aldimine intermediate from the *N*-trimethylsilylaldimine derivative following the addition of water, with a fast reaction of the aldimine with **1**.

Consequently, we repeated the reaction with dropwise addition of a molar equivalent of water in THF to the solution of **3** and **1** at -78°C (Table 1). Indeed, we obtained product amine **4** with 92 % *ee* and in 90 % yield (Scheme 2), which is



Scheme 2. Asymmetric allylboration of **3** as well as **5–8** with **1**.

considerably better than the 73 % *ee* and 70 % yield realized previously.^[6] Lowering the reaction temperature to -100°C improved the chiral induction to 94 % *ee*. In Itsuno's case, it is possible that the exothermic reaction during the aqueous workup raised the reaction temperature rapidly, resulting in an inferior enantioselectivity.

We then examined the allylboration of 2-chloro- (**5**) and 4-methoxybenzaldehydes (**6**), and obtained the products with 82 and 92 % *ee*, respectively (Table 1), in contrast to the values of 46 and 52 % *ee* reported earlier.^[6] In addition, the reaction of 2-furfuralaldimine (**7**) and 2-thiophenealdimine (**8**) with **1** furnished the corresponding homoallylamines with 86 and 81 % *ee*, respectively. Thus, we have shown that in the presence of one molar equivalent of H₂O, **1** is an excellent reagent for the allylboration of aldimines.

N-Trimethylsilylimines are not reactive toward **1**. However, our observation suggests that the reactive aldimines can be

Table 1. Asymmetric allylboration of some aromatic aldimines with D-Ipc₂BAl.

Imine ^[a]	<i>T</i> [$^{\circ}\text{C}$]	Yield [%] ^[b]	Product homoallylamines		
			<i>ee</i> [%] ^[b,c]	config. ^[d]	$[\alpha]_{\text{D}}^{22}$ (<i>c</i> , CHCl ₃)
3	-78	90 (73)	92 (73)	<i>S</i>	-43.2 (1.9)
3	-100	87	94	<i>S</i>	-44.0 (1.3)
5	-78	69 (62)	82 (46)	<i>S</i>	-71.3 (1.5)
6	-78	74 (65)	92 (52)	<i>S</i>	-34.7 (1.2)
7	-78	86	86	<i>S</i>	-30.4 (1.5)
8	-78	72	81	<i>S</i>	-20.2 (1.5)

[a] The *N*-trimethylsilylaldimines were prepared in 75–87 % yield based on the literature procedure.^[9] [b] The values in parentheses are from ref. [6]. [c] Determined by HPLC analysis of product amines on a Chiralcel OD-H column with hexanes/*i*PrOH/Et₂NH (95/5/0.05) as eluent. [d] Determined either by comparison with the rotation value reported in the literature,^[10] or by analogy.

generated in situ from *N*-trimethylsilylimines in a controlled manner upon addition of one equivalent of water, and then captured by the allylboration agent. We believe that this observation is significant for imine chemistry and should find wide applications in organic synthesis, extending considerably the range of applicability of the allylboration reaction.

Experimental Section

Representative example: asymmetric allylboration of **3** with **1**. All of the operations were conducted under a nitrogen atmosphere: To a stirred solution of **3** (0.89 g, 5.0 mmol) and **1** (1.79 g, 5.5 mmol) in THF (5 mL) at -78°C , was added dropwise H₂O (0.100 g, 5.5 mmol) in THF (0.50 mL). The mixture was stirred for 0.5 h, warmed to room temperature, and worked up with NaOH (6.0 mmol), H₂O₂ (12 mmol), and diethyl ether (20 mL). The product was extracted with 3*N* HCl (3 × 10 mL). The combined aqueous layer was washed with diethyl ether (2 × 10 mL), neutralized with Na₂CO₃, extracted with diethyl ether (3 × 10 mL), washed with brine (2 × 10 mL), and dried over anhydrous MgSO₄. Removal of the solvent provided a crude product, which was purified by column chromatography (hexanes/*i*PrOH/Et₂NH 80/20/0.5) to afford the product, 1-phenyl-3-butenamine, as a colorless liquid (0.66 g, 90 % yield). HPLC analysis of the amine on a Chiralcel OD-H column revealed it to be the *S* isomer (92 % *ee*); $[\alpha]_{\text{D}}^{22} = -43.2^{\circ}$ (*c* = 1.9 in CHCl₃); $[\alpha]_{\text{D}}^{22} = -42.6^{\circ}$ (*c* = 0.5 in CH₂Cl₂)^[10a].

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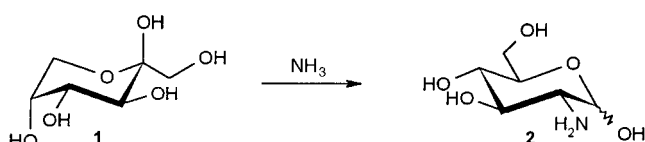
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The Heyns Rearrangement Revisited: An Exceptionally Simple Two-Step Chemical Synthesis of D-Lactosamine from Lactulose**

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*Dedicated to Professor Robin Ferrier
on the occasion of his 60th birthday*

In the 1950s, Heyns and Koch reported the formation of D-glucosamine (**2**) from the reaction of D-fructose (**1**) with ammonia (Scheme 1).^[1] Yields of isolated product for this reaction were found to be around 10 % with rare exceptions of 30 % depending on the reaction conditions employed.^[2] From



Scheme 1. Heyns rearrangement of D-fructose (**1**).

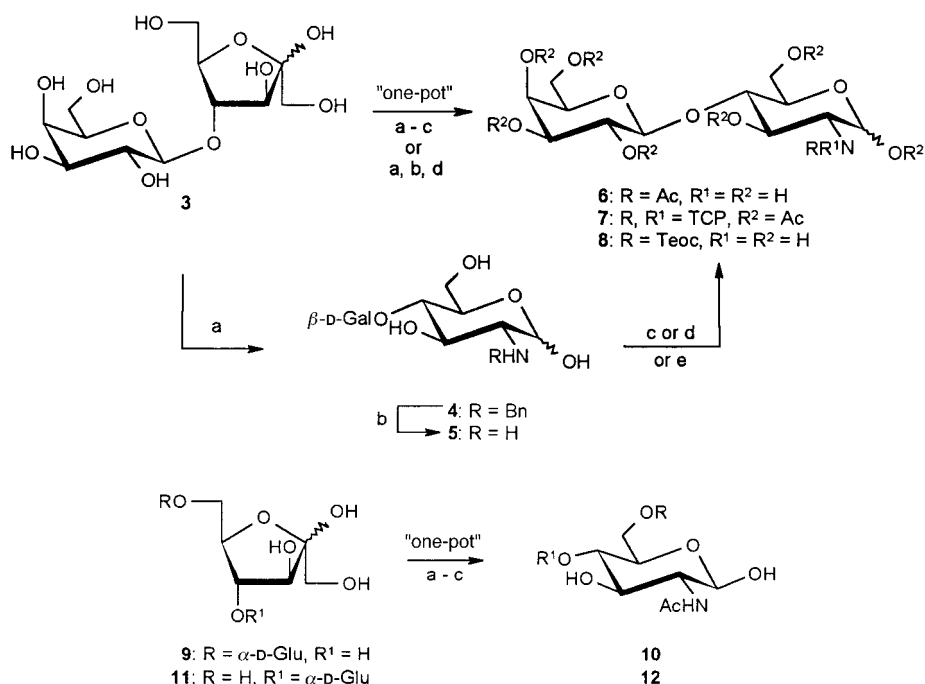
the reaction of D-fructose with primary or secondary aliphatic amines, N-substituted D-glucosamine derivatives were subsequently obtained by Carson^[3] as well as the Heyns group. The latter workers as well as Anet^[4] also examined the reactivities of the other ketohexoses^[5] and the condensation products of D-fructose with aromatic amines^[6] and amino acids.^[7] In all reported cases, yields rarely exceeded the 20 % mark.

Apart from these studies with simple ketoses, preparatively useful applications of the Heyns rearrangement have not been reported to date. We focussed our attention on this interesting and versatile reaction in the context of a demand for multi-gram quantities of various glycosylated N-acetyl-D-glucosamine derivatives, for example, N-acetyl-D-lactosamine (**6**, Scheme 2), which is a constituent of the sialyl Lewis^x epitope,^[8] a cell-type specific oligosaccharide. Disaccharides such as **6** have been available by either multistep chemical syntheses,^[9] with the inherent need for protecting group manipulations, or by enzymatic methods.^[10] The best chemical syntheses, in terms of yields of isolated product as well as scale-up potential, start from lac-

tose and allow access to **6** (Scheme 2) and/or suitable derivatives thereof in seven to nine steps and overall yields ranging from 10 to, at most, 30 %.^[11] Enzyme-catalyzed syntheses have employed D-galactosidases from various sources,^[10, 12–14] or D-galactosyl transferases^[15, 16] as components of multienzyme systems which serve co-factor recycling.

The Heyns rearrangement of lactulose (**3**) (Scheme 2) was achieved by reaction with commercial grade benzylamine followed by treatment of the resulting crude material with glacial acetic acid in methanol to give N-benzyl-D-lactosamine (**4**) in 65–70 % yield. A small proportion of the corresponding D-mannosamine derivative was also formed, as was shown by NMR spectroscopy. The N-benzyl group in **4** was removed by conventional catalytic hydrogenation over palladium hydroxide in methanol. Subsequent addition of acetic anhydride to the reaction mixture containing **5** led to **6**. Overall yields for this three-step procedure were found to range between 38 and 45 %. Changing the solvent in the hydrogenation step from methanol to dilute aqueous HCl allowed access to free D-lactosamine hydrochloride.

This sequence, which can be performed conveniently as a one-pot procedure, does not require any protecting group manipulations or elaborate chromatographic separation and is well suited for scaling-up. A wide range of N-modified derivatives suitable as intermediates en route to sophisticated oligosaccharides are available in good yields by employing



Scheme 2. a) BnNH₂, 40 °C, then AcOH/MeOH; b) H₂, Pd(OH)₂/C, MeOH; c) MeOH, Ac₂O, NaHCO₃; d) tetrachlorophthaloyl anhydride, NEt₃, MeOH, then Ac₂O, pyridine; e) (trichloroethyl)oxycarbonyl chloride, NaHCO₃, H₂O.

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different acylating reagents. For example, with tetrachlorophthaloyl anhydride^[17] and N-(2,2,2-trichloroethyl)oxycarbonyl chloride^[18] the corresponding N-tetrachlorophthaloyl (TCP) and N-(2,2,2-trichloroethyl)oxycarbonyl (Teoc) protected analogues of D-lactosamine, **7** and **8**, were obtained, demonstrating the versatility of the approach.

Other O-glycosylated ketoses reacted accordingly. By quenching at the point of maximum aldosaamine content, the formation of undesired side products, such as 1-aminodeoxy-D-fructose derivatives could be reduced to a minimum. As in the case of **4**, unoptimized yields of O-glycosylated free D-glucosamine derivatives obtained in the two-step approach based on benzylamine ranged between 40 and 70%. Consequently, N-acylated derivatives, for example, N-acetyl-6-O-(α -D-glucopyranosyl)-D-glucosamine (**10**) (40%) and N-acetylmaltosamine (**12**) (33%) could be prepared from isomaltulose (**9**) and maltulose (**11**), respectively, in satisfactory overall yields.

Generally, yields are superior to those obtained with unsubstituted D-fructose which can be rationalized by considering the destabilizing effects caused by unfavorable interactions of the large glycosyl residues at O-4 with other polar functional groups, particularly 5-OH, around the ketopyranose ring. This clearly contributes considerably to the driving force of the rearrangement reaction. The most telling example in this context is the ring enlargement of the highly crowded ketofuranose ring in isomaltulose (**9**) to the distinctly more relaxed 2-aminodeoxyaldopyranose chair in compound **10**.

Experimental Section

A mixture of the O-glycosyl-D-fructose (100 mmol) and benzylamine (700 mmol) was stirred at 40 °C until TLC (CHCl₃/MeOH/NH₄OH, 2:4:1) indicated that the starting material was largely converted into a slightly less polar main product. Conventional removal^[2] of excess amine gave a crude material which was dissolved in MeOH/glacial acetic acid (25:1) and the mixture was kept for 12 h at 25 °C. Pd(OH)₂/C (20%, 2 g) was added and the reaction mixture containing the 2-benzylaminodeoxyaldose was stirred under an atmosphere of H₂ at ambient pressure. After removal of the catalyst by filtration, excess Ac₂O and solid NaHCO₃ was added to the filtrate containing the free 2-aminodeoxy disaccharide. After 1 h, the reaction mixture was concentrated under reduced pressure. N-Acylated products were purified by filtration over a plug of silica gel (Merck 60) by employing a mixture of CHCl₃/MeOH/NH₄OH (8:4:1). Compound **6** was NMR spectroscopically identical with a commercial sample (Sigma, A 7791). The NMR spectra (300 MHz) of compounds **10** and **12** in D₂O matched previously reported data.^[19]

7: Compound **7** was prepared from **5**·HCl following the established procedure.^[17] ¹³C NMR (50.9 MHz, CDCl₃, 25 °C): δ = 162.4, 140.5, 129.8, 126.5 (TCP), 100.9 (C-1'), 90.3 (C-1), 76.5 (C-4), 71.1, 71.0, 70.8, 69.2, 67.2, 67.7 (C-3, C-5, C-2', C-3', C-4', C-5'), 60.8, 60.6 (C-6, C-6'), 53.5 (C-2), signals of N,O-acetyl groups were in the expected regions.

8: To a 5% aqueous solution of intermediate **5**·HCl containing excess sodium bicarbonate, 1.5 equivalents of (trichloroethyl)oxycarbonyl chloride^[18] were added and the mixture was stirred at 22 °C until all starting material had reacted. Conventional work-up^[18] and purification on silica gel (CHCl₃/MeOH/NH₄OH conc. 300:100:2) gave compound **8**: $[\alpha]_D^{25} = +18$ (c = 2.7 in MeOH); ¹³C NMR (50.9 MHz, CD₃OD, 25 °C): δ = 157.0 (C = O), 105.1 (C-1'), 97.2 (CCl₃), 92.6 (C-1), 81.2 (C-4), 77.2 (C-5'), 75.7 (CH₂), 74.8 (C-3'), 72.7 (C-2'), 71.6, 71.3 (C-3, C-5), 70.4 (C-4'), 62.7 (C-6'), 61.9 (C-6), 57.5 (C-2).

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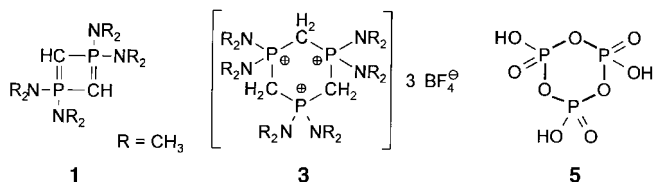
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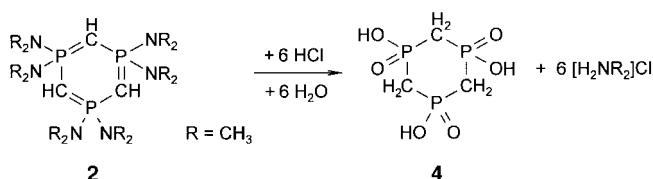
Cyclotrimethylenetriphosphinic Acid**

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The reaction of bis(dimethylamino)difluoromethylphosphorane with two equivalents of *n*-butyllithium gives 1,1,3,3-tetrakis(dimethylamino)-1 λ^5 ,3 λ^5 -diphosphete (**1**)^[1–3] and 1,1,3,3,5,5-hexakis(dimethylamino)-1 λ^5 ,3 λ^5 ,5 λ^5 -[1,3,5]triphosphinine (**2**).^[4–6] Whereas **1** readily hydrolyzes in water to give



water-soluble, acyclic products,^[7, 8] the water-insoluble compound **2** is stable to hydrolysis for a long period of time. In the reaction with HBF₄·Et₂O in diethyl ether, **2** forms crystalline 1,1,3,3,5,5-hexakis(dimethylamino)-1 λ^5 ,3 λ^5 ,5 λ^5 -triphosphininatrium tetrafluoroborate (**3**) in 79.3 % yield.^[9] The protonation of **2** can also be achieved by using other acids, for example with HCl in an aqueous medium, without cleavage of the six-membered ring of **3**. After heating the acidic solution for some time, the dimethylamino groups are successively hydrolytically eliminated resulting finally in the cyclotrimethylenetriphosphinic acid (**4**). The dimethylammonium chloride formed



in the course of the hydrolysis can be removed from the NaOH solution by heating the mixture and **4** can be isolated in the form of colorless sodium 1,3,5-trioxo-1 λ^5 ,3 λ^5 ,5 λ^5 -[1,3,5]triphosphinane-1,3,5-triolate (**4a**) in addition to NaCl.

The title compound **4** corresponds to the cyclotriphosphoric acid **5**,^[10a] in which the three endocyclic oxygen atoms are substituted by methylene groups. Similar heterocycles with alternating P–C sequences, but with λ^3 -phosphorus atoms, have been described in the form of 1 λ^3 -phospha-3,5-diphosphoniacyclohexanedihalogenides by Karsch.^[10b] Compound **4** is stable to hydrolysis both in acidic and in alkaline media, even on heating. The structures of **4** and **4a** have been determined by the multiple-resonance NMR spectra.

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The ¹³C{¹H} NMR spectrum, as expected, displays the twelve lines of the X part of an A₂BX spin system, which is characteristic for six-membered heterocycles with three symmetrically arranged P–C units (Figure 1).^[5, 9, 12, 13] The missing symmetry of this X-part, which is apparent in the four lines

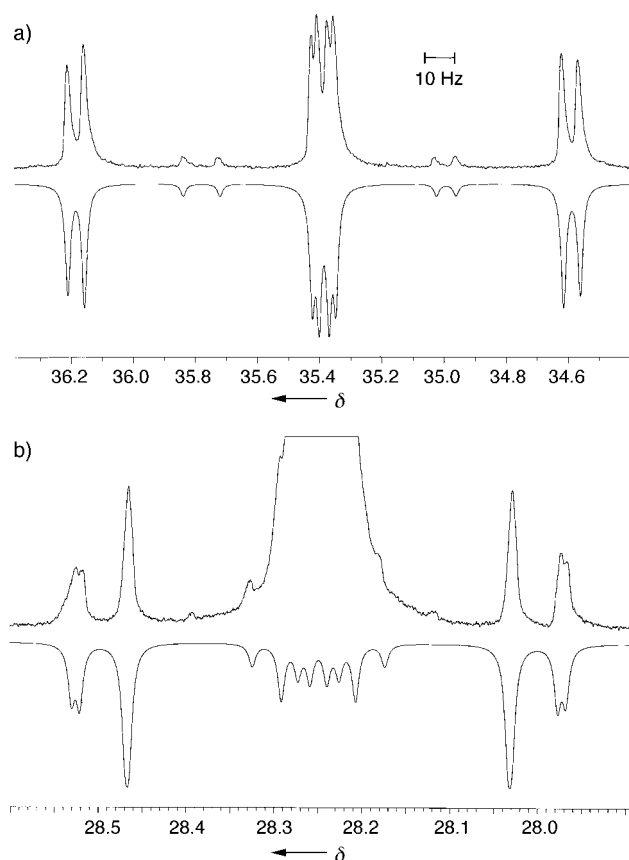
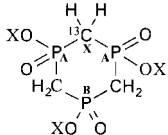


Figure 1. a) 100.614 MHz ¹³C{¹H} NMR spectrum of **4a** with simulated part of spectra and b) ¹³C satellites in the 161.983 MHz ³¹P{¹H} NMR spectrum of **4a** with corresponding simulation, in each case at 85 °C in aqueous NaOH (line width at the height of the two most intensive ¹³C satellites: 16 Hz).^[11]

with lowest intensity, allows the sign of the geminal coupling constant ²J(P_A,P_B) as well as the isotopic shift ¹Δ³¹P_A(¹³C^X) (see ref. [5]; for the numbering see Table 1) to be determined.^[11] The singlet in the ³¹P{¹H} NMR spectrum is flanked by ¹³C satellites, which can be assigned to the A₂B part (Figure 1). The ³¹P NMR signal of **4** and **4a**, respectively, is significantly shifted to higher field in comparison to the signals of the starting material **2** (δ = 65.6),^[5] of the cation **3** (δ = 45.2),^[9] of the cyclic acid 1,3-dioxo-1 λ^5 ,3 λ^5 -[1,3]diphosphinane-1,3-diol (which contains two 1,3-positioned phosphorus atoms; δ = 43.5),^[14] and of the central phosphorus atom P_α of the open-chain phosphinic acid O=P_α(OH)[CH₂P(O)(OH)₂]₂ (δ = 37.3);^[15] in comparison with the corresponding signal of **5** (δ = 20.7),^[16] the ³¹P NMR signal of **4** is, however, shifted downfield by about 15 ppm. Several salts of the acid 2,4,6-trioxo-2 λ^5 ,4 λ^5 ,6 λ^5 -[1,2,4,6]oxatriphosphinane-2,4,6-triol, which is bismethyleneanalogous to **5**, are known.^[15, 17, 18] NMR data are, however, only available for the sodium salt (CH₂ multiplet at δ(¹H) = 2.22).^[18]

Table 1. NMR data of **4** (in 37 % HCl) and **4a** (in aqueous NaOH); concentration in each case 0.1 mL⁻¹.^[11]



X = H: **4**

X = Na: **4a**

	δ			^nJ [Hz]		
	4	4a		4	4a	
³¹ P:	34.92	28.25	1	P _A C ^X	85.3	79.2
¹³ C:	31.56	35.38	1	P ¹⁷ O	—	89
¹⁷ O:	106	121	1	¹³ CH	127 ^[a]	117 ^[b]
¹ H:	2.177	1.637	2	P _A P _B	+14.3	+9.6
			3	P _B C ^X	−3.6	−3.2

[a] Determined from ¹³C satellites of the singlet in the ¹H{³¹P-CW} spectrum. [b] Determined from the ¹³C{¹H} multiplet of the monodeuterated methylene group (see text).

¹³C DEPT experiments on **4** and **4a** confirm that in each case only methylene carbon atoms are present in the heterocycle. The vicinal coupling constants of the PCPC ring units are negative as in **2** and **3**. The small value of $^2J(P_A, P_B) = +14.3$ Hz—compared with the significantly higher values of the geminal coupling in the P=CH–P triads of the trylide **2** (+73.0 Hz), of several diamino- λ^5 -[1,3]diphosphinines (44.3–164.2 Hz),^[19] and of the 1,1,3,3,5,5-hexakis(dimethylamino)-1,2-dihydro-3 λ^5 ,5 λ^5 -[1,3,5]triphosphininium cation (72.8 Hz)^[12]—indicates single bonds in the ring, as also confirmed by the similarly low $^2J(P, P)$ values of the λ^5 -PCH₂– λ^5 -P groups of **3** (4.0 Hz) and of the phosphorylphosphonylmethylene OP–[N(CH₃)₂]₂CH₂PCH₃(O)N(CH₃)₂ (5.7 Hz; hydrolysis product of **1**).^[7, 8, 20] The existence of O=P–O atom sequences in the title compound and its sodium salt were verified by ¹⁷O NMR experiments.^[11] The ¹⁷O NMR signals lie in the range of the signals of the phosphoryl oxygen atoms of the sodium phosphate as well as of the methyl-, dimethyl- and of the trimethylphosphate; in contrast to the singlet of the acid ($\Delta\nu_{1/2} = 410$ Hz), **4a**, as expected, displays a $^1J(^{31}\text{P}, ^{17}\text{O})$ splitting of 89 Hz.^[21] There are no signals of dimethylamino groups visible in the ¹H and ¹³C{¹H} NMR spectra.

Interestingly, when **4a** is heated to 90 °C in D₂O for 48 h, the three methylene groups of the sodium salt are partially deuterated; each of the methylene groups displays only one deuterium atom. The degree of deuteration is 90 %; doubly deuterated methylene groups were not detected. This extraordinary finding arose from ¹³C{¹H} and ¹H{³¹P} NMR experiments.^[11] The ¹³C{¹H} NMR spectrum displays a triplet structure ($^1J(P_A, C^X)$) at 365 K, which is due to C^XD coupling and within the limits of the precision of the measurement consists of broadened signals in the ratio 1:1:1:2:2:2:1:1:1 ($\Delta\nu_{1/2} \approx 8$ Hz); in each case the three outer lines show the same splitting as the two outer line pairs of the nondeuterated species (see Figure 1). This splitting pattern is only consistent with monodeuterated methylene groups in **4a**, as was also confirmed by simulations. The partly recognizable ¹³C lines of the nondeuterated isotopomer allow the determination of the ²H-induced isotope shift, whose value also indicated the presence of CHD groups.^[11] From the two ¹H{³¹P} NMR

spectra at 300 K and 363 K, which show a sharp singlet (CH₂ groups) and a second signal at 363 K, which is almost split into the triplet ($\Delta\nu_{1/2} = 3.8$ Hz; at 300 K: 1.8 Hz), an integral ratio of 1:9 was determined in each case by line form analysis; thus, a degree of deuteration of 90 % (± 2 %) can be assumed. The ²H-induced isotope shift is -0.02 ppm.^[11]

NMR spectroscopic studies of **4** and **4a** at lower temperatures, which could disclose information about the ring conformation and ring inversion of the saturated six-membered ring, were only possible to a limited extent due to the employed solvent (see Table 1) and did not produce any significant results. The width at half intensity of the singlet in the ³¹P{¹H} NMR spectrum of **4a** at 300 K is 1.4 Hz; at -10 °C only a broadening to 5.4 Hz was displayed.

Experimental Section

Compound **2** (1.00 g, 2.52 mmol) was treated with hydrochloric acid (20 mL, 37 %), and the reaction mixture was heated. The ³¹P{¹H} NMR spectrum of the resulting mixture only displayed the signals of **3**.^[9] The solution was heated under ³¹P{¹H} NMR control to 80 °C until all dimethylamino groups were eliminated from the cation of **3** (after ca. 48 h). After removal of the residual hydrochloric acid under vacuum, **4** remained in addition to [(CH₃)₂NH₂]Cl in the molar ratio 1:6 as a colorless powder. The powder was dissolved in water (30 mL), treated with sodium hydroxide solution (0.91 g, 22.70 mmol), and heated under reflux to remove the dimethylammonium ion and form the sodium salt **4a**. After about 48 h only the signals of the anion **4a** were visible in the ³¹P{¹H}, ¹³C{¹H}, and ¹H NMR spectra of the reaction solution. **4a**: IR (Nujol, CsBr plates, only significant absorptions; see ref. [24]): $\tilde{\nu} = 1193$ (ms, sh), 1164 (s, $\nu_{as}(\text{PO}_2)$), 1078 (ms, $\nu_s(\text{PO}_2)$), 1011 (s, $\delta(\text{CH}_2)$), 822 (s, $\nu_{as}(\text{P}_3\text{C}_3 \text{ ring})$), 485 cm⁻¹ (m, $\delta_s(\text{PO}_2)$); all bands are strongly broadened.

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- $\delta(^{17}\text{O})$ of the aqueous HCl (37%) for **4**: $\delta = 27.5$; [22] of the aqueous NaOH for **4a**: $\delta = 5.3$; $\Delta\nu_{1/2}$ in each case 55 Hz. $\delta(^1\text{H})$ of the HCl (37%) for **4**: $\delta = 7.00$; of the aqueous NaOH at **4a**: $\delta = 3.77$. Spectra simulations: Bruker WIN-DAISY, version 4.0. Deconvolution: Bruker WIN1D-NMR, version 6.0. Measurement temperature: 4300 K; **4a** 358 K; **4a** deuterated in D_2O 365 K. Isotope shifts: $^1\Delta^{31}\text{P}_\text{A}(^{13}\text{C}^\text{X}) = -0.009$ relative to [per- ^{12}C]-isotopomer; $^{13}\text{CH}_2/^{13}\text{CHD}$ $^1\Delta^{13}\text{C}(^2\text{H}) = -0.38$; CH_2/CHD $^1\Delta^1\text{H}(^2\text{H}) = -0.020$ [from $^1\text{H}\{^{31}\text{P}\}$ spectra]. [20, 23]
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Evolving Opportunities in Structure Solution from Powder Diffraction Data—Crystal Structure Determination of a Molecular System with Twelve Variable Torsion Angles**

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In general, determination of crystal structures from single-crystal diffraction data can be carried out in a routine and straightforward manner. However, many crystalline solids can be obtained only as microcrystalline powders and are not suitable for investigation by single-crystal diffraction methods. In the past, this problem has limited the ability to determine the structural properties of such materials. There is clearly a pressing need to develop and exploit techniques for solving crystal structures from powder diffraction data. [1–3] However, although traditional techniques for structure solution from powder diffraction data have been applied successfully in several cases, these techniques have certain

intrinsic limitations, [2] and organic molecular crystals represent a particularly challenging case. For these reasons, our recent research has focused on the development and implementation of new methodologies for structure solution from powder diffraction data, and has led to new “direct-space” approaches for structure solution which overcome some of these difficulties. Such direct-space techniques are particularly suited to the case of molecular crystal structures. Herein, we demonstrate the current state-of-the-art in the solution of molecular crystal structures from powder diffraction. To our knowledge, the structure reported here is the most complex molecular crystal structure, solved directly from powder diffraction data, that has so far been reported in the literature.

Direct-space approaches [2, 4, 5] for structure solution from powder diffraction data are based on sampling trial crystal structures in direct space. Structural models are generated independently of the powder diffraction data, with the “quality” of each structural model assessed by comparison between the powder diffraction pattern calculated for the structural model and the experimental powder diffraction pattern. In our work, this comparison is based on the profile R factor R_{wp} , [6] as used in Rietveld refinement. We emphasize that R_{wp} considers the whole digitized intensity profile, and thus uses the raw powder diffraction data directly “as measured”. Peak overlap is implicitly taken care of, and the need to extract integrated intensities $I(hkl)$ for individual reflections from the powder diffraction pattern is circumvented. In effect, our direct-space approach searches the $R_{\text{wp}}(\mathbf{X})$ hypersurface to find the best structure solution (lowest R_{wp}), where $\{\mathbf{X}\}$ represents the set of variables that define the structure. In direct-space strategies for structure solution, the structure is defined by a “structural fragment”, which represents an appropriate collection of atoms within the asymmetric unit. The variables in $\{\mathbf{X}\}$ represent the position, orientation, and intramolecular geometry of the structural fragment. The position is specified by the coordinates $\{x, y, z\}$ of the center of mass or a predefined pivot atom, the orientation is specified by rotation angles $\{\theta, \phi, \psi\}$ around a set of orthogonal axes, and the intramolecular geometry is specified by a set of variable torsion angles $\{\tau_1, \tau_2, \dots, \tau_n\}$. In general, the bond lengths, bond angles, and any known torsion angles (i.e. if aspects of the molecular conformation are known a priori) are fixed in the structure solution calculation, and are taken either from standard values for the type of molecule under study or from the known geometry of a similar molecule. Previous research has demonstrated the success of Monte Carlo, [4, 7–13] simulated annealing, [5, 14–16] and genetic algorithm [17–22] methods for searching R factor hypersurfaces to locate the global minimum (structure solution). Most of these direct-space approaches have considered hypersurfaces based on the profile R factor R_{wp} (as in this paper), although hypersurfaces based on other definitions of agreement factor have also been considered. [16, 20–22] In all of these methods for global optimization, the main limitation is the number of variables in $\{\mathbf{X}\}$ (the number of degrees of freedom which define the hypersurface) and early applications of direct-space approaches for structure solution focused on examples of essentially rigid molecules, with at most a few variable torsion angles. The generalization of direct-space

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approaches for structure solution of molecular crystals from powder diffraction data requires that structures of complex systems, defined by a significant number of torsional degrees of freedom, can be solved in a straightforward and reliable manner.

Here we report the application of our genetic algorithm approach to solve the crystal structure of heptamethylene-1,7-bis(diphenylphosphane oxide) $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ (Figure 1) from powder diffraction data. This compound is of importance within our systematic studies^[23, 24] of crystal packing and crystal engineering of phosphane oxides and their co-crystals.

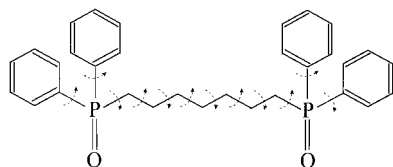
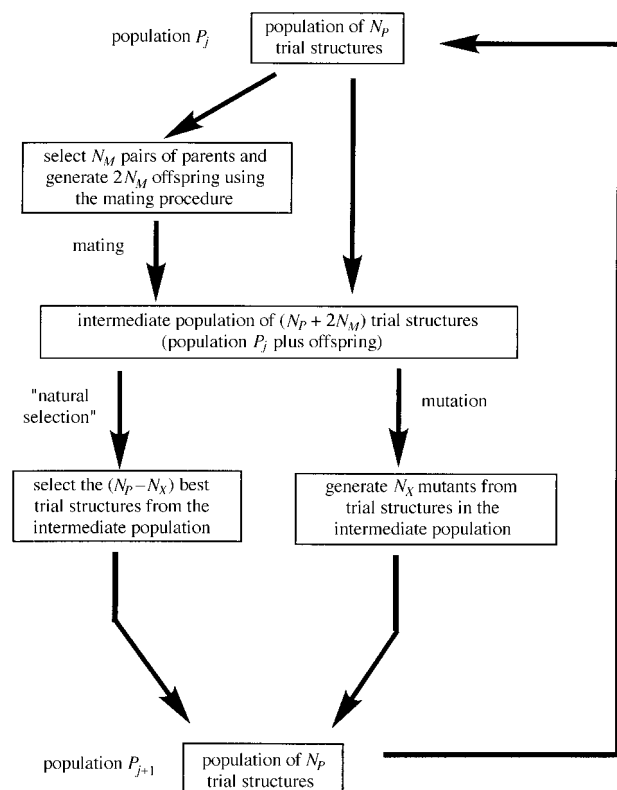


Figure 1. The structural formula of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$, showing the variable torsion angles considered in the genetic algorithm structure solution calculation.

In our genetic algorithm approach for structure solution (Scheme 1), a population of trial crystal structures is allowed to evolve subject to the normal rules and operations that govern evolutionary systems. Full details of our methodology are given in reference [19]. Initially, the population comprises a set of random structures (total number N_p). For the case with one molecule in the asymmetric unit, a given structure is



Scheme 1. Flow chart representing the evolution of the population from one generation (population P_j) to the next generation (population P_{j+1}) in our genetic algorithm approach for structure solution.

specified by the position $\{x, y, z\}$, orientation $\{\theta, \phi, \psi\}$, and intramolecular geometry $\{\tau_1, \tau_2, \tau_3, \dots, \tau_n\}$ of the structural fragment, and these variables represent the "genetic code" that uniquely characterizes each member of the population. The profile R factor R_{wp} is taken to indicate the quality ("fitness") of each structure in the population, where a low R_{wp} value (good agreement between experimental and calculated powder diffraction data) corresponds to high fitness and a high R_{wp} value corresponds to low fitness. It is advantageous to define a fitness function, and several different fitness functions have been considered in our work.^[19] In the present case, the linear fitness function $F(\rho) = 1 - \rho$ was used, where $\rho = (R_{wp} - R_{min}) / (R_{max} - R_{min})$ and R_{min} and R_{max} represent the minimum and maximum values of R_{wp} in the current population (the values of R_{min} and R_{max} are updated as the population evolves under the genetic algorithm). The use of ρ in the definition of the fitness function introduces the advantages of dynamic scaling. The population is allowed to evolve through a number of (typically a few hundred) generations (Scheme 1), using the operations of mating, mutation, and natural selection. In the *mating* procedure, a given number (N_M) of pairs of structures ("parents") are selected from the population, with the probability of selecting a given structure proportional to its fitness. New structures ("offspring") are then generated by combining genetic information from the two parents. Thus, mating does not introduce any new genetic material into the population but redistributes the existing genetic material. The *mutation* procedure, on the other hand, introduces new genetic material into the population. In each generation, a given number (N_X) of structures are selected at random from the current population and random changes are made to parts of their genetic code to create mutant structures. Note that the original structures from which the mutants are derived are retained within the population. The introduction of mutants is important to ensure that genetic diversity is maintained throughout the evolution of the population. The *natural selection* procedure ensures that only the best structures survive from one generation to the next, which leads to a continual improvement in the overall quality of the population. After the population has evolved for a sufficiently large number of generations, the fittest member of the population (the structure with lowest R_{wp}) should, in principle, be close to the correct crystal structure.

The powder X-ray diffraction pattern of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ was recorded using a conventional laboratory powder X-ray diffractometer,^[25] and the unit cell and space group were determined directly from the powder diffraction pattern.^[26]

In the genetic algorithm structure solution calculation, the structural fragment comprised all non-hydrogen atoms of the molecule. Standard bond lengths and bond angles were used,^[24, 28] and the atoms of each benzene ring and all atoms directly bonded to it were constrained to be planar. The molecule was subjected to translation and rotation within the unit cell, together with variation of all twelve torsion angles (Figure 1) that define the molecular conformation. Thus, each structure considered in the genetic algorithm calculation was defined by eighteen variables $\{x, y, z, \theta, \phi, \psi, \tau_1, \tau_2, \tau_3, \dots, \tau_{12}\}$.

The genetic algorithm structure solution calculation was carried out using the program GAPSS,^[19, 29] and involved the evolution of 500 generations of a population of 100 structures (N_p). In each generation, 100 offspring ($N_M=50$) and 20 mutations (N_X) were considered. For the mating and mutation procedures, the eighteen variables were considered in terms of fourteen groups $\{x, y, z\}$, $\{\theta, \phi, \psi\}$, $\{\tau_1\}$, $\{\tau_2\}$, $\{\tau_3\}$, ..., $\{\tau_{12}\}$. In carrying out a given mating operation between two parents, the fourteen groups from each parent were distributed between the two offspring, with no restriction on which combination of groups may come from each parent (in each mating operation, this was determined on a random basis). In carrying out the mutation procedure on a selected structure, seven groups were selected at random, and a new random value was assigned to one variable within each of the selected groups (thus, when the group $\{x, y, z\}$ or the group $\{\theta, \phi, \psi\}$ is selected, only one of the three variables was changed). The evolution of R_{wp} during the genetic algorithm structure solution calculation is shown in Figure 2, and demonstrates clearly that the overall quality of the population improves as the population evolves. The lowest value of R_{wp} in the population decreases significantly, with evidence for the occurrence of a particularly significant evolutionary event at generation 163.

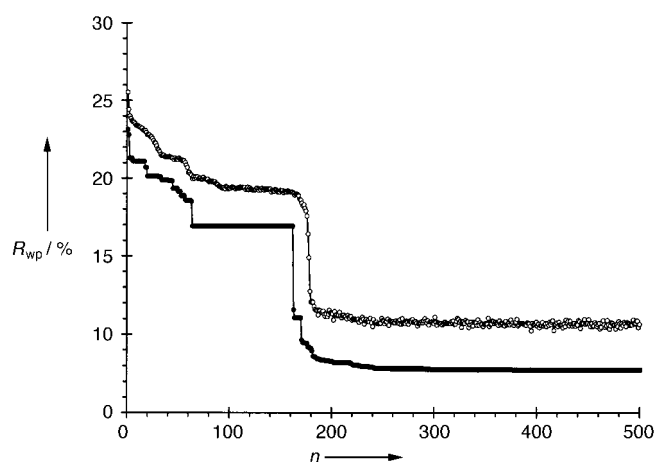


Figure 2. Evolutionary progress plot showing the evolution of R_{wp} for the best structure in the population (filled circles) and the average R_{wp} for the structures in the population (open circles) as a function of generation number (n) in the genetic algorithm calculation.

The best structure (the structure with lowest R_{wp} in the final generation) was taken as the starting structural model for Rietveld refinement using the GSAS program package.^[30] The positions of all non-hydrogen atoms were refined, with standard geometric restraints applied to the bond lengths and bond angles. A common isotropic displacement parameter was fixed at $U_{iso}=0.025 \text{ \AA}^2$ for all atoms. In the final stages, a preferred orientation parameter was refined. The final Rietveld refinement (Figure 3) gave $R_{wp}=0.050$ and $R_p=0.038$. Details of the final refined structure are given in Table 1.

The successful structure solution of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ reported here using experimental data collected on a conventional laboratory powder X-ray diffractometer emphasizes

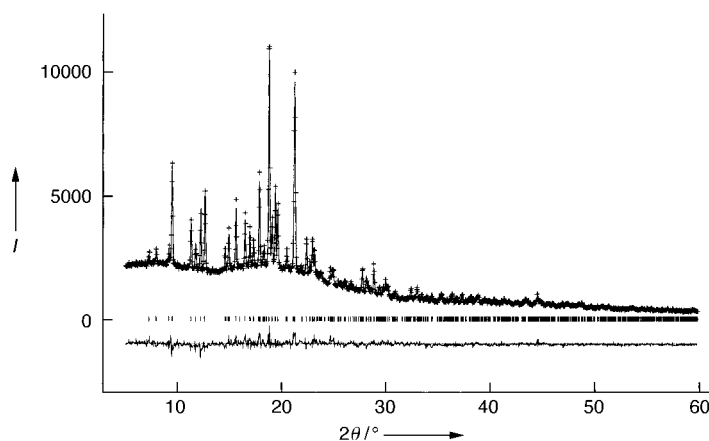


Figure 3. Experimental (+ marks), calculated (solid line) and difference (lower line) powder X-ray diffraction profiles for the Rietveld refinement of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$. Reflection positions are marked. The calculated powder diffraction profile is for the final refined crystal structure, details of which are given in Table 1.

Table 1. Fractional coordinates for the non-hydrogen atoms in the final refined crystal structure of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ ($P2_1/n$; $a=12.560(1)$, $b=10.203(1)$, $c=22.889(2) \text{ \AA}$, $\beta=105.53(1)^\circ$).

Atom	x/a	y/b	z/c
C(1)	0.224(2)	0.940(2)	0.6306(5)
C(2)	0.299(3)	0.989(4)	0.6830(6)
C(3)	0.290(3)	0.953(4)	0.7404(5)
C(4)	0.209(3)	0.862(4)	0.7457(7)
C(5)	0.137(3)	0.809(4)	0.694(1)
C(6)	0.146(3)	0.845(3)	0.6361(7)
C(7)	0.205(1)	1.173(1)	0.558(1)
C(8)	0.097(1)	1.223(1)	0.549(2)
C(9)	0.080(2)	1.356(2)	0.557(2)
C(10)	0.171(2)	1.442(1)	0.573(3)
C(11)	0.277(2)	1.394(1)	0.577(2)
C(12)	0.294(1)	1.261(1)	0.570(2)
P(1)	0.227(1)	0.998(1)	0.5565(6)
O(1)	0.144(2)	0.932(2)	0.5067(6)
C(13)	0.366(2)	0.977(1)	0.553(1)
C(14)	0.413(2)	0.844(2)	0.573(1)
C(15)	0.486(2)	0.790(2)	0.536(1)
C(16)	0.509(3)	0.645(2)	0.5459(7)
C(17)	0.562(5)	0.610(2)	0.611(1)
C(18)	0.568(5)	0.464(2)	0.623(1)
C(19)	0.612(3)	0.418(1)	0.687(1)
P(2)	0.628(1)	0.245(1)	0.6950(5)
O(2)	0.542(1)	0.170(2)	0.6507(7)
C(20)	0.766(1)	0.209(3)	0.6889(7)
C(21)	0.782(2)	0.164(4)	0.634(1)
C(22)	0.889(2)	0.147(5)	0.628(1)
C(23)	0.980(1)	0.168(5)	0.677(1)
C(24)	0.965(1)	0.208(5)	0.733(1)
C(25)	0.858(1)	0.226(5)	0.739(1)
C(26)	0.631(2)	0.207(1)	0.7726(6)
C(27)	0.663(4)	0.303(2)	0.8179(6)
C(28)	0.668(4)	0.273(2)	0.8782(6)
C(29)	0.635(4)	0.149(2)	0.8929(7)
C(30)	0.597(4)	0.055(2)	0.847(1)
C(31)	0.596(5)	0.084(2)	0.787(1)

that, in order to achieve successful structure solution from powder diffraction data, it is not essential that synchrotron X-ray powder diffraction data are used. This fact has also been demonstrated by several previous examples (see reference [2] for a more detailed discussion).

In the crystal structure of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ (Figure 4 top), the molecule adopts an unexpected (but completely rational) conformation (Figure 4 bottom), with one *gauche* bond in the $(\text{CH}_2)_7$ chain and the other parts of the chain close to all-*trans* conformations. It could not have been predicted in

tions of our techniques to solve molecular crystal structures of even greater complexity.

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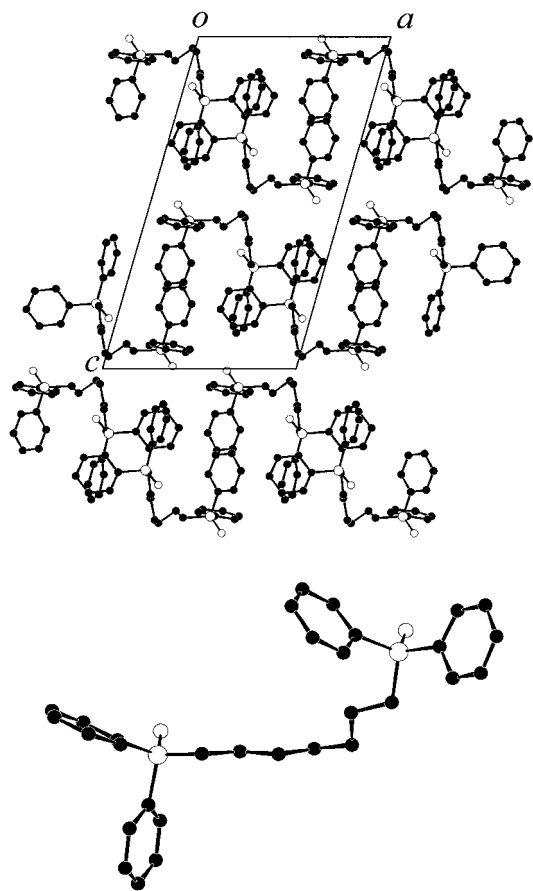


Figure 4. Top: Final refined crystal structure of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ (hydrogen atoms not shown) viewed along the *b* axis. Bottom: Conformation of the $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$ molecule in the crystal structure.

advance that this specific conformation would be adopted in the crystal structure, which emphasizes the importance of allowing complete conformational flexibility in the structure solution calculation. In this conformation, the two $\text{P}=\text{O}$ groups in a given molecule are brought into an almost parallel alignment, and the $\text{P}(\text{O})\text{Ph}_2$ end-groups of neighboring molecules interact through a series of $\text{C}-\text{H}\cdots\text{O}$ and $\text{C}-\text{H}\cdots\pi$ interactions. This structure exhibits very interesting contrasts to the structures of other members of the homologous family $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_n\text{P}(\text{O})\text{Ph}_2$. A full description and rationalization of the crystal structure of $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_7\text{P}(\text{O})\text{Ph}_2$, and comparison with others in the $\text{Ph}_2\text{P}(\text{O})(\text{CH}_2)_n\text{P}(\text{O})\text{Ph}_2$ family, will be given in a future paper.^[24]

The successful determination of a molecular crystal structure defined by eighteen structural degrees of freedom demonstrates clearly the increasing scope and potential of techniques for the solution of molecular crystal structures from powder diffraction data. Indeed, the opportunities demonstrated here augur well for future successful applica-

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$$R_{\text{wp}} = 100 \times \left(\frac{\sum_i w_i (y_i(\text{obs}) - y_i(\text{calcd}))^2}{\sum_i w_i (y_i(\text{obs}))^2} \right)^{1/2} \quad (1)$$

profile, $y_i(\text{calcd})$ is the intensity of the *i*th data point in the calculated powder diffraction profile, and w_i is a weighting factor for the *i*th data point. Thus, R_{wp} considers the whole digitized intensity profile, as measured, rather than the integrated intensities of individual diffraction maxima.

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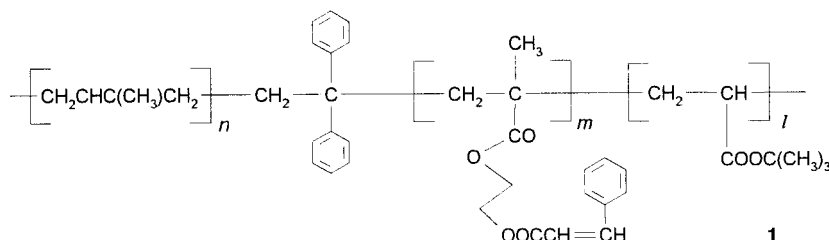
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Preparation and Properties of Nanoporous Triblock Copolymer Membranes**

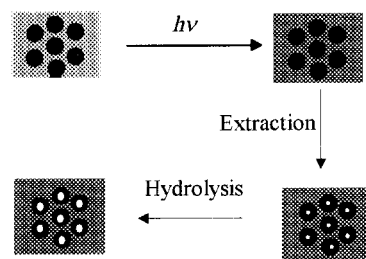
Guojun Liu,* Jianfu Ding, and Sean Stewart

A block copolymer is a linear macromolecule consisting of different monomers in long sequences. $(A)_n(B)_m(C)_l$, for example, denotes a triblock consisting of n units of monomer A, m units of B, and l units of C in sequence. The different blocks of most block copolymers are incompatible. They segregate in bulk, and each block forms domains with a specific shape. At block segregation equilibrium, the domain shape of a given block in a diblock copolymer, the simplest type of block copolymer, is determined by the composition of the diblock, and the domain shape/composition relation is well understood.^[1] The morphologies of triblocks are, however, far more complex and less well understood because they are determined by two independent composition variables, the sequence of block linkage and the interfacial tensions between the different blocks. The domain shape of the minor component, which exists in the least amount in a triblock, ranges from spheres^[2, 3] to cylinders,^[3–9] dumbbells,^[10] and layers.^[2, 3, 6] The cylinders can be straight^[3–6] or folded into loops^[6, 7] or spirals.^[7] The cylinders can also be tortuous and interconnected.^[8, 9] The domain size of the minor component can be tuned by varying the molar mass of the block and has a very narrow distribution. The domains can be regularly packed. Because of these properties, films of block copolymers are ideal precursors for membranes with nanometer-sized channels or nanochannels. The nanochannels can be produced by full^[11, 12] or partial decomposition^[13, 14] of the cylindrical domains formed from one of the blocks, as was demonstrated for diblock films.^[11–14]

Here we report the first preparation of thin films with nanochannels from a triblock copolymer, polyisoprene-*block*-poly(2-cinnamoyl ethyl methacrylate)-*block*-poly(*tert*-butyl acrylate) or PI-*b*-PCEMA-*b*-PtBA **1**. The polymer consists of 3.7×10^2 units of isoprene, 4.2×10^2 units of CEMA, and



5.5×10^2 units of *t*BA. Ten weight portions of this triblock were mixed with one portion of a homopolymer PtBA (HPtBA) with approximately 1.0×10^2 units of *t*BA. At this mixing ratio, the PtBA block of the triblock and HPtBA form a seemingly novel continuous phase in the matrix of PI-*b*-PCEMA (Scheme 1). The PCEMA block was crosslinked by



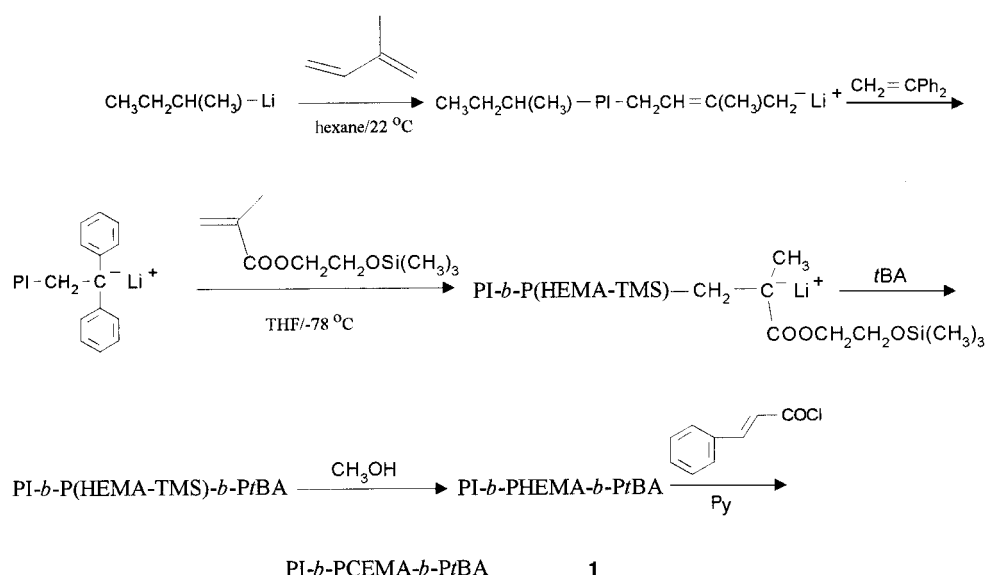
Scheme 1. Strategy for the preparation of nanoporous membranes. PtBA/HPtBA domains (black circles) do not form hexagonally packed cylinders in the system studied, and the cross-sectional view of hexagonally packed cylinders is shown here for convenience in drawing only. UV irradiation crosslinks the PCEMA domains in the PI-*b*-PCEMA matrix (gray rectangle). Extraction of HPtBA out of the PtBA/HPtBA domains yields membranes with nanochannels (white dots). Larger nanochannels (white circles) are produced by both extracting HPtBA and removing the *tert*-butyl groups in the PtBA block.

UV photolysis. Instead of generating nanochannels by the traditional block degradation method, we prepared membranes more conveniently in this study by extracting HPtBA out of the cast films with methylene chloride. Such extracted films had gas-permeability constants roughly 6 orders of magnitude higher than that of low-density polyethylene films. Membranes with even greater gas permeability were obtained by both extracting HPtBA and also removing the *tert*-butyl groups from the PtBA block by hydrolysis (see Scheme 1).

The polymer PI-*b*-P(TMS-HEMA)-*b*-PtBA (TMS = trimethylsilyl, HEMA = 2-hydroxyethyl methacrylate), the precursor to triblock **1**, was synthesized by combining procedures we used for the synthesis of PI-*b*-P(TMS-HEMA)^[15, 16] and P(TMS-HEMA)-*b*-PtBA.^[13, 14, 17] The exact reactions are outlined in Scheme 2. Isoprene polymerization was carried out in dry hexane at room temperature for two days with *sec*-butyllithium as the initiator. The other two blocks were

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Scheme 2. Preparation of triblock **1**. Py = pyridine.

polymerized in sequence at -78°C in THF/hexane. The polyisoprene anions were first converted into the sterically more hindered 1,1-diphenylethylene terminal anions to minimize side reactions between the polymer initiator and HEMA-TMS. The TMS groups were hydrolyzed by adding about 20% methanol to the polymerization mixture to yield PI-*b*-PHEMA-*b*-PtBA. The cinnamoyl groups were attached by treating PI-*b*-PHEMA-PtBA with excess *trans*-cinnamoyl chloride in pyridine. The polydispersity indices of the isoprene block and the triblock were 1.05 and 1.16, respectively. The molar mass of the PI block was $2.5 \times 10^4 \text{ g mol}^{-1}$ as determined by light scattering, which yielded an isoprene repeat unit number of 3.7×10^2 . The ratio of the isoprene, CEMA, and *t*BA repeat units as determined by NMR spectroscopy was 1.00/1.14/1.49. The CEMA and *t*BA repeat unit numbers are thus 4.2×10^2 and 5.5×10^2 , respectively.

Films 20- to 50- μm thick were prepared by casting a solution of triblock **1** and HPtBA, 20% by mass, in toluene on glass slides. The films were dried at room temperature for 2 h and at 70°C for about 5 h, and were then annealed at 100°C for 1 d. The colorless films were softened by soaking in methanol for 20 min before they were peeled off the glass slides with the help of a razor. Since the densities of PI, PCEMA,^[18] and PtBA^[19] are 0.91, 1.25, and 1.00 g cm^{-3} , respectively, the volume fractions of PI, PCEMA, and PtBA/HPtBA in the triblock **1**/HPtBA mixture are 0.13, 0.43, and 0.44, respectively.

For transmission electron microscopic (TEM) studies, a small piece of such a film was embedded in an epoxy resin, and the epoxy resin was cured at 60°C overnight. Slices, 50-nm thick, were obtained from the embedded film by microtomy. The thin slices were stained with OsO_4 vapor overnight before they were viewed under a Hitachi-7000 transmission electron microscope operated at 100 kV.

Figure 1(top) shows the image of a typical slice cut approximately parallel to the plane of the bulk film. Since OsO_4 was used to stain the sample, the dark and gray domains represent the locations of PI and PCEMA, which are not differentiated well here. The light regions must correspond to

the locations of PtBA/HPtBA domains. The image clearly shows that no macroscopic phase separation occurred for PtBA/HPtBA. The good mixing between HPtBA and the PtBA block is expected because the molar mass of HPtBA is much lower than that of PtBA.^[1] The shape of the PtBA/HPtBA domains seems to change from location to location. The typical shapes of the PtBA domains as revealed by TEM are circles (one is marked by a short arrow) and tortuous stripes (two are marked by long arrows). This suggests that PtBA

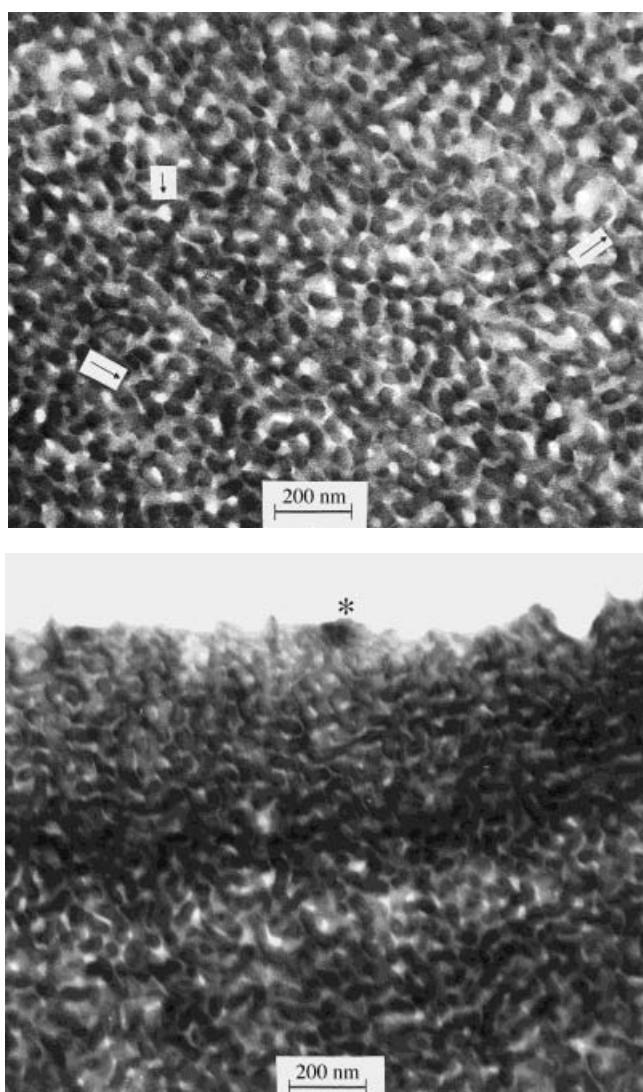


Figure 1. TEM images of 50-nm-thick films of triblock **1**/HPtBA stained with OsO_4 . The thin sections were obtained by microtomy approximately parallel to the film plane (top; several PtBA domains are marked with arrows) and at an angle of roughly 30° to the film plane (bottom; air-polymer interface marked with an asterisk).

probably forms interconnected tortuous cylinders in the PI-*b*-PCEMA matrix. The tortuous cylinders may appear different depending on their different orientations relative to the electron beam.

Figure 1 (bottom) displays the image of a slice cut at an approximately 30° angle to the plane of the bulk film. Because of the different microtoming direction, more tortuous light stripes are seen. Also the morphology at the air–polymer interface (marked with an asterisk) seems to be fairly similar to that in bulk. The TEM image patterns shown here do not resemble any found for triblock copolymers but bear resemblance to those known for the gyroid morphology of diblocks.^[1] While the exact morphology of such a sample remains to be elucidated, the suggested continuity of the P*t*BA/HP*t*BA domain is, however, reasonable for the P*t*BA/HP*t*BA volume fraction of 0.44.

The bulk films were irradiated for 10 min on each side in a UV beam from a 500-W mercury lamp passed through a 310-nm cut-off filter. This caused crosslinking of roughly 40 % of the PCEMA double bonds, as verified by FT-IR analysis. HP*t*BA was extracted out of the films by soaking them in CH₂Cl₂ for three days. The solvent was then gradually changed to methanol before the films were collected and dried at room temperature. The fact that the films did not disintegrate in CH₂Cl₂ suggests that PCEMA, at the volume fraction of 0.43, formed a continuous skeleton as well. The removal of HP*t*BA can be qualitatively judged from the fact that the extracted films appeared somewhat cloudy. More conclusive evidence for nanopore formation was obtained from TEM analysis of the microtomed slices of the extracted films.

Figure 2 shows a TEM image of nanochannels generated by both extracting HP*t*BA and removing the *tert*-butyl groups in the P*t*BA/HP*t*BA domains. The bright ovals must represent the channels. Some of the channels (marked by arrows) are obviously not normal to the film plane but tortuous as judged from the contrast variations surrounding the bright ovals. Not

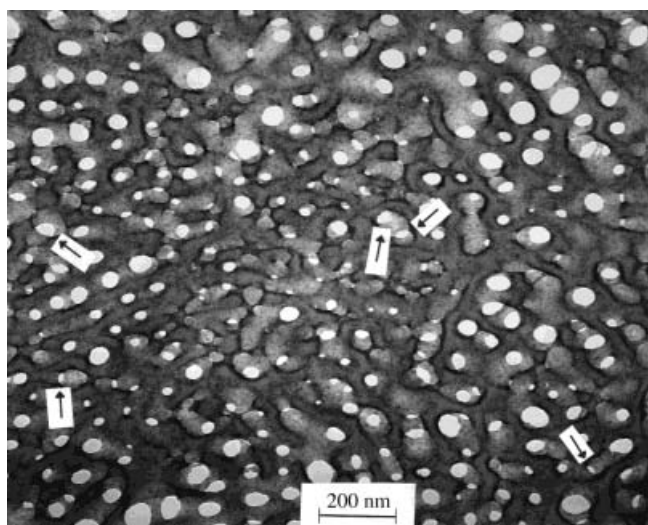
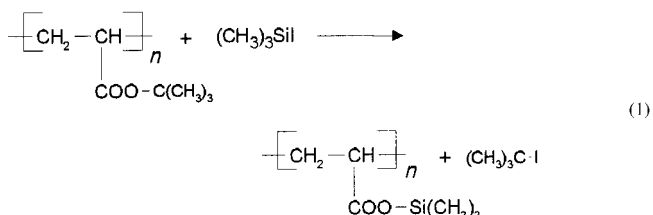


Figure 2. TEM image of a 50-nm-thick film of triblock 1/HP*t*BA after extraction of HP*t*BA and removal of the *tert*-butyl groups. The sample was not stained. The bright circles must represent holes where polymer density is close to zero. Several tortuous channels are marked with arrows.

seen in Figure 2 are bright tortuous stripes. This might be caused by the particular direction along which microtoming was performed to obtain this particular thin film, because bright tortuous stripes were seen in other images.

The simultaneous removal of HP*t*BA and the *tert*-butyl groups of the P*t*BA block was achieved by soaking the films in 0.10 M (CH₃)₃SiI solution in dichloromethane for two weeks. The *tert*-butyl esters were transformed into TMS esters [Eq. (1)]. The TMS groups were then removed in methanol/water (95/5), yielding chains of poly(acrylic acid) (PAA) in the nanochannels.^[13, 14] The quantitative cleavage of the *tert*-butyl groups was confirmed by comparing the FT-IR spectra of the films before and after the hydrolysis step.



To test the gas permeability of this material, a membrane, typically 25- to 50-μm thick, was sandwiched at the interface between the arms of a U-tube. A gas pressure drop (Δp) of $\sim 1.96 \times 10^3$ Pa was applied across the membrane. After the thickness (l) and area (A) of the membrane and the gas permeation rate (F) had been determined (Table 1), Equa-

Table 1. Permeation rates F [cm³s⁻¹] of different gases across the two types of membranes measured at 22 °C.

H ₂	N ₂	O ₂	Ar	CO ₂
Membrane without HP <i>t</i> BA, $A = 0.071$ cm ² , $l = 2.0 \times 10^{-3}$ cm				
0.0350	0.0125	0.0112	0.0093	0.0076
Membrane without <i>tert</i> -butyl groups, $A = 0.049$ cm ² , $l = 2.5 \times 10^{-3}$ cm				
0.0248	0.0123	0.0111	0.0082	0.0135

tion (2) was used to evaluate the gas-permeability constant (P). Plotted in Figure 3 are the variations in P as a function of the square root of the molar masses (M) of different gases for

$$P = \frac{Fl}{A\Delta p} \quad (2)$$

the membranes from which HP*t*BA and both HP*t*BA and *tert*-butyl groups had been removed. The gas-permeability constants are on the order of 10^{-7} cm²s⁻¹cm⁻¹Pa⁻¹, which is about 6 orders of magnitude higher than those found for low-density polyethylene.^[20] The high gas permeability confirms the highly porous nature of the films.

We also tested water permeability across such membranes by filling the arms of a U-tube with water to different heights. Regardless of the ionic strengths or pH of the aqueous medium, no water permeation was noticed. The lack of water permeability across the HP*t*BA-free membranes was understandable, because the channels are hydrophobic and cannot be easily wetted by water. The lack of water permeability across the membranes without *tert*-butyl groups was more surprising, because the nanochannels with PAA chains are

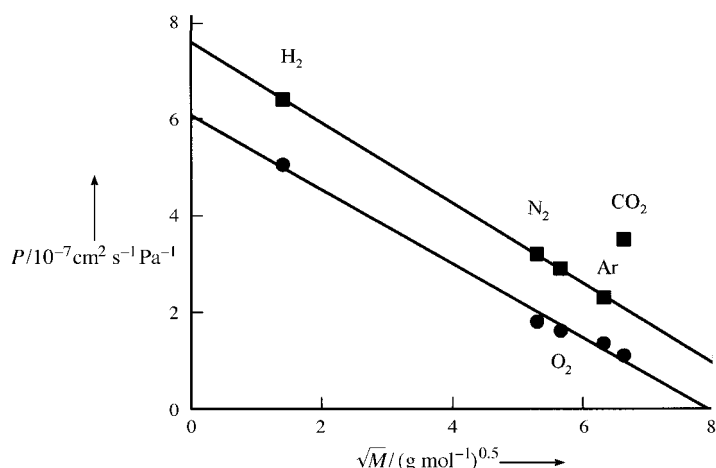


Figure 3. Plot of the permeability constants (P) of different gases across membranes from which HPtBA (●) and *tert*-butyl groups (■) have been removed as a function of the square root of the molar masses of the gases.

hydrophilic. The lack of water permeability and high gas permeability suggest that the PAA nanochannels are probably continuous in a grain, which may be of the size of several micrometers, and are discontinued at the boundaries between different grains. A grain here would be similar to a single crystal in which the different polymer domains are packed with order and the same orientation. From grain to grain, the domain orientations may change. Such grain boundaries may be very narrow. While gas molecules can permeate through such boundaries with ease, water flow would be stopped there. This model is supported by the observation of water permeability across thinner films without *tert*-butyl groups, for instance 2- μm thick, obtained by microtoming a 50- μm -thick film.

The gas-permeability constants are higher for the membranes without *tert*-butyl groups. This is reasonable, because the channels and the pore volume fraction are supposed to be larger here owing to the removal of *tert*-butyl groups of the PtBA block. The linear decrease of P with increasing $\sqrt{M}$ indicates that the gas molecules permeate through the nanochannels due to Knudsen flow^[21] and that the gas molecules have no specific interactions with the channel walls. The only exception to this is the permeation of CO_2 across these membranes. This abnormality is reproducible and suggests some specific interactions between the nanochannel walls and CO_2 . The membranes without *tert*-butyl groups were not gas permeable immediately after their preparation. They became gas permeable after they were soaked in 0.10 M NaOH solution, rinsed in water, and dried. The treatment with base converts PAA to poly(sodium acrylate), and the electrostatic force between the different polyelectrolyte chains presumably pushes the channels open. While the exact mechanism for this specific interaction is not known, it must be between CO_2 and poly(sodium acrylate).

In summary, we have discovered a seemingly new morphology for triblock copolymers. A convenient method was demonstrated for preparing membranes that are gas- but not liquid-permeable. Such membranes may be permeable to water vapor as well and thus should behave similarly to Gore-

Tex films, which reject liquid water because the channels cannot be wetted by water. The membranes without *tert*-butyl groups reject water probably due to channel discontinuity at grain boundaries. The methods proposed for membrane preparation are general and should be useful in many other systems. A detailed study aimed at examining the effect of varying the nanochannel size on gas permeability is under way.

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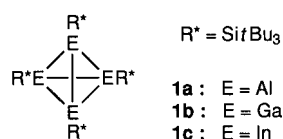
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Dodecaindane (*t*Bu₃Si)₈In₁₂—A Compound with an In₁₂ Deltapolyhedron Framework**

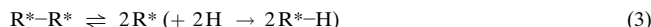
Nils Wiberg,* Thomas Blank, Heinrich Nöth, and Werner Ponikwar

Dedicated to Professor Helmut Werner on the occasion of his 65th birthday

We recently reported that the thermolysis of tetrasupersilyldialane R₂*Al–AlR₂* (“supersilyl” R* = tri-*tert*-butylsilyl) in heptane at 100 °C initially gives trisupersilyldialanyl R₃*Al₂, which is metastable at room temperature, and then leads to the red-violet tetrasupersilyl-*tetrahedro*-tetraalane **1a**.^[1] Analogously, trisupersilyldigallanyl R₃*Ga₂ thermolyzes in heptane at 100 °C to give the deep violet tetrasupersilyl-*tetrahedro*-tetragallane **1b**.^[2] The enlargements of the element

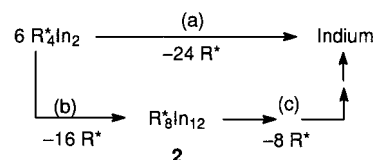


clusters according to Equations (1), and (2), accordingly are initiated by thermal dissociations of element–supersilyl bonds, which in the case of the digallane R₂*Ga–GaR₂* are so facile that so far instead of R₄*Ga₂ only the supersilyl-poorer R₃*Ga₂ compound could be isolated.^[3] The resulting supersilyl radicals R* dimerize *quickly* to give “superdisilane” R*–R* [Eq. (3)], which is *slowly* converted at 100 °C in heptane into “supersilane” R*–H by H-uptake.^[4]



As a continuation of our studies we set out to synthesize tetrasupersilyl-*tetrahedro*-tetraindane **1c** as a further indate-tetrahedrane in addition to the two known tetraorganyl-*tetrahedro*-tetraindanes R₄In₄ (R = C(SiMe₃)₃ and C(SiMe₂Et)₃)^[6] by thermolysis of tetrasupersilyldiindane R₂*In–InR₂*.^[5] The diindane in question, which is readily accessible from InCl₃ and R*Na in THF (see Experimental Section), indeed completely thermolyzes in boiling heptane (ca. 100 °C) after 22 h to give supersilane R*H and an indium-containing product. From the dark reaction solution black-violet, water- and air-sensitive crystals precipitate at room temperature in the course of days after substituting benzene for the heptane. These crystals are thermally comparably stable and are almost insoluble in heptane, benzene, or

toluene even at 100 °C. Surprisingly, according to X-ray structural analyses^[7] these crystals are not of **1c** but of octasupersilyldodecaindane **2**. Thus, thermolysis of the diindane R₄*In₂ at 100 °C is accompanied by elimination of more supersilyl radicals than that of the dialane R₄*Al₂ or that of the digallanyl R₃*Ga₂. Consequently the former case leads to a more pronounced enlargement of the element cluster than the latter two. In other words, the thermal decomposition of the diindane, which finally leads to indium (Scheme 1, Reaction (a)), initially stops at an element-rich cluster level (Scheme 1, Reaction (b)) than that of the dialane or that of the digallanyl.



Scheme 1. Thermal cluster enlargements starting from R₄*In₂ (R* = Si*t*Bu₃).

Figure 1 shows the X-ray crystal structure of **2** (local symmetry approximately S₄), in which all H atoms have been omitted for clarity. It reveals a closed polyhedron framework of 20 triangular faces and 12 In atoms, which does not exhibit a spherical form, such as the icosahedrally shaped B₁₂H₁₂²⁻,^[8] but

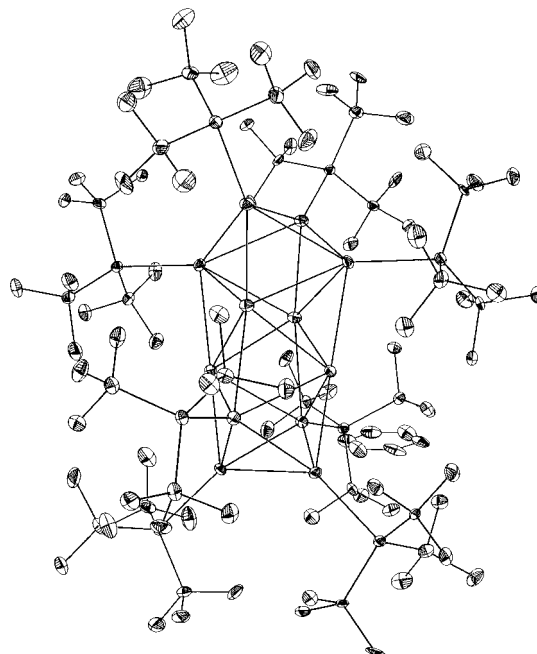


Figure 1. View of the crystal structure of R₈*In₁₂ (ORTEP plot, 25% thermal probability ellipsoids, hydrogen atoms are omitted for clarity).

resembles a stretched ellipsoid. The four In atoms at the ends of the ellipsoid each carry one supersilyl group and the four In atoms in the middle of the ellipsoid carry no supersilyl group. The four spherical Si*t*Bu₃ groups at the end of the ellipsoid display distorted-tetrahedral packing. This illustrates the tendency (attributed to van der Waals interactions^[4]) of the

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[**] Compounds of Silicon, Part 125: Supersilyl Compounds of the Triels, Part 8. This work was supported by the Deutsche Forschungsgemeinschaft. Part 124: N. Wiberg, H. Auer, H. Nöth, J. Knizek, K. Polborn, *Angew. Chem.* **1998**, *110*, 3030; *Angew. Chem. Int. Ed.* **1998**, *37*, 2869; Part 7: ref. [2].

supersilyl groups to form the densest possible packing arrangement. The arrangement of the In atoms at the ends of the In_{12} cluster has to "submit" to this tendency. The $\text{Si}t\text{Bu}_3$ groups play a structure-determining role in the structure of the In_{12} cluster, which can be described as a section of elemental indium (distorted cubic closest packing^[8, 9]). The longitudinal diameter of the In_{12} cluster is about 750 pm. Thus cluster **2**, which is formed in the course of the thermolysis (b), approaches the realm of nanostructured materials; this area is then passed through in the course of the thermolysis (c) (Scheme 1), which has still to be studied in detail.

The presentation of the In_{12} polyhedron shown in Figure 2 gives a better insight into the present structures. The In_{12} cluster consists of two distorted octahedral In_6 building blocks, which when considered as separate entities can be classified as

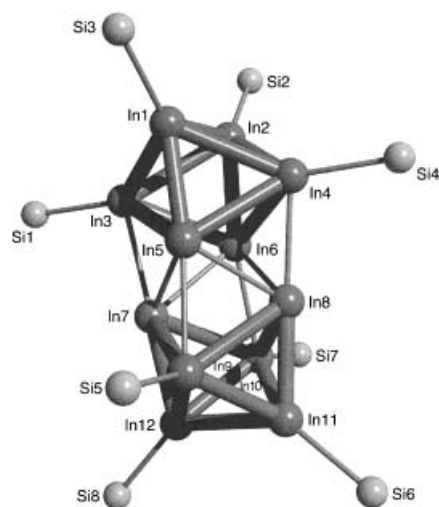


Figure 2. View of the crystal structure of $\text{R}_8^*\text{In}_{12}$, which is divided into two R_6^*In_6 octahedra, and atom numbering system used (local symmetry approximately S_4 ; SCHAKAL plot; atoms with arbitrary radii; $t\text{Bu}$ groups are omitted for clarity). Selected bond lengths [Å] and angles [°] (in each case only one distance and angle are given for one set of bond and angles; however, the underlying S_4 symmetry is merely an approximation): In1–In2 3.091(2), In1–In3 2.948(1), In1–In4 3.173(2), In1–In5 2.814(2), In3–In5 3.191(1) (the equivalent distances In4–In6/In7–In10/In9–In12 are 3.192(2)/3.267(1)/3.307(2)), In3–In6 3.141(2), In3–In7 3.037(2), In5–In7 3.004(1), In5–In8 3.009(1), In5–In9 3.059(2), In1–Si3 2.668(3), In3–Si1 2.685(3), Si–C (mean) 1.94; In5–In1–In3 67.21(3), In5–In1–In2 94.41(3), In3–In1–In4 93.40(5), In3–In1–In2 64.06(3), In5–In1–In4 62.49(3), In2–In1–In4 55.81(3), In1–In3–In7 111.63(4), In1–In3–In6 90.82(4), In7–In3–In5 57.62(3), In7–In3–In2 109.25(4), In5–In3–In2 85.37(4), In7–In3–In6 58.32(3), In1–In3–In5 54.38(3), In6–In3–In5 68.73(4), In1–In3–In2 60.13(4), In1–In5–In7 116.62(4), In7–In5–In8 72.42(4), In7–In5–In9 61.59(3), In1–In5–In4 64.41(3), In1–In5–In8 121.77(4), In1–In5–In9 173.68(4), In8–In5–In9 64.07(3), In7–In5–In4 109.27(4), In8–In5–In4 59.06(4), In1–In5–In3 58.41(3), In8–In5–In3 107.68(4), In4–In5–In3 89.87(4), In9–In5–In4 121.84(4), In7–In5–In3 58.61(4), In9–In5–In3 118.73(4), Si3–In1–In2 135.62(7), Si1–In3–In1 136.22(8), C–Si–C (mean) 111.3.

closo compounds.^[10] Thus, the dodecaindane **2** can be described as a *conjuncto* indane (cf. the boron hydride $\text{B}_{20}\text{H}_{16}$ ^[8]). Therefore it seems possible that **2** is formed from the diindane R_4^*In_2 via hexaindane as an intermediate product R_6^*In_6 , which dimerizes to give the dodecaindane **2**. Indeed, in the synthesis described above insoluble **2** is formed from a soluble precursor, which is initially detectable in the reaction mixture, and which we are currently trying to isolate.^[11]

The In–In distances in **2** range from almost 2.80 to 3.30 Å. On average they are shorter than the In–In bond lengths in indium metal and longer than those in other low-valent uncharged indium compounds (Table 1). The In–In distances differ slightly within the two In_6 clusters; in contrast, the In–In distances between the two In_6 octahedra are both about

Table 1. Bond lengths $d(\text{In}–\text{In})$ in indium metal and in several uncharged indium compounds ($\text{Dsi} = \text{CH}(\text{SiMe}_3)_2$, $\text{Tsi} = \text{C}(\text{SiMe}_3)_3$, $\text{Tip} = 2,4,6\text{-}i\text{Pr}_3\text{C}_6\text{H}_2$, $\text{R}^* = \text{Si}t\text{Bu}_3$).

Compound	$d(\text{In}–\text{In})$ [Å]	Ref.
In metal	3.252/3.377	[9]
Dsi_4In_2	2.828(1)	[12]
Tip_4In_2	2.775(2)	[13]
R_4^*In_2	2.922(1)	[5]
$(\text{Tip}_2\text{In})_3\text{In}$	2.696(2)	[13]
Tsi_4In_4	3.002(1)	[6]
$\text{R}_8^*\text{In}_{12}$	2.80–3.30	this work

3.00 Å. The Si–In distances of about 2.68 Å are shorter than those in the diindane R_4^*In_2 (ca. 2.78 Å^[5]), which indicates that the supersilyl groups in the dodecaindane sterically inhibit one another less than in the diindane. The same tendency is indicated by the Si–C distances and the C–Si–C angles of the supersilyl groups, which are on average 1.94 Å and 111.3°, and lie in the normal range of 1.94–1.95 Å and 110–112°^[4] (cf. R_4^*In_2 : 1.95 Å and 109.7°^[5]).

To date there are only a few donor-free, uncharged molecular compounds with clusters from boron homologues (cf. ref. [5]). In the case of indium, these include diindanes R_4In_2 with In_2 frameworks (cf. among others ref. [5, 12, 13] and Table 1), the tetraindanes $(\text{Tip}_2\text{In})_3\text{In}$ with an acyclic In_3In framework (cf. ref. [13] and Table 1), and tetraindanes R_4In_4 with a tetrahedral In_4 framework (cf. ref. [6] and Table 1). The In–In interactions are only very weak (cf. ref. [5]; $\text{Cp}^* = \text{C}_5\text{Me}_5$; $\text{Cp}^{**} = \text{C}_5(\text{CH}_2\text{Ph})_5$) in the compounds $(\text{Cp}^*\text{In})_6$ and $(\text{Cp}^{**}\text{In})_2$. The *conjuncto*- $\text{R}_8^*\text{In}_{12}$ compound represents a unique E_{12} polyhedron structure for boron and its homologues and will naturally stimulate the search for compound homologues R_8E_{12} ($\text{E} = \text{B}, \text{Al}, \text{Ga}, \text{Tl}$) as well as for other donor-free and uncharged polyhedral compounds $\text{R}_{<n}\text{E}_n$ of the boron homologues.

According to the space-filling model the surface of the central In_{12} cluster of $\text{R}_8^*\text{In}_{12}$ is almost completely covered by eight supersilyl groups. This probably plays a major role in determining the outcome of the thermolysis of R_4^*In_2 . Owing to their smaller surface the corresponding Ga_{12} and Al_{12} polyhedra offer less space for eight supersilyl groups, thus compounds of the type $\text{R}_8^*\text{Ga}_{12}$ and $\text{R}_8^*\text{Al}_{12}$ should be more thermolabile than $\text{R}_8^*\text{In}_{12}$ or only accessible with sterically less demanding substituents such as $i\text{Pr}_3\text{Si}$.

Experimental Section

R_4^*In_2 : $t\text{Bu}_3\text{SiNa}(\text{THF})_2$ (10.5 mmol) in THF (10 mL) was added dropwise to a suspension of InCl_3 (0.778 g, 3.50 mmol) in THF (30 mL), which had been cooled to -78°C . The reaction mixture was stirred for 20 h at -78°C . On slowly allowing the mixture to warm to room temperature, the solution turned deep violet. The mixture was then stirred for 5 h at room temperature. According to the ^1H NMR spectrum (C_6D_6) the solution

contained exclusively $R_4^*In_2$ and R^*-R^* in the molar ratio 1:1 ($2InCl_3 + 6R^*Na \rightarrow R_4^*In_2 + R^*-R^* + 6NaCl$; identification of the products by comparison with authentic samples^[2,5]). After removal of all volatile components in an oil pump vacuum, the residue was taken up in toluene (80 mL), the insoluble components (NaCl) were filtered, and the filtrate was concentrated to 20 mL, deep violet $R_4^*In_2$ (1.12 g, 1.09 mmol; 63 %) crystallized from the solution at $-23^\circ C$.^[5]

$R_8^*In_{12}$: A solution of $R_4^*In_2$ (0.370 g, 0.360 mmol) in heptane (45 mL) was heated under reflux (ca. $100^\circ C$) for 22 h. According to the ^1H , $^{13}C\{^1H\}$, and ^{29}Si NMR spectra of the reaction solution, the diindane completely decomposed after replacement of C_6D_6 for heptane, leading to supersilane tBu_3SiH (identified by comparison with authentic sample^[2]) and an indium- and supersilyl-containing product (two different types of SiR_3 groups in the ratio 1:1); ^1H NMR: $\delta = 1.419$ and 1.271 (ratio of areas ca. 1:1); $^{13}C\{^1H\}$ NMR: $\delta = 33.72/25.47$ and $32.60/26.59$ (each CMe_3/CMe_3); ^{29}Si NMR: $\delta = 64.2$ and 50.9). After removal of all volatile components in an oil pump vacuum, the residue was taken up in benzene (40 mL), and the solution was concentrated to 10 mL, $R_8^*In_{12}$ (0.05 g, 0.02 mmol; ca. 30 %) crystallized from the solution in the course of a few days at room temperature. Crystals suitable for the X-ray structural analysis were grown from C_6D_6 . They do not dissolve in heptane, benzene, or toluene, either at room temperature or at $95^\circ C$ (no NMR signals). Thus the original thermolysis solution evidently did not contain dodecaindane but precursors of this cluster, from which $R_8^*In_{12}$ was formed.

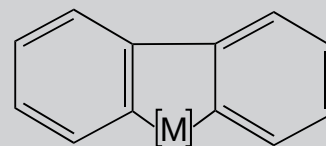
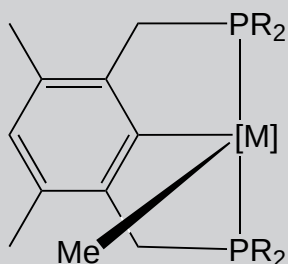
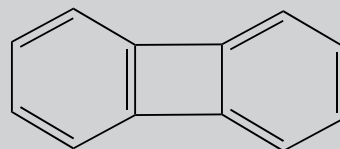
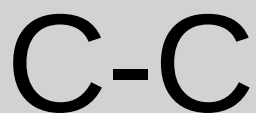
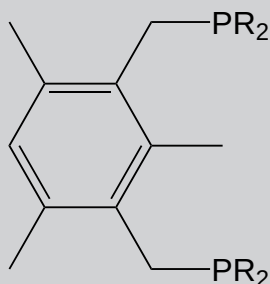
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Keywords: cluster compounds • indium • silicon

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dimensions $0.02 \times 0.3 \times 0.4$ mm³. The crystal was mounted in perfluoro ether oil, $T = 183(2)$ K, $C_{102}H_{216}D_6In_{12}Si_8$, $M_r = 3051.36$, black-violet platelets, monoclinic, $a = 18.329(7)$, $b = 32.02(2)$, $c = 22.454(8)$ Å, $\beta = 97.74(1)^\circ$, $V = 13059(10)$ Å³, $Z = 4$; space group $P2_1/n$, $\rho_{\text{calcd}} = 1.552$ Mg m⁻³, $\mu = 2.185$ mm⁻¹, $F(000) = 6136$. Data collection: 2θ from 2.98 to 49.42° , $-21 \leq h \leq 19$, $-37 \leq k \leq 37$, $-26 \leq l \leq 25$; of 64586 reflections, 18449 were independent and 11876 were observed ($F > 4\sigma(F)$), semiempirical absorption correction with SADABS (max./min. transmission 1.000/0.684), $R_1 = 0.0622$, $wR_2 = 0.1033$ ($F > 4\sigma(F)$), $GOF(F^2) = 1.076$; maximum residual electron density 1.098 e Å⁻³. The weighting factor was $w^{-1} = \sigma^2 F_o^2 + (0.0279 P)^2 + 53.5075 P$ with $P = (F_o^2 + 2F_c^2)/3$. The structure was solved by direct methods (SHELXTL, version 5). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in calculated positions and refined using fixed isotropic U_i values and a riding model. 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-112178. 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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- [10] If the four R^*In groups each donate two electrons, and the two In atoms each donate three electrons or—more probably—each only one electron (inert s electron pair!) to the In_6 framework of **2**, $14 = 2n + 2$ or $8 = 2n - 2$ framework electrons (n = number of framework atoms) can be calculated. According to the framework electron counting rules of Wade and Mingos^[8] in the first case $R_4^*In_6$ should be classified as a *closo* compound (In_6 octahedron), in the latter case as a double-capped *closo* compound (“*hypoprecloso*” compound; double-capped In_4 tetrahedron).^[11] $R_4^*In_6$ can be described as the supersilyl derivative of the *closo*-hexahydridoheptahexa- $In_6H_6^{2-}$, which has two hydride ions less and is a homologue of the known *closo*-hexahydridoheptaborate $B_6H_6^{2-}$.^[8]
- [11] By employing $R_8^*In_8$ we recently isolated an indane, which in terms of its framework electron number^[10] is related to $R_4^*In_6$. According to the X-ray structural analysis in the structure of the present In_8 cluster (double-capped In_6 octahedron) the octa-indane is rather classified as a *hypoprecloso* compound in accordance with the discussion in ref. [10]: N. Wiberg, T. Blank, H. Schnöckel, A. Purath, unpublished results.
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Transition metal insertion into C–C bonds in solution is a relatively unexplored reaction, particularly when strong C–C bonds are involved. The understanding of the reaction mechanisms, reactivity patterns, and factors controlling the competition between C–C and C–H activation may lead to the design of new selective processes for the utilization of hydrocarbons.

Metal Insertion into C–C Bonds in Solution

Boris Rybtchinski and David Milstein*

Dedicated to Professor Helmut Werner on the occasion of his 65th birthday

Metal-promoted activation of C–C bonds in homogeneous media under mild conditions may lead to the design of new selective processes for the utilization of hydrocarbons. However, examples of C–C bond activation in solution are much more scarce than those of C–H bond activation. We looked at the available data on metal insertion into C–C bonds in solution and tried to answer some important questions regarding the C–C bond activation. What kind of ligated metal

center is necessary in order to cleave the “hidden” C–C bond? What are the possible mechanisms of the C–C activation in various reaction systems, and how do different metal centers influence the reactivity? How can one tune the metal center for C–C bond cleavage by varying the steric and electronic properties of ligands? Thermodynamically, insertion into a C–C bond in solution is not a forbidden process, even if no strain relief or aromatization driving force is involved. Moreover,

with an appropriate system C–C activation may prevail thermodynamically and kinetically over the competing C–H activation. The relative thermodynamics of C–C versus C–H cleavage is influenced by the electron density on the metal center. When kinetics is concerned, coordinative unsaturation appears to be crucial for C–C oxidative addition.

Keywords: C–C activation • C–H activation • homogeneous catalysis

1. Introduction

Metal complex promoted activation of C–H and C–C bonds in homogeneous media is a field of much current interest since it can lead to the design of new selective and efficient processes for the utilization of hydrocarbons.^[1] Beginning in the early 1960s, much effort has been invested in the field of C–H bond activation in solution. Landmarks involving metal insertion into C–H bonds include various cyclometalation processes,^[2] intermolecular activation of aromatic C–H bonds,^[1, 3] and insertion of a metal into C–H bonds of saturated hydrocarbons, including methane.^[4] While C–H bond activation in solution is relatively well studied both synthetically and mechanistically, examples of C–C bond activation by soluble metal complexes are much less common, with the exception of strained C–C bonds (see Section 3).

There are several factors, mainly kinetic in nature, that favor C–H over C–C bond activation. These include 1) the

generally easier approach of the metal center to C–H bonds, 2) the statistical abundance of C–H bonds, and 3) a substantially higher activation barrier for C–C versus C–H oxidative addition due to the more directed nature of the C–C bond (see Section 2). Nevertheless, in some cases these factors can be overcome. “Naked” metal atoms both in the gas phase and in matrices are capable of activating C–C bonds in alkanes (see Section 6). C–C activation in saturated hydrocarbons induced by superacids, such as $\text{HSbF}_6 \cdot \text{SbF}_5$, in solution is also known.^[5] In this review we survey C–C bond activation processes in solution, confining ourselves to metal insertion into C–C single bonds. Alkene metathesis and β -alkyl elimination are beyond the scope of this review, as are C–C activations on surfaces and in the gas phase.

We believe that an understanding of the factors influencing metal insertion into C–C bonds and knowledge of the C–C bond activation mechanisms are key to the future development of this important field of organometallic chemistry. Therefore, in this review we pay special attention to studies which provide mechanistic insight regarding the C–C bond activation process.

2. Thermodynamic and Kinetic Considerations

C–H bond activation is in general thermodynamically more favorable than C–C bond activation due to the fact that in

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solution, unlike in the gas phase (see Section 6), M–H bonds are generally considerably stronger than M–C bonds in alkyl metal complexes.^[6] Nevertheless, C–C bond cleavage is not forbidden thermodynamically, as testified by several stable dialkyl metal complexes. Based on the known M–C bond strengths one can find systems in which metal insertion into a C–C bond will be not only thermodynamically feasible (i.e., $\text{BDE}(\text{M–C} + \text{M–C}) > \text{BDE}(\text{C–C})$; BDE = bond dissociation energy), but also more favorable than insertion into a C–H bond. For example, M–C_{aryl} bonds are quite strong in the cases of rhodium and iridium, sometimes stronger than M–H bonds.^[6, 7] Thus, substrates containing C_{aryl}–R and C_{aryl}–C_{aryl} bonds may be advantageous targets for iridium- or rhodium-mediated activation, although these bonds are stronger than C_{sp}³–C_{sp}³ bonds. However, the limited information regarding M–C bond strengths and the expected sensitivity of these bonds to steric effects interfere with the estimation of M–C bond energy in a specific metal complex, making the thermodynamic evaluations very rough. Energy gain from strain relief or aromatization appears to be a strong driving force for C–C cleavage (see Sections 3 and 4.2).

In cases where the C–C activation step is thermodynamically unfavorable, it may be accomplished by employing a subsequent “energy-releasing” step. For example, addition of H₂ may result in CH₄ elimination following the C–C activation step, making the overall process thermodynamically favorable even when very strong C–C bonds are involved.

The kinetic barrier is thought to be higher for C–C activation than for C–H activation. The common occurrence of stable polyalkyl metal complexes, in contrast to the rarity of alkyl metal hydrides, suggests that if thermodynamic factors favor reductive elimination, by microscopic reversibility arguments, there is a substantially higher kinetic barrier for C–C than for C–H activation. The “hidden” character of C–C bonds makes them kinetically much less attractive targets for activation by metals than the corresponding, more accessible, external C–H bonds. Still, although C–H activation is reversible in many systems, C–C oxidative addition is not generally observed.

The ease of approach of the active metal center to the bond to be cleaved is strongly influenced by the repulsive steric interaction of the ligands with the substrate. Thus, in the case of ligated metal complexes, C–C bonds are not only “chemically hidden” by C–H bonds, they are also sterically less accessible, unlike in the case of naked metals in a gas phase or matrices, or in the case of the naked proton of superacids.

Theoretical calculations point at another intrinsic problem in C–C activation.^[8] With an implied three-centered nonpolar transition state, it was calculated that for first-row (3d) late transition metal atoms, the activation energies for insertion into the C–C bond of ethane and the C–H bond of methane are 40–45 and 20–25 kcal mol^{–1}, respectively. In the case of second-row (4d) late transition metals the barriers are substantially lower: 0–9 kcal mol^{–1} for C–H insertion and 13–27 kcal mol^{–1} for C–C insertion. The difference between the kinetic barriers for the C–C and C–H insertions is independent of the metal. This suggests that the insertion is largely influenced by the different nature of the C–C and C–H bonds and not by the nature of the metals. The key is the difference in orbital directionality between the C–H and C–C bonds. In the transition state leading to C–H oxidative addition the spherically symmetrical 1s orbital of the hydrogen atom can bind to the metal and carbon atom at the same time. The sp³-hybridized carbon atom of the alkyl group has only one optimal binding direction, and in the course of C–H or C–C activation the alkyl groups have to rotate into a position that is no longer optimal for the R–H or R–R bond (Figure 1). Since in the case of C–C activation two alkyl groups are involved, the activation barrier for this process should be higher than that for C–H bonds, in which only one alkyl group participates.

3. Activation in Strained Systems

Metal-promoted C–C activation of strained alkanes has been known since the report by Chatt et al. in 1960,^[9] in which the structure of a platinacyclobutane complex obtained by

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D. Milstein



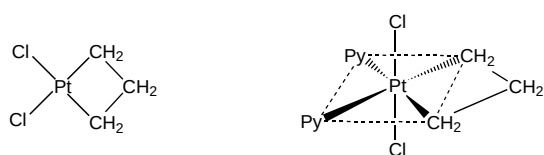
B. Rybtchinski

Boris Rybtchinski, born in Kiev in 1971, studied chemistry at Kiev State University between 1988 and 1992. In 1992 he moved to Israel, and since 1993 he has been a graduate student at The Weizmann Institute of Science. He received his M.Sc. degree in chemistry for research in the group headed by Professor D. Milstein and is currently a Ph.D. student in the same group.



Figure 1. Schematic representation of R–H (left) and R–R bond cleavage (right) showing the influence of C–C and C–H orbital directionality on the oxidative addition process.

Tipper in 1955^[10] as a product of cyclopropane oxidative addition was elucidated by NMR and IR studies of its bis(pyridine) derivative (Scheme 1). Since then a great deal of



Scheme 1. A platinacyclobutane complex (left) obtained by oxidative addition of cyclopropane and its bis(pyridine) derivative (right).

work on this topic has been published. The field has been reviewed by Bishop (on small ring rearrangements catalyzed by transition metals)^[11] and by Jennings and Johnson (on the synthesis and properties of metalacyclobutanes).^[12] Here we review only cases in which insertion of a transition metal into strained C–C bonds was clearly demonstrated and/or those that provide mechanistic insight regarding the C–C activation step.

Strain relief in the product and in the transition state provides an important thermodynamic and kinetic driving force for C–C activation in strained systems. The HOMO of cyclopropane and cyclobutane are largely p orbitals in character, forming “banana bonds” somewhat similar to the bonds in olefins (the same is true for the LUMO; it is similar to the π^* orbital of olefins). Thus, the symmetry and availability of the cyclopropane and cyclobutane HOMO and LUMO allow interaction with a metal HOMO (normally p_y) and LUMO (normally d_{xy}) in a way similar to a metal–olefin interaction. This lowers the kinetic barrier of the C–C cleavage reaction (Figure 2).

In an important early example, Cassar and Halpern demonstrated that $[\{\text{Rh}(\text{CO})_2\text{Cl}\}_2]$ undergoes insertion into a strained C–C bond of quadricyclene by oxidative addition (Scheme 2).^[13] In a similar way $[\{\text{Rh}(\text{CO})_2\text{Cl}\}_2]$ oxidatively adds to the C–C bond of cubane.^[14] In two other early reports, Ir^[15] and Fe complexes^[16] were demonstrated to insert into a C–C bond of cyclopropane to form structurally characterized oxidative addition metallacyclobutane products.

Various cyclopropanes react with Zeise’s Pt^{II} dimer $[\{\text{Pt}(\text{C}_2\text{H}_4)\text{Cl}_2\}_2]$ to form tetrameric platina(IV)cyclobutanes, which upon addition of nitrogen donors provide the monomers in high yields, as was shown by McQuillin et al. (Scheme 3).^[17]

Several reports refer to intermediacy of an “edge” platinum–cyclopropane complex, although such an intermediate has not been observed.^[12] The existence of such a species en

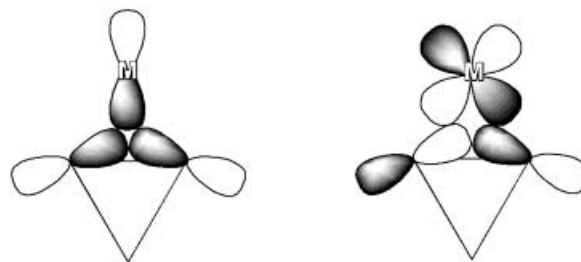
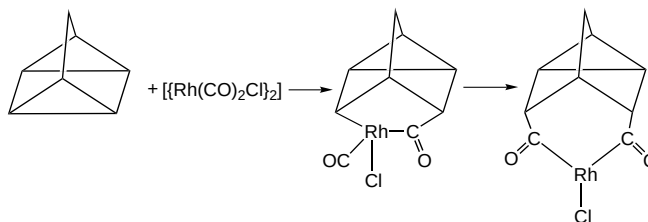
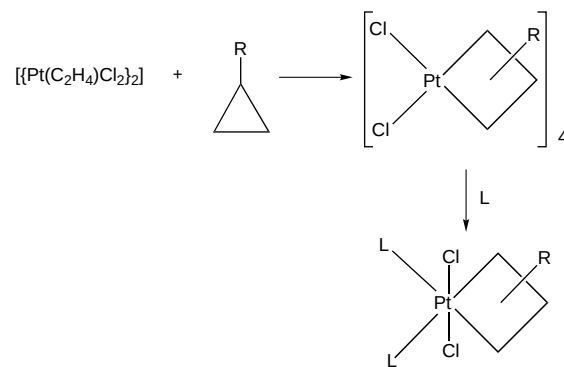


Figure 2. Cyclopropane–metal orbital interactions. Left: σ bond formed by the interaction of metal p_y and cyclopropane σ orbitals. Right: π bond formed by the interaction of metal d_{xy} and cyclopropane σ^* orbitals.



Scheme 2. Reaction of quadricyclene with $[\{\text{Rh}(\text{CO})_2\text{Cl}\}_2]$.



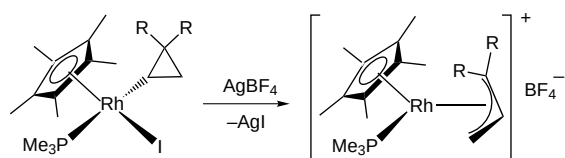
Scheme 3. Insertion of Pt^{II} into C–C bonds of cyclopropanes.

route to C–C activation seems reasonable for orbital symmetry reasons, since the banana bond is available for σ and π interaction with the HOMO and LUMO of the reactive metal center (Figure 2). Importantly, there is evidence that oxidative addition of cyclopropane to platinum occurs at an edge rather than at a corner of the ring.^[12]

The oxidative addition of cyclopropanes to Zeise’s dimer is limited in scope; with cyclopropanes bearing electron-withdrawing groups no direct platinacyclobutane formation is observed. This suggests that electrophilic attack of the Pt^{II} center on the cyclopropyl moiety is involved. On the other hand, Graziani et al.^[18] and Ibers et al.^[19] showed that Pt^0 and Pd^0 centers insert effectively into cyclopropanes substituted with electron-withdrawing groups, the low-valent metal center reacting as a nucleophile. Thus, as expected, the metal oxidation state strongly influences the affinity of a metal complex for various substrates, changing the mechanism of the reaction.

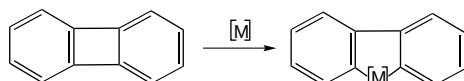
The mechanism of cyclopropane activation by the in situ generated $[\text{Cp}^*\text{RhL}]$ ($\text{Cp}^* = \text{C}_5\text{Me}_5$) fragment was studied in detail by Periana and Bergman.^[20] Initially, C–H oxidative

addition takes place to yield the kinetic product $[\text{Cp}^*\text{Rh}(\text{L})(\text{cyclopropyl})(\text{H})]$, which rearranges to rhodacyclobutane. The rearrangement was shown to be intramolecular (without alkane dissociation from the metal center) and regioselective—the $[\text{Cp}^*\text{RhL}]$ moiety inserts only into the α C–C bond of the cyclopropyl ring. Steric constraints are important: In the case of substituted cyclopropanes insertion into the less substituted α C–C bond takes place. Importantly, when coordinative unsaturation was created by iodide abstraction with AgBF_4 , a facile C–C cleavage reaction took place, resulting in formation of a π -allyl complex (Scheme 4).



Scheme 4. C–C bond activation in cyclopropane induced by iodide abstraction.

Metal insertion into the strained C–C bond of biphenylenes is another class of reactions which can be clearly identified as oxidative addition. This reaction is driven not only by strain relief but also by formation of two strong $\text{M}-\text{C}_{\text{aryl}}$ bonds (Scheme 5).



Scheme 5. Metal insertion into the strained C–C bond of biphenylene.

Eisch et al. showed that the reactivity of nickel complexes toward biphenylene increased in the sequence $[\text{Ni}(\text{cod})(\text{bpy})] < [\text{Ni}(\text{Ph}_3\text{P})_4] < [\text{Ni}(\text{C}_2\text{H}_4)(\text{Ph}_3\text{P})_2] < [\text{Ni}(\text{Et}_3\text{P})_4]$ (cod = 1,5-cyclooctadiene, bpy = 2,2'-bipyridyl), demonstrating that higher electron density on the metal center promotes the C–C cleavage reaction, as expected for an oxidative addition process. Tetraphenylene derivatives were catalytically formed from biphenylene derivatives.^[21]

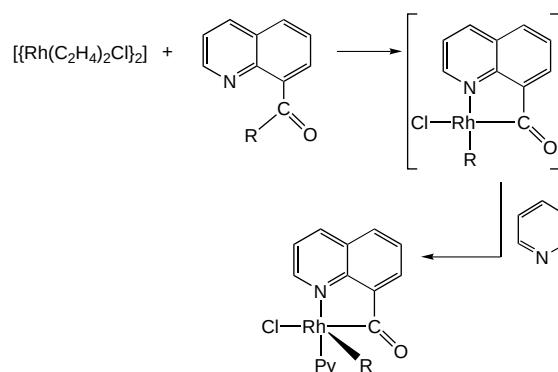
Jones et al. suggested that insertion of the $[\text{Cp}^*\text{Rh}(\text{PMe}_3)]$ fragment into the central ring of biphenylene proceeds intramolecularly via an η^2 coordinated species,^[22] similarly to cyclopropane activation by this reactive metal fragment, as demonstrated by Periana and Bergman (see above).^[20] $[\text{Cp}^*\text{Rh}(\text{C}_2\text{H}_4)_2]$ and $[\text{Cp}^*\text{Co}(\text{C}_2\text{H}_4)_2]$ were also shown to give C–C insertion products in the reaction with biphenylene.^[23a] As reported by Jones et al., $[\text{Pt}(\text{PET}_3)_2]$ and $[\text{Pd}(\text{PET}_3)_2]$ insert into the biphenylene C–C bond to provide Pd^{II} and Pt^{II} complexes $[\text{M}(\text{PET}_3)_2(2,2'\text{-biphenyl})]$, which are also capable of biphenylene C–C cleavage with prior phosphane dissociation. In this work C–C bond activation and formation result in a catalytic reaction to generate tetraphenylene.^[23b] Insertion of Ir^{I} into the strained C–C bond of biphenylene was demonstrated by Crabtree et al.^[24] Recently, Ito, Murakami et al. have demonstrated catalytic C–C cleavage of strained spirocyclobutanones with a Rh^{I} catalyst, $[\text{Rh}(\text{cod})(\text{dppe})]\text{BF}_4$ (dppe = 1,2-bis(diphenylphosphanyl)ethane).^[25]

Even though a strong driving force for C–C activation is present in strained hydrocarbons, precoordination of the substrate is a necessity in most of the reported reaction systems. Recently it has been established that in many cases oxidative addition of an alkane C–H bond proceeds through precoordination of the alkane C–H bond to the metal.^[1c] Taking into account the hidden and directed nature of the C–C bond, metal insertion into C–C bonds seems to be a kinetically (sterics and unfavorable orientation of the reactive orbitals) very demanding process, more so than that of the C–H bond.

4. Activation in Unstrained Systems

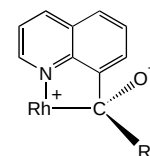
4.1. Activation of C–C Bonds Adjacent to a Carbonyl Group

Insertion of Rh^{I} into the α -keto C–C bond of 8-quinolynyl alkyl ketones takes place readily with $[\{\text{Rh}(\text{C}_2\text{H}_4)_2\text{Cl}\}_2]$, as demonstrated by Suggs et al. (Scheme 6).^[26] The α -keto C–C bond is slightly weaker than other C–C single bonds, and, as



Scheme 6. Reaction of 8-quinolynyl alkyl ketones with $[\{\text{Rh}(\text{C}_2\text{H}_4)_2\text{Cl}\}_2]$.

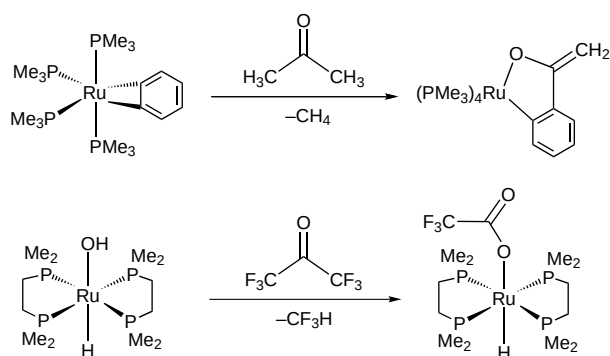
was pointed out by the authors, upon coordination to the nitrogen atom in the substrate the metal is somewhat directed at this bond, making the C–C activation both thermodynamically and kinetically feasible. Indeed, it was shown by labeling studies that direct C–C cleavage without prior C–H activation takes place.^[26a] The carbonyl functionality not only weakens the C–C bond, but also plays an important role in the C–C cleavage process: Chiral induction at the Rh center suggests that a tetrahedral intermediate (Scheme 7) is on the path to



Scheme 7. Tetrahedral intermediate proposed for cleavage of the C–C bond in 8-quinolynyl alkyl ketones.

C–C cleavage in 8-quinolynyl alkyl ketones. Thus, the reaction mechanism is similar to that of the Baeyer–Villiger reduction of ketones.^[26c]

Bergman et al. demonstrated that ruthenium complexes capable of producing unsaturated species cleave the C–C bond in acetone^[27] and hexafluoroacetone^[28] (Scheme 8).

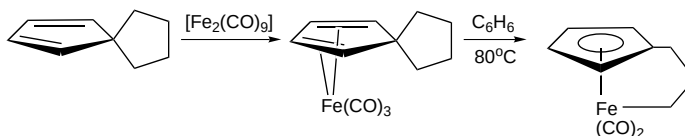


Scheme 8. Ruthenium-promoted cleavage of the C–C bond in acetone and hexafluoroacetone.

Cleavage of the C–C bond in cyclic aliphatic ketones with $[\text{Rh}(\text{PPh}_3)_3\text{Cl}]$ has been reported by Murakami, Ito et al. Exclusive activation of the less substituted C–C bond α to a carbonyl group has been observed. Catalytic C–C bond activation in cyclic ketones using hydrogen has been also demonstrated.^[29]

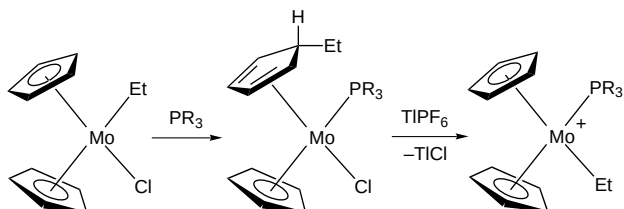
4.2. Activation of C–C Bonds Driven by Aromatization

Eilbracht et al. showed that C–C single bonds within tricarbonyliron complexes of 1,1-dialkyl-substituted spiro cyclopentadienes were cleaved in boiling benzene, leading to π -cyclopentadienyl- σ -alkylcarbonyliron complexes.^[30] Cross-over experiments demonstrated that the *endo*-alkyl group is intramolecularly transferred from the diene ligand to the metal (Scheme 9).



Scheme 9. Cleavage of C–C bonds in tricarbonyliron complexes of 1,1-dialkyl-substituted spiro cyclopentadienes.

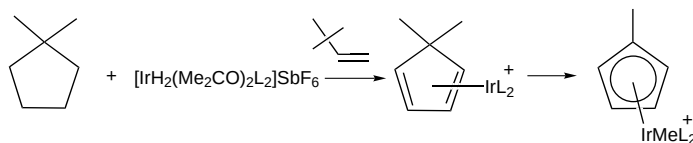
Green et al. reported a similar molybdenum system in which a reversible metal-to-ring transfer of an ethyl group occurs when a free coordination site on the metal center is generated by chloride abstraction (Scheme 10).^[31]



Scheme 10. C–C bond activation by a reversible metal-to-ring transfer of an ethyl group. $\text{PR}_3 = \text{PEt}_3, \text{PMe}_2\text{Ph}, \text{PMePh}_2$.

Crabtree et al. reported C–C bond cleavage in cationic 1,1-dialkyl-substituted cyclopentadienyliridium complexes. An elegant two-step route with prior dehydrogenation of the alkane was developed, leading to an overall selective C–C

bond breaking in disubstituted cyclopentanes (Scheme 11).^[32] Interestingly, no C–C cleavage was observed with the analogous saturated 18e complex, containing a dialkylcyclohexadienyl ligand, under various reaction conditions. Thus, coordinative unsaturation was suggested to play a key role in the process of C–C activation.^[32b]



Scheme 11. C–C bond cleavage in cationic 1,1-dialkyl-substituted cyclopentadienyliridium complexes. $\text{L} = \text{P}(p\text{-FC}_6\text{H}_4)_3$.

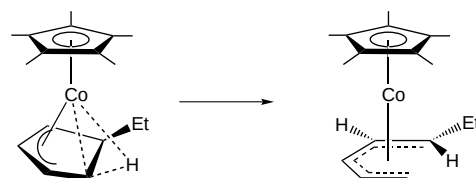
The energy gain from aromatization in the systems described above renders the processes thermodynamically feasible. The orientation of the alkyl group towards the metal center in the rigidly coordinated cyclopentadiene is favorable for C–C cleavage, making the process also kinetically feasible. The *endo*-alkyl group which is closer to the metal center is always the one transferred to the metal.

Maitlis et al. described an unusual rhodium-assisted C–C cleavage in Dewar benzene to produce a cyclopentadienyl-rhodium dimer.^[33] The driving force for the overall process is clearly aromatization to form two Cp rings. Palladium-promoted isomerization of Dewar benzene into hexamethylbenzene was also observed, aromatization being a strong driving force for this reaction.^[34]

Chaudret et al. demonstrated that reaction of the electrophilic fragment $[\text{Cp}^*\text{Ru}]^+$ with the A rings of steroids resulted in aromatization through cleavage of various bonds, including C–C bonds.^[35] A driving force for these reactions is the remarkably high affinity of the cationic ruthenium fragment for aromatic hydrocarbons. Significantly, the aromatization occurs when the ruthenium moiety and the methyl group being activated are located on opposite faces of the steroid substrate. Based on this, and on the observation of ethane in the gas phase of the reaction mixture, presumably formed by coupling of two methyl radicals, it was suggested by the authors that the C–C cleavage process proceeds by a radical mechanism.

4.3. C–C Bond Cleavage in Unsaturated Cobalt Complexes Stabilized by an Agostic Interaction

Bennett and Spencer et al. have demonstrated an interesting case of C–C cleavage in unsaturated cationic cobalt complexes stabilized by an agostic interaction^[36] (Scheme 12).



Scheme 12. C–C cleavage in unsaturated cationic cobalt complexes stabilized by an agostic interaction.

In these complexes the diene-hydride form is of higher energy than the species stabilized by the agostic interaction with the C–H bond. Thus, the product of C–H activation is not stable, making C–C activation thermodynamically more favorable. A thermodynamic driving force such as relief of strain by opening of a cyclic five-membered allyl is present. No C–C cleavage was observed in six-membered rings either due to thermodynamic factors (stability of six-membered rings) or kinetic factors (poor accessibility of C–C bonds to the metal), as suggested by the authors.

4.4. C–C Activation Involving More than One Metal Center

Cluster-promoted scission of a coordinated alkyne in solution providing alkylidene fragments was demonstrated with a variety of metal carbonyl clusters. Alkyne migration to the edge of the cluster is required, and subsequent CO loss triggers the C–C bond cleavage. Coordinative unsaturation appears to be a prerequisite for the reaction.^[37]

C–C bonds of cumulenes and diynes were also successfully cleaved in carbonyl clusters or in bimetallic complexes. The C–C bonds in these compounds are more accessible due to the absence of C–H bonds.^[38]

An interesting example of selective C–C bond cleavage in cyclopentadiene by an unsaturated trinuclear ruthenium cluster has been reported by Suzuki et al. It was suggested that the three metal centers cooperate in the activation.^[39]

Recently Johnson et al. have shown that the trinuclear cluster $[\text{Ru}_3(\text{CO})_{12}]$ is capable of activating C–C bonds in cyclohexene and cycloocta-1,3-diene upon heating under reflux in octane.^[40] However, the product of C–C activation was obtained in low yield.

4.5. Electrochemically Induced Oxidative Addition of C–C Bonds

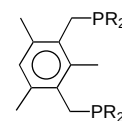
As shown by Geiger et al., electrochemical oxidation of the pseudo-triple-decker complex $[\text{Cp}_2\text{Ru}_2(\mu\text{-cyclo-C}_8\text{H}_8)]$ results in the flyover dication $[\text{Cp}_2\text{Ru}_2(\mu\text{-cat-C}_8\text{H}_8)]^{2+}$, in which the C_8H_8 group is an open chain. Thus, the redox process induces insertion of both metal atoms into a C–C bond of cyclooctatetraene.^[41] The C–C bond being cleaved bridges the two Ru centers in $[\text{Cp}_2\text{Ru}_2(\mu\text{-cyclo-C}_8\text{H}_8)]$. As a consequence it is fairly long (1.570(6) Å) and can be defined as “preactivated”. The orientation of the bond is ideal for C–C cleavage, since only electron redistribution is needed for the C–C rupture, which is induced by an electrochemical two-electron oxidation.

4.6. Metal Insertion into an Unstrained C–C Bond in PCP and PCN Systems

As discussed above, most of the examples of C–C bond activation by insertion of soluble metal complexes include a drive to aromatic character in prearomatic systems, the presence of a carbonyl group, or strain. When we set the goal of studying the activation of strong, unstrained C–C bonds, we

chose an intramolecular process in order to bring a metal center close to the “hidden” C–C bonds. For an unequivocal demonstration and mechanistic evaluation of metal insertion into a C–C bond, it is desirable that this process would be irreversible, and that the C–C activation product would be stable and readily characterized. The substrates of our choice, PCP ligands (Scheme 13), possess the required properties. They can be viewed as a mesitylene molecule with two attached phosphane “arms”. The $\text{C}_{\text{Me}}\text{--C}_{\text{aryl}}$ bonds of these ligands, targeted for metal insertion, are very strong (e.g. $\text{BDE}(\text{C}_6\text{H}_5\text{--CH}_3) = 101.8 \pm 2 \text{ kcal mol}^{-1}$), stronger than the competing benzylic C–H bonds (e.g. $\text{BDE}(\text{C}_6\text{H}_5\text{CH}_2\text{--H}) = 88 \pm 1 \text{ kcal mol}^{-1}$).^[42] The presence of two types of $\text{C}_{\text{Me}}\text{--C}_{\text{aryl}}$ bonds which are potentially available for C–C activation in these systems can provide additional information regarding this process.

The process of C–C activation in a PCP ligand can be viewed as being similar to the well-known cyclometalation of the C–H bond situated between the phosphane arms. The expected competing reaction, metal insertion into the benzylic C–H bond of a PCP ligand, may be reversible and would allow a comparison between the two processes.

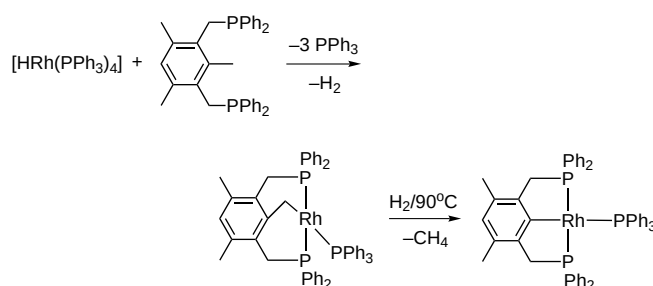


Scheme 13. Schematic representation of the PCP ligands.

4.6.1. Insertion into a Strong C–C Bond in Solution: C–C versus C–H Activation

4.6.1.1. C–C Activation—Demonstration of the Process

The first example of metal insertion into an unstrained, unactivated C–C bond in solution was demonstrated with $[\text{HRh}(\text{PPh}_3)_4]$.^[43] The use of a metal hydride complex was intended to provide a substantial driving force to the overall C–C activation process by the expected methane elimination following insertion into the C–C bond. Reaction of $[\text{HRh}(\text{PPh}_3)_4]$ with the Ph-PCP ligand at room temperature resulted in elimination of H_2 and formation of the kinetic C–H activation product (Scheme 14). Significantly, the C–H



Scheme 14. Reaction of $[\text{HRh}(\text{PPh}_3)_4]$ with Ph-PCP.

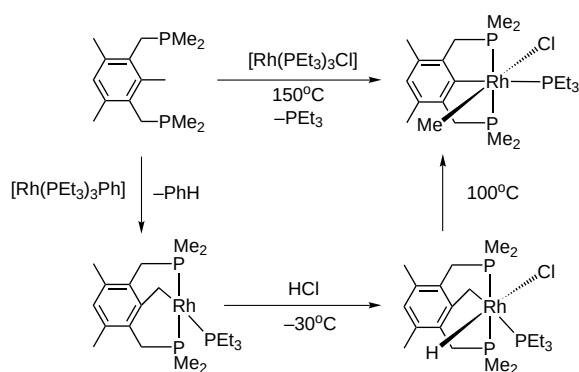
activation process can be reversed by heating of this product under a mild hydrogen pressure, resulting in quantitative C–C cleavage and methane elimination.

As the complex with the activated C–C bond does not react with methane, the overall C–C activation process is irreversible and thermodynamically more favorable than the overall C–H activation sequence. The use of hydrogen to drive the reaction towards C–C activation could potentially

be utilized as a general strategy to overcome the problem of C–H activation which is expected to accompany C–C activation in hydrocarbons. While the intimate mechanism of C–C activation was unclear at that stage, a three-centered transition state and/or an η^2 -arene intermediate were suggested to be on the reaction pathway.

4.6.1.2. C–C versus C–H Activation—Thermodynamics

Employment of hydrogen in the above-mentioned process masks the relative thermodynamic stability of the C–H and C–C activation products, leaving open the questions of the thermodynamic feasibility of the oxidative addition of a strong C–C bond in the absence of added reagents and the thermodynamic interplay between C–C and C–H oxidative addition. Direct C–C insertion in the absence of hydrogen proved to be thermodynamically feasible with Me-PCP. Heating this ligand with $[\text{Rh}(\text{PET}_3)_3\text{Cl}]$ led to the product of direct C–C activation quantitatively (Scheme 15, Figure 3).^[44]



Scheme 15. Insertion of Rh into C–C and C–H bonds of the Me-PCP ligand.

The C–H activation product, which was prepared by an independent route, is quantitatively converted into the C–C activation product upon heating (Scheme 15), unambiguously proving that Rh^{I} insertion into the C–C bond in this system is thermodynamically more favorable than insertion into the C–H bond.

The $\text{C}_{\text{Me}}\text{--C}_{\text{aryl}}$ bond in PCP ligands is much stronger than the $(\text{aryl})\text{CH}_2\text{--H}$ ($\text{C}_{\text{benzyl}}\text{--H}$) bond ($\text{BDE}(\text{C}_6\text{H}_5\text{--CH}_3) =$

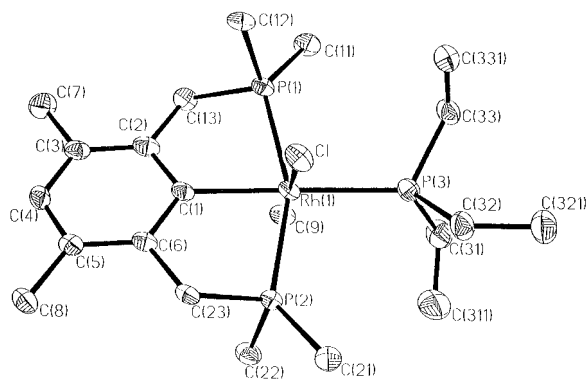


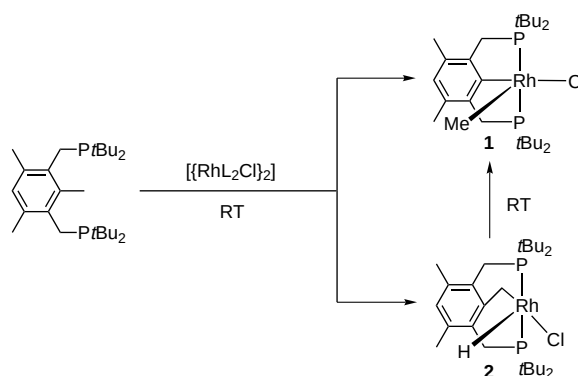
Figure 3. ORTEP diagram of the C–C activation complex $[\text{Cl}(\text{Me-PCP})\text{Rh}(\text{CH}_3)(\text{PET}_3)]$ (see Scheme 15). Hydrogen atoms are omitted for clarity.

101.8 ± 2 versus $\text{BDE}(\text{C}_6\text{H}_5\text{CH}_2\text{--H}) = 88 \pm 1 \text{ kcal mol}^{-1}$ ^[42]), indicating that conversion of the C–H activation product into the C–C activation product is product-controlled and that $\text{BDE}(\text{Rh--C}_{\text{aryl}} + \text{Rh--CH}_3) > \text{BDE}(\text{Rh--CH}_2(\text{aryl}) + \text{Rh--H})$. This is in accord with the observation that the strongest bonds to metals are usually formed by breaking of the strongest bonds in the substrates.^[7, 45] Formation of the strong $\text{Rh--C}_{\text{aryl}}$ bond most probably is a driving force for the reaction (see above). The expected slightly lower stability of the six-membered chelate ring in the C–H activation complexes, as compared to the five-membered ring in the C–C activation complexes, may also influence their relative stability. Importantly, decreasing the electron density on the metal, when Ph instead of Me substituents are attached to a phosphane, results in stabilizing the product of C–H activation, which does not undergo C–C activation even upon heating to 150° .^[44]

4.6.1.3. C–C versus C–H Activation—Mechanism

After it was demonstrated that Rh^{I} insertion into the C–C bond in the PCP systems is thermodynamically feasible and can be preferable to insertion into the C–H bond, the question of the reaction mechanism was addressed. Is the mechanism polar or nonpolar? What is the role of the aromatic ring in the process? Is it a direct process or does it require prior C–H activation followed by some rearrangement to the C–C activation product? Mechanistic studies were performed on the reaction of the bulky *t*Bu-PCP ligands with Rh^{I} and Ir^{I} .

Bulky phosphanes are advantageous ligands for the study of oxidative addition processes, especially cyclometalations, since upon coordination to a metal center they generate a species having a shielded vacant coordination site and a congested conformation. The latter assists the cyclometalation process due to favorable entropy.^[2a] Remarkably, when the *t*Bu-PCP ligand was allowed to react with rhodium olefin dimers at *room temperature*, direct rhodium insertion into one of the strong aryl–carbon bonds took place, yielding the product of C–C oxidative addition **1**, the structure of which was elucidated by X-ray analysis (Scheme 16).^[46a] At the beginning of the reaction the product of C–C oxidative addition was formed concurrently with the product of C–H oxidative addition, the latter being converted into the former

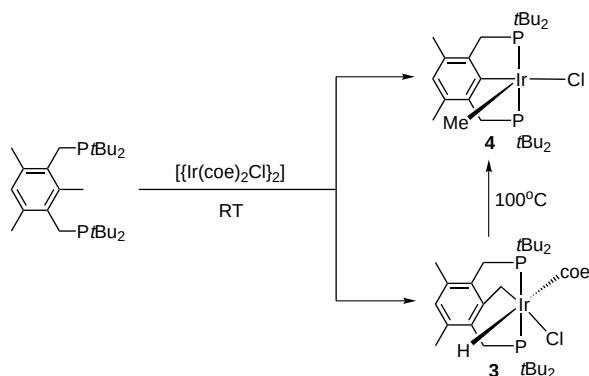


Scheme 16. Reaction of *t*Bu-PCP with $[\text{RhL}_2\text{Cl}]_2$. L = cyclooctene, ethylene, *tert*-butylethylene.

within several hours. These observations indicate that the “methylene bridged” compound **2** undergoes slow C–H reductive elimination, followed by rapid metal insertion into the C–C bond.

Increasing the steric bulk of the alkene ligand in the $[\{\text{RhL}_2\text{Cl}\}_2]$ complex resulted in a large decrease in the overall reaction rate. The reaction is very fast with the ethylene complex and slow with the *tert*-butylethylene one. This reactivity order indicates that associative displacement of the alkene by the phosphane takes place and that the initial coordination of the diphosphane ligand to the rhodium olefin complex is the rate-determining step for the entire process rather than the C–C or C–H activation steps. Nevertheless, important mechanistic conclusions could be obtained from a comparison of these steps (see below).

Concurrent formation of the C–H and C–C activation products took place in the reaction of $[\{\text{Ir}(\text{coe})_2\text{Cl}\}_2]$ (coe = cyclooctene) with the *t*Bu-PCP ligand at room temperature. The product of C–H activation, complex **3**, is quantitatively converted into the C–C activation product, complex **4**, upon moderate heating (Scheme 17).^[46a] However, in contrast with the rhodium complex **2**, **3** is stable under the reaction conditions, making the study of the process easier.



Scheme 17. Reaction of *t*Bu-PCP with $[\{\text{Ir}(\text{coe})_2\text{Cl}\}_2]$.

The above-mentioned reactivity unequivocally proves that the insertion of iridium and rhodium into the C–C bond is thermodynamically more favorable than insertion into the C–H bond, as observed in the Me-PCP system (see above). In the case of iridium the ratio between the products of C–C and C–H activation was constant at different temperatures during the course of the reaction and remained the same after the reaction was complete (C–H:C–C is 1.75 ± 0.07 in benzene and 2.29 ± 0.08 in THF, Scheme 17). The C–H and C–C insertion products were formed irreversibly within the temperature range of 20–60 °C, indicating that the C–C and C–H activation processes are kinetically controlled; the constant ratio demonstrates that the complexes are formed in two independent, concurrent processes. Thus, the C–H activation product **3** is not an intermediate in the C–C activation process, as verified by the observation that it does not convert into the C–C activation product under the reaction conditions. Both the C–C and C–H activation processes were shown to proceed through a common intermediate with the two phosphane arms coordinated to the

metal center. Thus, taking into account that the product ratio is temperature-independent and that three C–H bonds per one C–C bond are accessible for activation, $\Delta\Delta H^\ddagger_{\text{CH-CC}} \approx 0 \text{ kcal mol}^{-1}$ and $\Delta\Delta S^\ddagger_{\text{CH-CC}} = -1.07 \pm 0.05 \text{ eu}$. Surprisingly, the kinetic barrier for C–C oxidative addition is slightly lower than that for C–H ($\Delta\Delta G^\ddagger_{\text{CH-CC}}(293) = 0.342 \text{ kcal mol}^{-1}$, Figure 4).

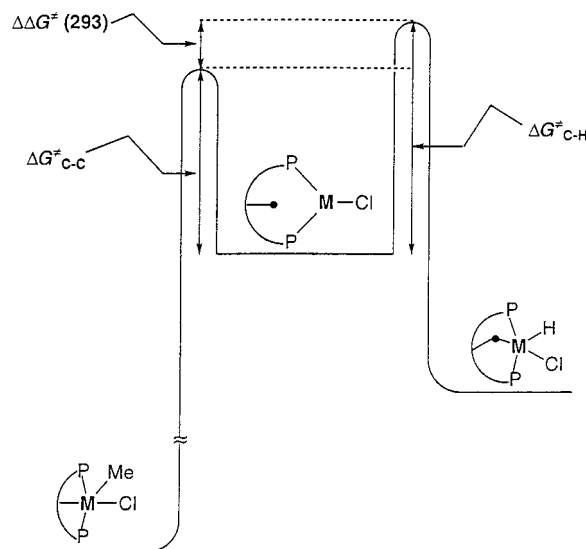


Figure 4. Reaction profile for C–C versus C–H oxidative addition. $\Delta\Delta G^\ddagger(293) = 0.3$ (M = Ir), $0.5 \text{ kcal mol}^{-1}$ (M = Rh).

The mechanistic aspects of C–H bond activation by low-valent late transition metals were extensively studied. It was shown that C–H bond breaking involves an early three-centered, nonpolar transition state.^[1] The striking similarity of the activation parameters for C–C and C–H activation processes, and the fact that the activation parameters are not much affected by variation in solvent polarity (benzene and THF) or by the use of a *para*-methoxy-^[46a] and *para*-carboxy-substituted derivatives^[46b] of the *t*Bu-PCP ligand, indicates that similar nonpolar transition states are involved in both processes. The lack of substituent effect also suggests that a η^2 -arene complex is not involved in the C–C activation process. The η^2 -arene complex is generally thought to be an intermediate which brings the metal into the proximity of the C–H^[3a] or C–C bond^[22] to be cleaved. Taking into account that in the metal–PCP system the metal center is already held in the vicinity of the C–C bond by coordination to the two phosphane moieties, precoordination of the aromatic ring does not seem to be required in the reaction course. Thus, oxidative addition of the C–C bond in our system most probably proceeds through a three-center, nonpolar transition state similar to that postulated for aliphatic C–H bond activation.

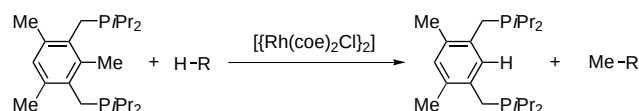
The observed similarity in the kinetic barriers for the C–H and C–C activation processes is in striking contradiction with the common belief that the barrier for C–C activation is much higher than that of C–H activation. Theoretical calculations predict that due to the difference in directionality between bonds to methyl groups and to the hydrogen atom, the activation energy of oxidative addition of the C–C bond

should be substantially higher (by about 10–20 kcal mol^{−1}) than that of the C–H bond (see Section 2). The surprising kinetic preference for the C–C bond activation in our system can be accounted for in terms of the specific directionality of the reactive metal orbitals towards the C–C and C–H bonds, favoring the oxidative addition of the former.

It is remarkable that direct insertion into a very strong C–C bond in solution can take place even at room temperature. Moreover, the insertion is not the rate-determining step. This can be attributed to factors favoring oxidative addition in the *t*Bu-PCP system. The two *tert*-butylphosphane groups bring the metal into the vicinity of the C–C bond to be activated and enhance the electron density on the metal center, while the bulky *tert*-butyl substituents are capable of shielding a vacant coordination site and imposing a conformation that favors cyclometalation.^[2a] Unsaturation in the reactive intermediate seems to be very important, since heating to 150 °C was required in order to achieve C–C oxidative addition with Me-PCP (see above). This may be a result of the much lower steric bulk of the methylphosphane ligands, which permits the formation of a four-coordinate Rh^I complex prior to the C–C activation process.

4.6.1.4. Catalytic C–C Activation

An unstrained, strong C_{aryl}–C bond can be catalytically cleaved by a metal complex in solution. In this unprecedented transformation, reaction of [[Rh(cod)₂Cl]₂] with an excess of 1,3-bis(diisopropylphosphanylmethyl)mesitylene in dioxane under a mild H₂ pressure or with an excess of HSi(OEt)₃ results in catalytic, selective cleavage of one of the C–C bonds in the diphosphane (Scheme 18). Although the catalytic



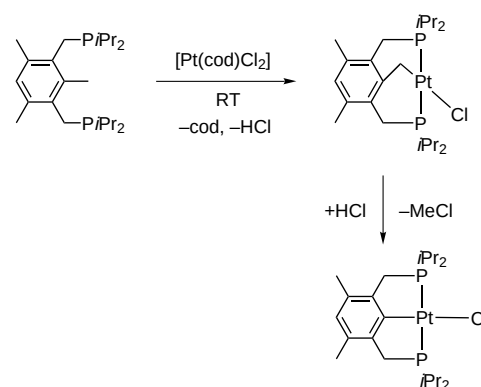
Scheme 18. Reaction of *i*Pr-PCP with H–R (R = H, Si(OEt)₃).

reactions were not optimized, more than 100 turnovers were observed in the case of H₂. A mechanistic scheme has been proposed.^[47]

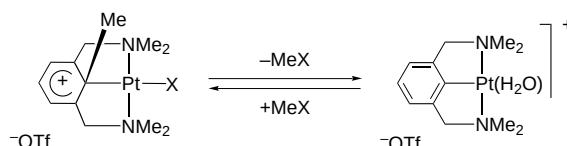
4.6.1.5. C–C versus C–H Activation by Pt

The C–C bond in PCP ligands was also cleaved by Pt^{II} (Scheme 19).^[48] The kinetic C–H activation product was quantitatively converted into the C–C activation product by addition of HCl. Pt^{II} is known to activate C–H bonds both by electrophilic and nucleophilic mechanisms.^[2b] C–C activation in the PCP system can proceed by an electrophilic attack on the aromatic ring with a subsequent 1,2-methyl shift from the coordinated alkyl group to Pt in an arenium cation intermediate.

Importantly, in the closely related NCN system such a 1,2-methyl shift is a plausible mechanistic step, as shown in the work of van Koten et al.^[49] A Pt–NCN arenium complex was demonstrated to promote reversible C–C bond formation and activation (Scheme 20). C–C bond activation in this system appears to be driven by aromatization (see Section 4.2)



Scheme 19. Cleavage of the C–C bond in *i*Pr-PCP by Pt^{II}.

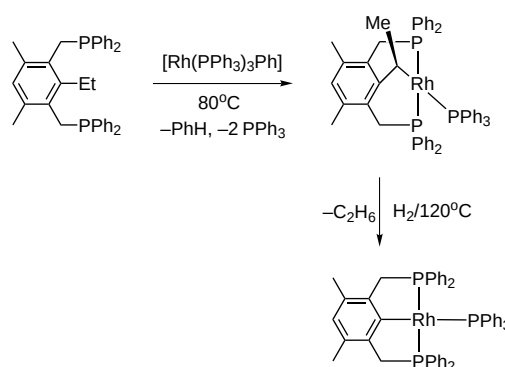


Scheme 20. Reversible C–C bond activation in a Pt–NCN arenium complex. X = Cl, Br, I; OTf = trifluoromethanesulfonate.

4.6.1.6. C_{aryl}–CF₃ and C_{aryl}–Et Activation: C–C versus C–F and C_{sp³}–C_{sp²} versus C_{sp³}–C_{sp²}

When the CH₃ group between the phosphane arms of the *t*Bu-PCP ligand is changed for CF₃, selective oxidative addition of the very strong C_{aryl}–CF₃ bond takes place in the reaction with [[RhL₂Cl]₂] (L = ethylene, cyclooctene). No (aryl)CF₂–F activation product was observed.^[50] Both non-polar and polar (nucleophilic attack) mechanisms are possible, and further studies are required.

The possibility of competitive activation of C_{sp³}–C_{sp³} versus C_{sp³}–C_{sp²} bonds was studied with a PCP ligand containing an aryl–Et moiety. Upon reaction with Rh^I complexes, oxidative addition of C–H initially takes place. Heating of the C–H activation product at 120 °C under H₂ results in selective C_{sp³}–C_{sp²} cleavage, yielding the C–C activation product and ethane (no methane was detected, Scheme 21).^[51] Direct



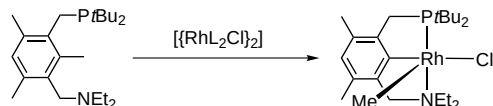
Scheme 21. Reaction of a PCP ligand containing an aryl–Et moiety with [Rh(PPh₃)₃Ph].

C_{sp³}–C_{sp²} bond activation in *t*Bu-PCP ligand containing an aryl–Et moiety was also observed.^[51b] Significantly, a pathway, involving consecutive C_{sp³}–C_{sp³} and C_{sp³}–C_{sp²} bond cleavages, generating methane and the same C–C activation complex

would have been thermodynamically much more favorable (by about 28 kcal mol⁻¹) than the observed C_{sp³}-C_{sp²} cleavage. The reason for the exclusive ethane formation is clearly kinetic. We believe that the specific orientation of the reactive metal orbitals is responsible for the observed preference.

4.6.1.7. The PCN System

A unique preference for C-C activation has been recently demonstrated in the new PCN system.^[52] Upon reaction of a PCN ligand with [RhL₂Cl]₂ (L = ethylene, cyclooctene) at room temperature or below, exclusive C-C activation took place and no C-H activation products were observed (Scheme 22). This may be due to the sterically less crowded metal center in the reactive intermediate (PrBu₂ is bulkier than NEt₂; compare the cone angles of EtPrBu₂ (162°) versus NEt₃ (150°)). The electronic influence of replacing the soft phosphane ligand by a hard amine may



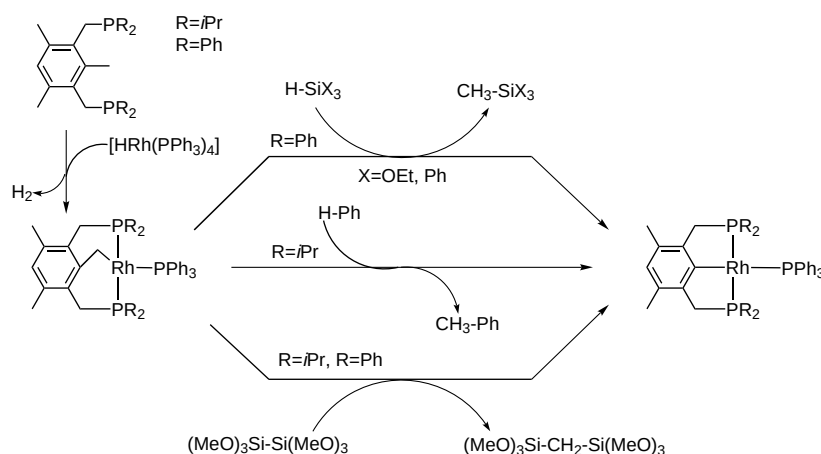
Scheme 22. Reaction of a PCN ligand with [RhL₂Cl]₂. L = ethylene, cyclooctene.

also influence the reactivity of the metal center in the C-C versus C-H oxidative addition. The possibility of a rapid, reversible C-H activation process involving a short-lived (on the NMR time scale), unobserved, C-H activated complex cannot be discounted. A likely reason for fast C-H reductive elimination in this system (in comparison to the PCP system) may be a rapid, reversible on-off dissociation of the amine arm.

In the first example of metal insertion into an unstrained, unactivated C-C bond in solution, we used hydrogen and heating to suppress C-H activation. Then we demonstrated that direct C-C activation can be achieved at elevated temperatures and at room temperature or lower with concurrent C-H activation. In the case of the PCN ligand system direct C-C insertion takes place even at -30 °C, with no formation of C-H activation product. This ligand seems to be perfectly designed for C-C cleavage.

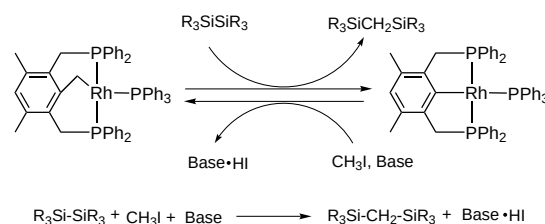
4.6.2. The Methylene Transfer Reaction

The ability to insert a metal into an unactivated C-C bond raises the possibility that a hydrocarbon could serve as a source of methylene groups. If the inserted metal center is capable of binding and activating an additional substrate, the result could be selective insertion of the methylene group into another chemical bond. Indeed, the methylene group in the "methylene-bridged" complexes can be abstracted not only by H₂ but by a variety of reagents. The methylene group was inserted into Si-H, Si-Si, and C_{aryl}-H bonds (Scheme 23).^[53] This represents a conceptually new process in organometallic



Scheme 23. Insertion of a methylene group into Si-H, Si-Si, and C_{aryl}-H bonds.

chemistry and an unusual combination of reactions: C-C bond cleavage, methylene transfer, and selective incorporation into other bonds. Interestingly, the methylene group can be regenerated by treatment of complex whose C-C bond was cleaved with methyl iodide and a base. In this two-step process a methylene group is extruded from MeI and selectively incorporated into a Si-Si bond (Scheme 24). A mechanism



Scheme 24. Regeneration of the methylene group. The overall reaction is given at the bottom.

accounting for the methylene group transfer may include oxidative addition of the substrate with subsequent C-C bond cleavage by a three-coordinate Rh^I fragment and, finally, product release by reductive elimination.

Remarkably, in a one-pot reaction it was possible to combine selective C-C cleavage with the activation of other strong bonds to form products of methylene group transfer. As expected, the electron density on the metal center is an important tool in adjusting the reactivity towards various substrates. Thus, hydrosilylation of a C-C bond was demonstrated with a complex containing phenylphosphane moieties. In the case of methylene group insertion into benzene, a higher electron density on the metal is needed in order to oxidatively add the less reactive C-H bond, and, hence, a complex with isopropylphosphane moieties was used. The reactivity of the Si-Si bond is intermediate to that of Si-H and C-H; therefore both phenyl- and isopropylphosphane complexes transfer the methylene group to the disilane.

4.6.3. C-C versus C-H Activation: Substituent Effects

There is a remarkable influence of the phosphane substituents of PCP ligands on the thermodynamics and kinetics of C-C and C-H activation. Tuning the electron density and

sterics on the metal center by choice of different substituents in the PCP ligands or use of the PCN ligand made possible regulation of the reaction preferences, and selective C–C or C–H activation was achieved.

Thus, Me-PCP, *i*Pr-PCP, and *t*Bu-PCP ligands result in more stable C–C insertion products (Section 4.6.1), whereas the less basic Ph-PCP thermodynamically favors C–H activation (Section 4.6.1.2). This demonstrates the importance of electron density on the metal center in the thermodynamic balance between C–C and C–H oxidative addition (Table 1). Steric factors are apparently of minor importance here, taking

Table 1. Influence of phosphane substituents on the activation of C–C versus C–H bonds in the PCP and PCN ligands.

Metal	Ligand		C–C activation		C–H activation	
	phosphane substituent	additional phosphane	thermo. product	kinetic product	thermo. product	kinetic product
Rh	Me	PEt ₃	+			+
Rh	Ph	PPh ₃			+	+
Rh ^[a]	<i>i</i> Pr	–	+	+		
Rh	<i>t</i> Bu	–	+	+		
Ir	<i>t</i> Bu	–	+	+		
Rh	PCN ^[b]		+	+		

[a] S.-Y. Liou, B. Rytchinski, D. Milstein, unpublished results. [b] *t*Bu on P, Et on N.

into consideration the wide variation in the alkylphosphane size. When higher electron density is involved, metal insertion into the C_{aryl}–CH₃ bond becomes thermodynamically more favorable than insertion into the (aryl)CH₂–H bond. This is perhaps due to the possibility of backbonding from the metal in C_{aryl}–M into the aryl π^* system, which stabilizes the C_{aryl}–M bond relative to the (aryl)CH₂–M bond. Supporting this, M–C_{aryl} bonds are known to be strong for the late transition metals, and M–C_{aryl} bond dissociation energies are comparable to or higher than those of M–H bonds.^[6]

The role of the size of the substituents on the reaction kinetics in C–C versus C–H activation was discussed above. Bulky *t*Bu substituents shield a vacant coordination site and impose a congested conformation in a reactive intermediate that drastically lowers the kinetic barrier of C–C activation. The smaller Ph and Me substituents kinetically favor C–H activation (Table 1), which is likely to be a consequence of forming a four-coordinate intermediate.

5. Tendencies in C–C Bond Cleavage: Metals and Substrates

It is tempting to make a direct comparison between various metals regarding their reactivity in the oxidative addition of a C–C bond. However, in solution chemistry this is a task of significant difficulty, since the reported examples of C–C oxidative addition reactions involve metals in different ligand environments. Valuable information can be obtained from gas-phase chemistry.^[54–57] Schwarz et al. showed that in the gas phase various naked metal ions bind molecules of the type R–X, where R is an alkyl chain and X is a functionality with a donor atom (NH₂, OH, CN), and cleave one of the alkyl C–C bonds.^[55] The investigation of the product distribution using isotope labeling provides an insight into the reactivity prefer-

ences of metals for oxidative addition of C–C and C–H bonds. Thus, reactivity towards C–C bonds increases and selectivity decreases in the row Fe⁺, Co⁺, Ni⁺. A variety of alkyl C–C bonds can be activated such as γ – δ , β – γ , and α – β C–C bonds in the R–X–M moiety. In the work by Freiser et al. a comparison of titanium and iron ions in reaction with various alkanes reflected an exclusive preference of Ti⁺ for C–H activation, whereas Fe⁺ was shown to be active both in C–C and C–H activation.^[56] The Fe⁺ ion readily inserted into any C–C bond of the alkane. So, late transition metals appear to be more active in C–C oxidative addition, the reactivity increasing on going from left to right in the Periodic Table. One should be very careful, however, in applying the gas-phase reaction aptitudes to solution chemistry. In the gas phase, unlike in solution, the M–R bond strength can be higher than for M–H.^[57] Clearly, the naked metal center in the gas phase is very different from that of metal complexes in solution.

In solution C–C oxidative addition was observed for a variety of late transition metals. Most of the examples of oxidative addition of unstrained unactivated C–C bonds were demonstrated in reactions with rhodium and iridium complexes. Electron-rich Rh^I and Ir^I complexes possessing a vacant coordination site seem to be suitable for oxidative addition of unstrained unactivated C–C bonds. The only example in which a direct comparison between the reactivity of different metals with the same ligand system in solution was made is C–C oxidative addition to rhodium and iridium centers in the *t*Bu-PCP–metal system (see Section 4.6.1). Insertion into a C–C bond of the ligand was thermodynamically more favorable than into a C–H bond in the case of both metals. Kinetically, C–C activation was also slightly preferable over C–H activation in the case of both metals, rhodium being more selective for C–C activation than iridium.

No C–C cleavage was observed when various Pd precursors were used in the case of PCP and PCN ligands. It is also noteworthy that in the case of the PCN–Pt system exclusive C–H bond activation takes place.^[52]

In most of the reported cases, only C–C bonds in “special” substrates can be activated by a metal complex in solution. Strained C–C bonds oxidatively add to a wide variety of the metals (see Section 3). C–C bonds adjacent to a carbonyl group (see Section 4.1) or preactivated by potential aromaticity (see Section 4.2) can be activated by various metal centers. Agostic interaction with the metal center could be supportive for C–C cleavage if the substrate is appropriately coordinated to the metal (see Section 4.3). Unstrained, unactivated C–C bonds in PCP and PCN ligands are readily cleaved due to the favorable coordination mode imposed by these ligands (see Section 4.6). Polymetal species such as clusters allow one to widen the spectrum of the substrates targeted for C–C activation (see Section 4.4).

6. Summary and Outlook

Thermodynamically, insertion into a C–C bond in solution is not a forbidden process even if no strain relief or aromatization driving force is involved (C_{aryl}–C_{Me} bonds in PCP and PCN ligands, carbonyl group adjacent to C–C,

cluster-supported C–C activation). Moreover, it can be even more favorable than competing C–H activation processes, as shown in the case of alkyl-PCP and PCN-type systems. In cases where the primary C–C oxidative addition is unfavorable, C–C cleavage may be accomplished by the use of a subsequent energy-releasing reaction, such as hydrogenolysis. C–C activation can proceed directly and does not require prior C–H activation. The competition between C–H and C–C activation in aryl-alkyl substrates is very sensitive to the electron density on the metal center, so metal complexes designed to prefer one process over the other may be designed. Coordinative unsaturation appears to be crucial for C–C oxidative addition. The “hidden” character of the C–C bonds is undoubtedly a contributing factor to this requirement. An increased naked character of ligated metal centers should be sought to achieve C–C bond cleavage in solution. An approach utilizing multimetal species (clusters and polynuclear complexes) seems promising in light of the possibility to create more orientation modes disposed for C–C bond cleavage. Another promising approach is the one based on methylene transfer.

There is only a small difference in the kinetic barriers for C–C and C–H bond activation in a system in which complexes of late transition metals possessing a vacant coordination site are brought in a close vicinity of these bonds (*t*Bu-PCP and PCN). Therefore, the kinetic reluctance of C–C bonds to insertion of transition metal complexes seems to be predominantly steric in nature. However, since C–C oxidative addition can be thermodynamically favorable, an important question is whether it is possible to bring unstrained, unactivated C–C bonds of substrates that are not bound to a metal through heteroatoms in close proximity of the metal center in order to achieve metal insertion, the entire process being thermodynamically driven. Such a process (inter- or intramolecular) would be of primary importance in the light of possible catalytic applications to simple organic molecules.

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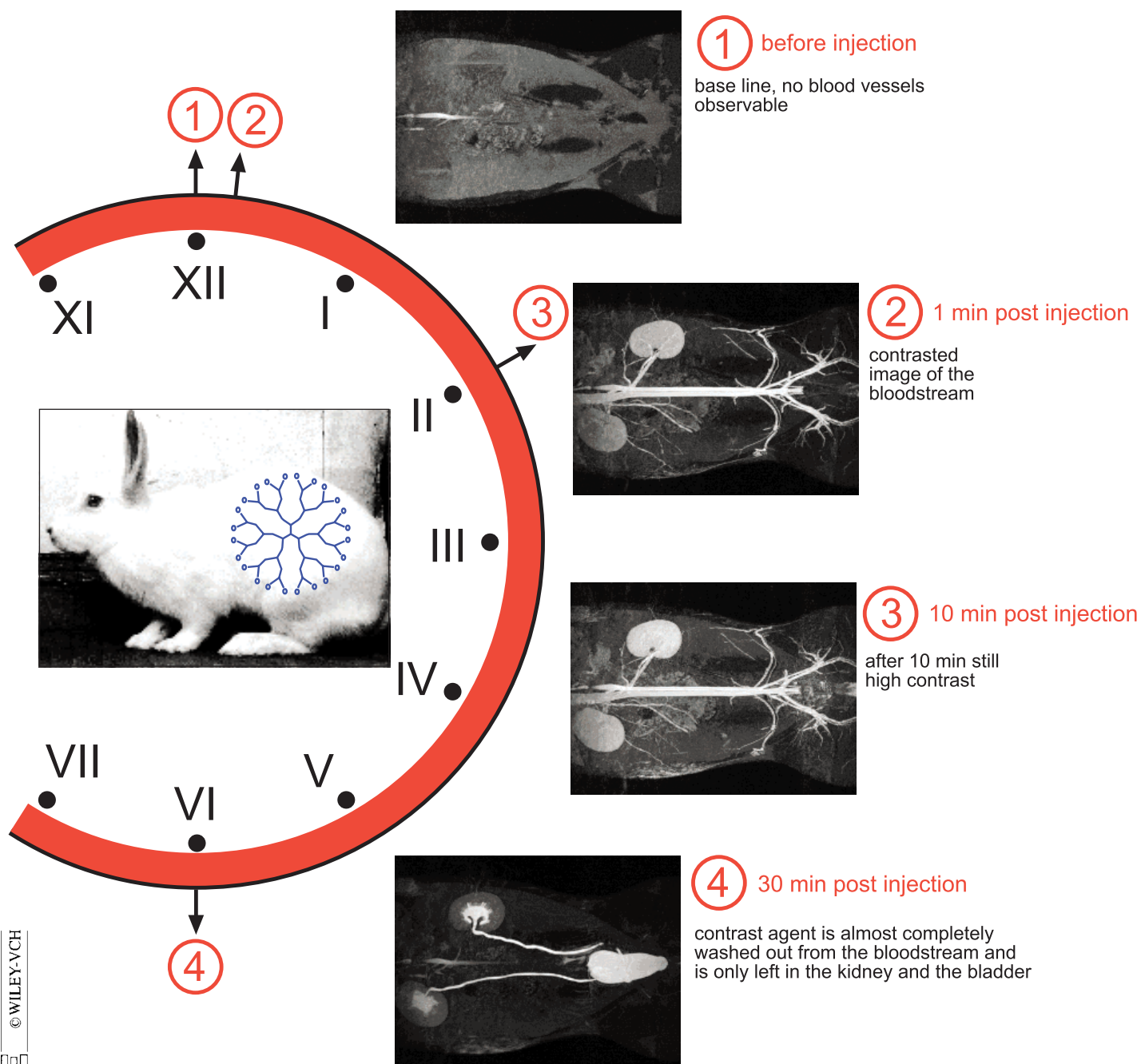
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- Dendrimers -

From Design to Application

Dendrimers as Magnetic Resonance Imaging (MRI) Contrast Agents



Dendrimers: From Design to Application—A Progress Report

Marco Fischer and Fritz Vögtle*

Two decades ago a repetitive strategy for the synthesis of cascade molecules succeeded for the first time, and a rapid increase in the chemistry of dendrimers began. In the beginning the work was concentrated on the synthesis of dendrimers with new skeletons in high generations, but the tailoring of dendrimers to certain applications soon started. Here we would like to take stock and examine the latest developments in this research field. Therefore, we report on topical works to show the many facets of modern dendrimer chemistry. Dendrimers have also become commercially available, and the first applications have emerged. A contrast agent for magnetic resonance

imaging (MRI) to visualize the bloodstream in the body is probably the most spectacular progress. Since specific properties of dendrimers were used for this process, this development could be at the leading edge of new applications. In the future dendrimers will generally be used more as anchor groups than they are hitherto, since dendritic building blocks allow the attachment of many substituents unlike the carbon tetrahedron (four substituents) and the benzene core (six substituents). Thus it is possible to enhance physical properties. As a tree can be full of blossoms and fruit, dendrimers can multiply functions. Undoubtedly it is more difficult to

develop new functionalities for desired effects than to obtain new properties by multiplying known functions. When the orderly synthetic attachment of particular, many, selected, or different moieties on fractal central units can be controlled better than hitherto, and when analytical methods can distinguish between very similar dendritic isomers ("dendrimer libraries") and detect structure defects more easily and more precisely, then the progress ought to be even more ingeniously tempestuous than it is currently.

Keywords: chirality • dendrimers • nanostructures • supramolecular chemistry

1. Introduction

Dendrimers, originally referred to as cascade molecules and arborols, have now been known for two decades.^[1] After the publication of the first papers,^[2] which dealt with the development and the realization of this fundamentally new molecular architecture but which were limited by the little developed analytical methods, the progress in this research field proceeded slowly at first and then from the early 1990s more rapidly (Figure 1).

In the meanwhile a variety of dendrimers with different cores, branching units, and end groups have been synthesized (Figures 2–4). The best known representatives, poly(amidoamine) dendrimers (PAMAM) and poly(propyleneimine) dendrimers,^[3] are produced in kilogram scale and distributed commercially.

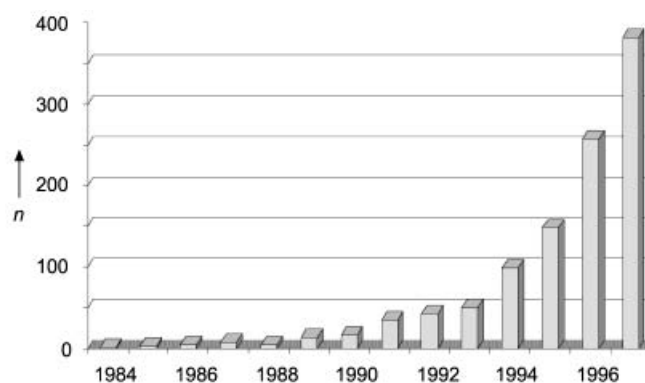


Figure 1. Number *n* of publications on dendrimers obtained from a CAS-Online search.

In accordance with the great attention that this new type of molecule has recently attracted, reviews that summarize the whole field^[1, 4a–c, i–l] and important parts^[4d–h] have been published. Therefore we would like to deal mainly with the most recent works, topical structure investigations, as well as trends and applications. We hope to convey the latest developments in this still-progressing research field.

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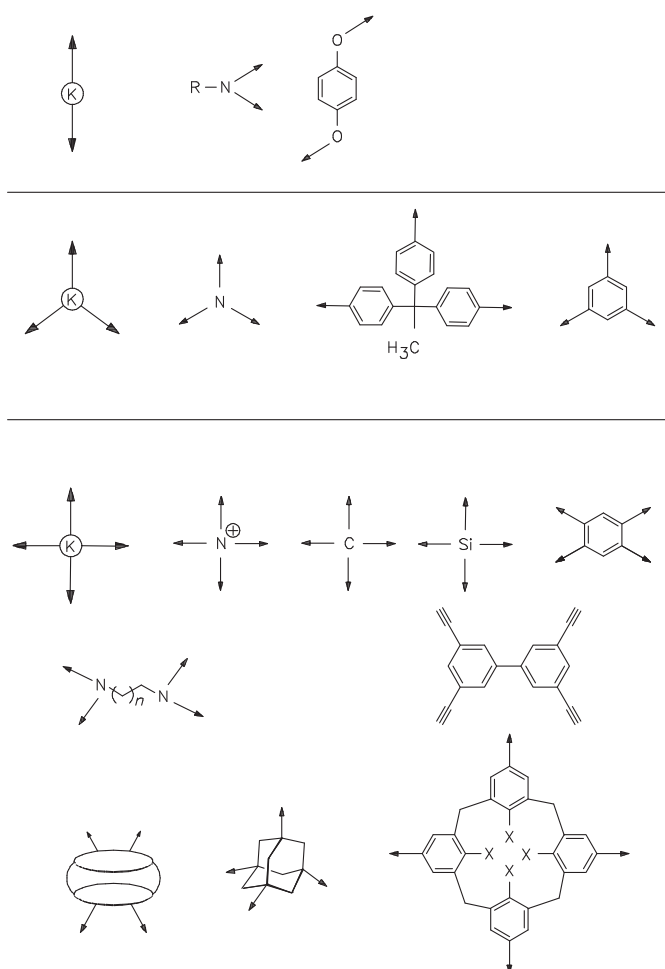


Figure 2. Overview of di- to tetravalent core units K used in synthesis.

2. General

The term “dendrimer” (greek: dendron = tree, meros = part) graphically describes the architecture of this new class of molecule. Although the earlier name “cascade molecule” is more suitable to design their own nomenclature,^[1] the expression “dendrimers” has been established in the meantime. Generally these molecules emanate from a core (Figure 2–3), and like a tree they more and more ramify with each subsequent branching unit (generation, Figure 4). There are two fundamentally different construction concepts:

- 1) The divergent method^[2] in which one branching unit after another is successively attached to the core molecule, hence the multiplication of the number of peripheral groups is dependent on the branching multiplicity (usually 2 or 3). This way the dendrimer can be built up step by step until steric effects prevent further reactions of the end groups (starburst effect, see section 3).
- 2) The convergent method,^[5] which takes the opposite course. The skeleton is constructed stepwise starting from the end groups towards the inside and is finally treated with a core molecule to yield the dendrimer.

Problems occur in the divergent synthesis from an incomplete reaction of the end groups, since these structure defects accumulate with the build-up of further generations. Because the by-products reveal similar physical properties, chromatographic separation is not always possible. Hence, higher generations of divergently built-up dendrimers always contain certain structure defects (see Section 3).^[9] In the convergent course, however, a segment growing with each reaction step is coupled with only one branching unit. Thus, this approach facilitates the removal of undesired by-products, for example,

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Marco Fischer was born in Zell an der Mosel (Germany) and studied chemistry in Bonn between 1990 and 1996. During this time he received a scholarship from the Professor-Rhein-Stiftung, Königswinter. He did his diploma thesis in the group of F. Vögtle and worked on the synthesis of new functional poly(propyleneimine) dendrimers. He is currently doing his Ph D in the same group on the synthesis, structural analysis, and supramolecular interactions of new functional dendrimers. He worked for some months on dendrimers with Professor Kari Rissanen, University Jyväskylä, and with Professor Kazuhisa Hiratani, University Tsukuba, Japan.

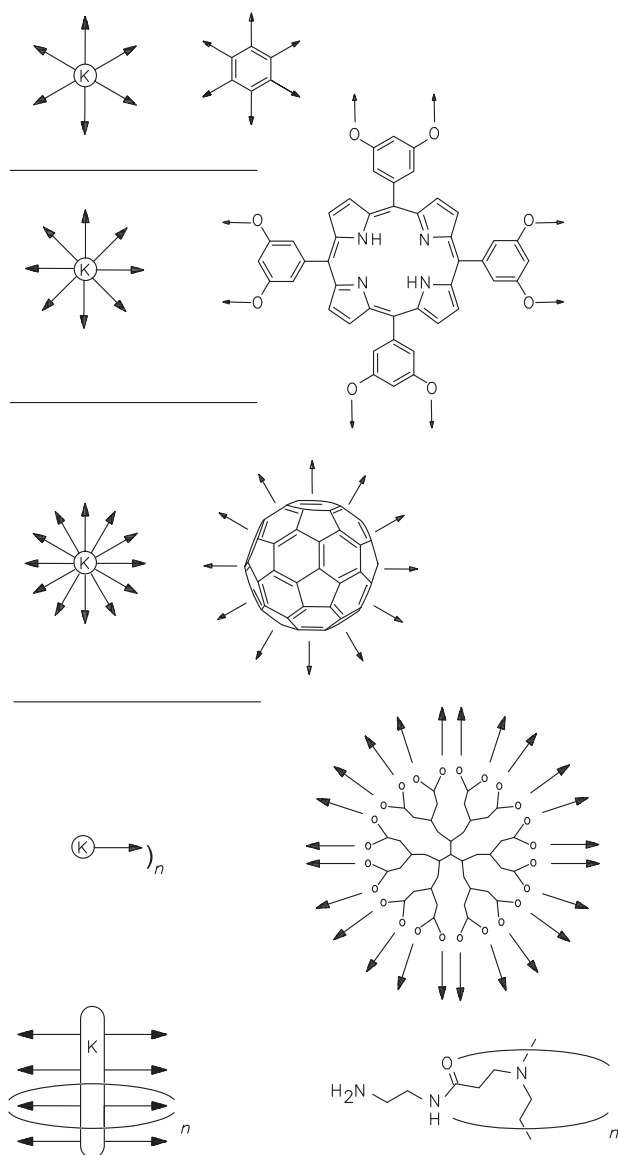


Figure 3. Overview of hexacovalent and larger core units **K** used in synthesis.

by means of gel permeation chromatography (GPC), though it does not allow the formation of as many generations as the divergent method because of steric problems that arise from the reaction of the segments and the core molecule.

After 20 years, a variety of dendrimer skeletons, organic, inorganic as well as organometallic ones, has now been produced; a selection of core and branch cells is given in Figures 2–4 to give a general idea of the wealth of possibilities.

3. Structural Analysis

Higher generation dendrimers reach molecular masses that in earlier days had not been accessible through directed organic reactions, apart from through polymerization reactions, which lead to products with a broad distribution of molecular masses (polydisperse).^[6] They are modelled on natural globular biomacromolecules that are able to perform certain functions as a consequence of their defined three-

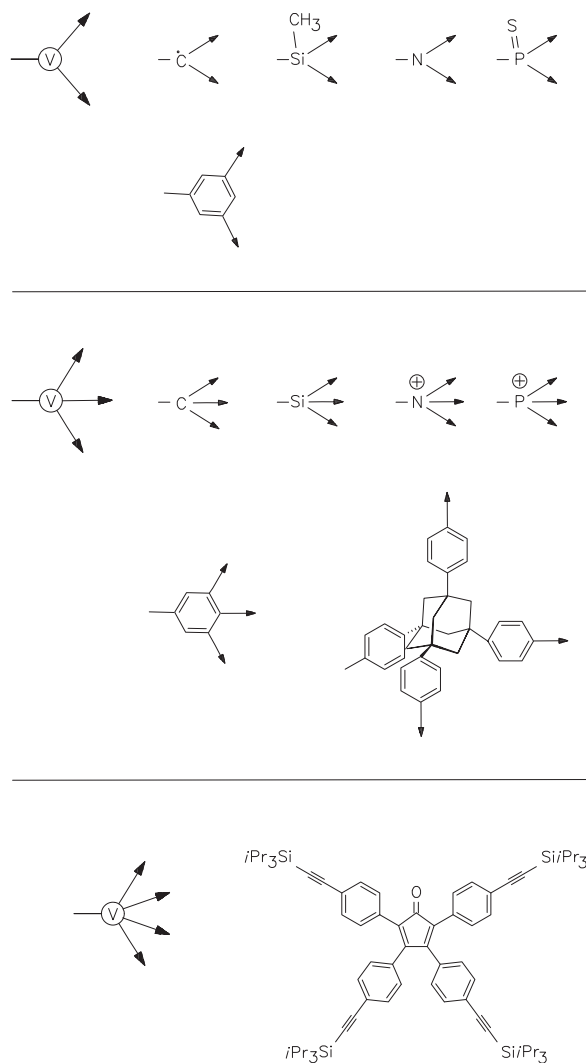


Figure 4. Overview of branch units V used in synthesis.

dimensional formation through hydrogen bonds. Hence, the three-dimensional structure of high-molecular compounds, such as dendrimers, is of great interest, since the dendrimers differ from biological macromolecules, such as proteins, in their three-dimensional covalently linked skeleton. According to an early theoretical work^[7] on idealized structures, it was postulated that dendrimers of lower generations take a rather flat, ellipsoidal shape. Assuming that in divergently synthesized dendrimers each branch is directed radially towards the outside and that the end groups lie on the surface of an ellipsoid, the macromolecules transform into a more spherical shape from a certain generation upwards (depending on the core molecule, branching multiplicity, and the length of the branch segment). Simple calculations^[41] have shown that the area of an end group on this ellipse becomes continually smaller with an increasing number of generations until a critical branched state, the so-called “Starburst dense packing”, is reached and prevents any further reaction. Hence, the molecules are meant to be spherical constructions with a dense exterior and a loose interior with channels and cavities. Experimental results, for example, the inclusion of guest molecules and the viscosity, confirm this assumption. Thus, dendrimer chemistry has become a part of supramolecular chemistry.

More recent methods of structural analysis have revealed additional information that has revised this idea of dendrimers in some respect. In fact X-ray and neutron small-angle scattering experiments^[8] have shown that the higher generations of PAMAM and poly(propyleneimine) dendrimers have a spherical shape. The electron density distribution, however, differs significantly from the model of a dense exterior and a loose interior. The electron density profile reveals a high electron density near the center and a continuous decrease towards the outside. The extension of the high density area is dependent on the generation number. These results instead correspond to the picture of a spherical construction that has the highest density in the inside and a fractal surface. Therefore, the inclusion of guest molecules can be explained by changes in the flexible dendritic arms, the terminal groups of which are observable not only on the surface, but, according to more recent calculations, also in the whole volume of the dendrimer.^[8c]

Meijer et al.^[9] succeeded in obtaining detailed information on the structure of poly(propyleneimine) dendrimers by means of ESI mass spectrometry. These experiments have shown that the fifth generation dendrimer is of comparatively high purity, but structural defects arise from statistical accumulation after 248 reaction steps so that in principle only 20 % of the molecules are structurally perfect. In contrast to polymers, these dendrimers with a polydispersity of 1.002 can be considered as approximately monodisperse. Consequently, a higher generation dendrimer cannot be regarded as absolutely structurally perfect, but rather as a highly ordered system with a high number of easily accessible functional groups.

4. New Types of Dendrimers

More recently most of the research groups have started to synthesize functional representatives of some established dendritic skeletons. They are particularly interested in new molecular properties that arise from the multiplicative effect of peripheral functionalities that are easily accessible or from steric shielding of the reactive center by a dendritic shell.

Nevertheless, compounds with extraordinary properties could also be produced with new molecule skeletons. Majoral et al. have shown that the preparation of particularly high generations (up to generation ten) of a dendrimer is possible based on divalent phosphor branch junctures.^[10a] This molecule has the remarkable property of reacting with a large variety of functional groups. By coupling the tenth generation (3072 end groups) with a diphenylphosphanyl unit and complexing gold(I) chloride a complex could be obtained that contains up to 3072 gold ions; the size of the complex has been determined by high resolution electron transmission microscopy to be about 15 nanometers (150 Å). Correspondingly, they managed to synthesize multipalladium, -platinum, and -rhodium complexes by grafting diphosphane units onto lower generations of the dendrimer.^[10b] The free accessibility to the individual binding sites of the metal complexes could be proved by means of complete ligand exchange reactions. Experiments to investigate the catalytic activity of these compounds are still required. New prospects arise from the possibility of supplying each terminal group of these den-

drimers with three different functionalities so that several active units with variable properties result.^[10c] Polyalkylations that were successfully carried out to yield polycationic compounds have revealed the interior of the molecule to still be freely accessible, despite the high generation numbers.^[10d] Very recently the same group even succeeded in divergently building up new dendrons inside the skeleton (Figure 5).^[10e]

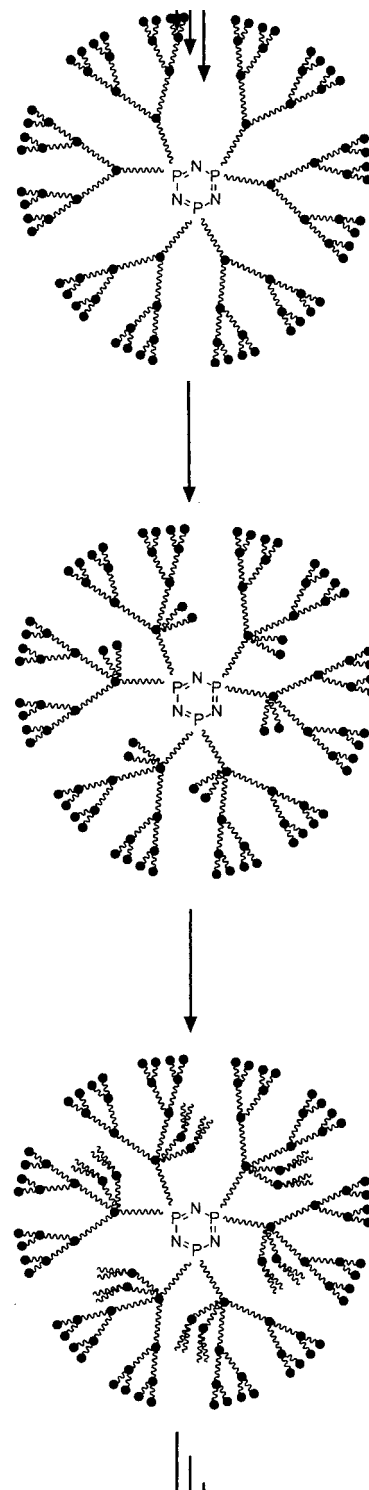


Figure 5. Divergent construction of dendrons inside a dendrimer skeleton.

This fact demonstrates the flexibility of the phosphorus dendrimer skeleton and its easily accessible interior. Therefore, a functionalization inside the covalent skeleton can be carried out in this way.

The possibility of grafting different functionalities onto existing dendritic systems has also been demonstrated by our own group.^[11] The reaction of all primary amino groups on the periphery of poly(propyleneimine) dendrimers has been achieved by coupling with different sulfonic acid chlorides. The sulfonamide units obtained permit the remaining SO₂NH protons to be simply substituted by benzyl halides under mild conditions so as to variably functionalize the surface of the dendrimer. The application of various substituted sulfonic acid chlorides has been tested, and hence this way represents a method for the specific, doubly different multifunctionalization of a thoroughly examined dendritic system.

Müllen et al.^[12] developed dendritic hydrocarbon skeletons of nanometer size that consist exclusively of linked benzene rings and act as interesting precursors to polycyclic aromatic systems that are difficult to access by any other way. The hydrocarbon dendrimers were constructed by Diels–Alder reactions of a substituted cyclopentadienone derivative and an acetylene unit (as the dienophile). In the beginning they succeeded in the preparation of dendrimers up to the second generation with 62 benzene rings^[12a] and later even the construction of dendrimers up to the third generation with 142 benzene units and a molecular mass of about 16600 amu succeeded.^[12b] Further research concentrated on even using a cyclopentadienone derivative as a tetravalent branch juncture (Figure 6).^[12c] This multiplicity had only been achieved previously by moieties based on heteroatoms.^[13] The second generation benzene dendrimer obtained in this way already consists of 102 benzene rings, and according to force-field calculations it is of a rigidly spherical shape. Its thermal stability is remarkable, even in the presence of atmospheric oxygen it only decomposes at temperatures above 580 °C.

4.1. Synthetic Strategies

In addition to the common synthetic concepts described in the second section for constructing dendrimers there are some other approaches. In all cases the dendrimer skeleton is built up in a repetitive manner, namely, after one reaction a further

step for the deprotection or activation has to be carried out.^[14] The “orthogonal coupling” method is an effective synthesis that alternately uses two different branch cells with complementary coupling functionalities (Figure 7).^[15] For this strategy the functionalities and the coupling products have to be inert under the reaction conditions of the second functionality pair. This way the dendrimer skeleton can be constructed either divergently or convergently depending on the target molecule.

Bradley et al.^[16] reported on a solid-phase synthesis analogous to the Merrifield peptide synthesis,^[17] which had earlier been applied to polylysine dendrimers by Tam.^[18] This method allows the use of a large excess of reagents to achieve complete reaction without causing any purification problems. By connecting a group that was unstable to acid to the resin, the separation was guaranteed, and it was possible to synthesize a polyamide dendrimer up to the fourth generation.

Reinhoudt et al. have reported on an entirely new synthetic concept. They have carried out the synthesis of an organo-metallic dendrimer up to the third generation as a one-pot reaction by the controlled association of the building blocks through coordinative bonding.^[19] This method is based on the self-assembly of the branching units in which an unstable coordinative cyano group and two terdentate diorganysulfide-palladium complexes are covalently linked (Figure 8). A chloride ion that occupies the fourth coordination site of the palladium atom prevents the cyano group from being bound. After the building block has reacted with the core cell, however, the chloride ion can be removed in situ by the addition of silver tetrafluoroborate. Hence, by repeating the sequence stepwise a dendrimer of higher generation can be prepared without the usual work-up. Comparable investigations on the one-pot synthesis of polyamide dendrimers by melt polymerization of an acrylic acid derivative merely resulted in hyperbranched polymers with a broad molecular mass distribution.^[20] Such a one-pot reaction is desirable for a commercial application for economic reasons, since the high expenses of a dendrimer being synthesized in many repetitive steps restricts the possible applications to a few special fields such as medicine and diagnosis (see Section 7).

4.2. Nano Structures

Nanometer-sized molecular architectures prepared by “dendronizing” linear polymers have been examined by

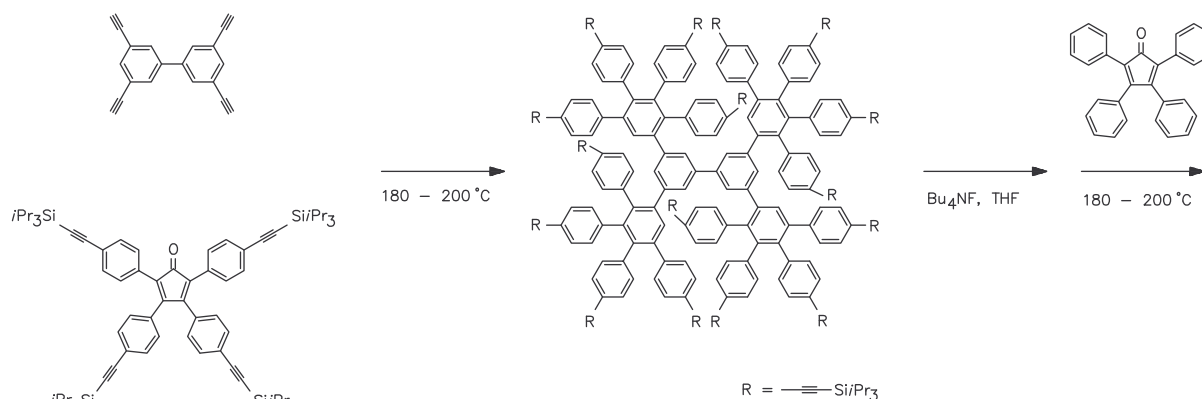


Figure 6. Divergent synthesis of a dendrimer that consists only of benzene units.

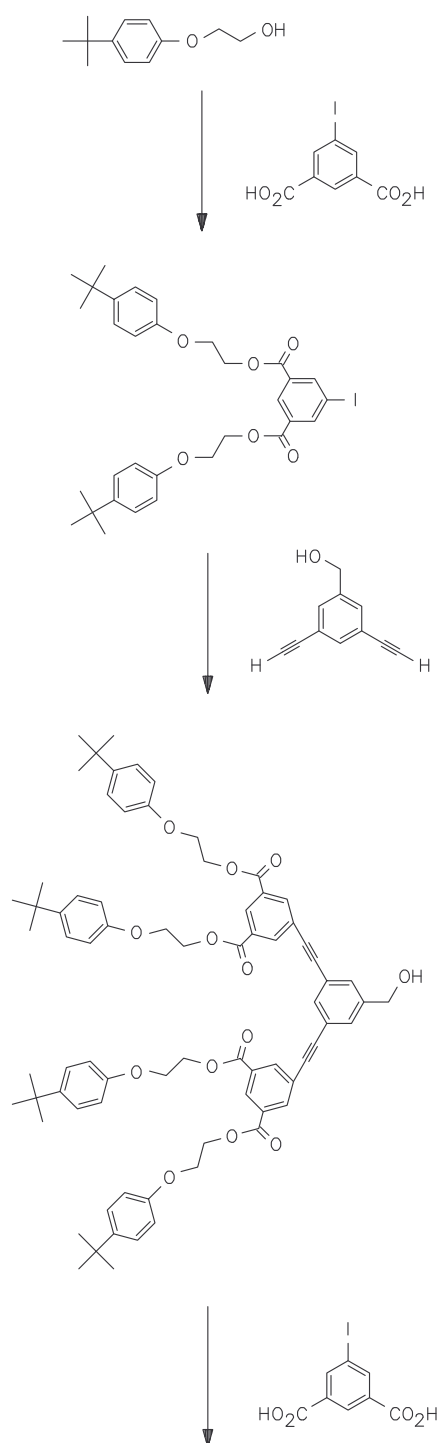


Figure 7. Orthogonal synthetic strategy (convergent and divergent).

several groups.^[21] These macromolecules can be considered as dendrimers with a polymeric initiator core or—from a different point of view—as comb polymers with dendritic side arms attached to it. Two synthetic routes are employed to produce these molecules. Either a convergently prepared dendrylic substituent is coupled with each monomer moiety of the polymer

chain or a dendron carrying a monomer moiety at the focal point is polymerized by standard methods. Schlüter et al. demonstrated the advantages and disadvantages of the two concepts by synthesizing poly(*p*-phenylenes) with third generation Fréchet-type dendrons (Figure 9).^[22] This work has shown that third generation dendrons cannot be homogeneously connected to each monomer moiety of the polymer chain because of steric effects. Generally, this problem did not occur in the polymerization of dendritically substituted mono-

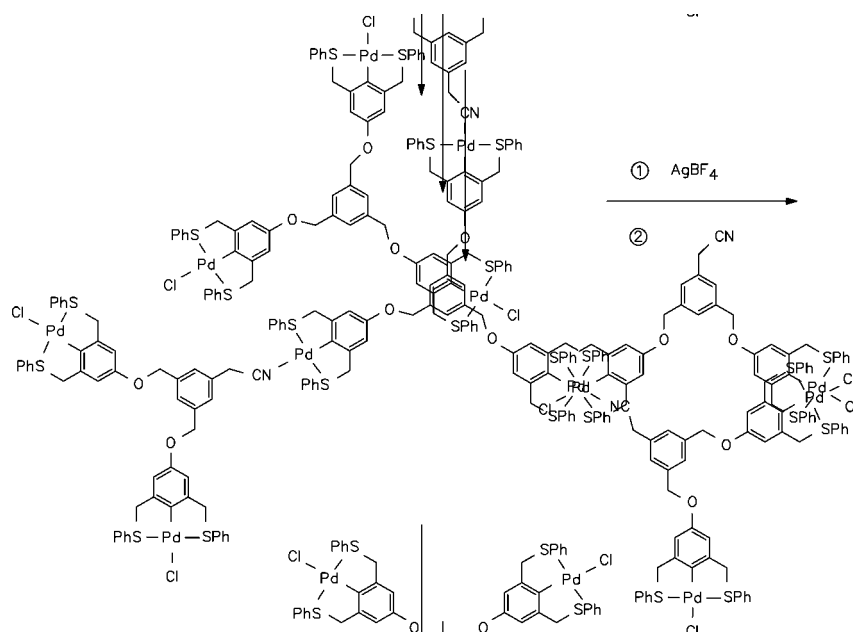
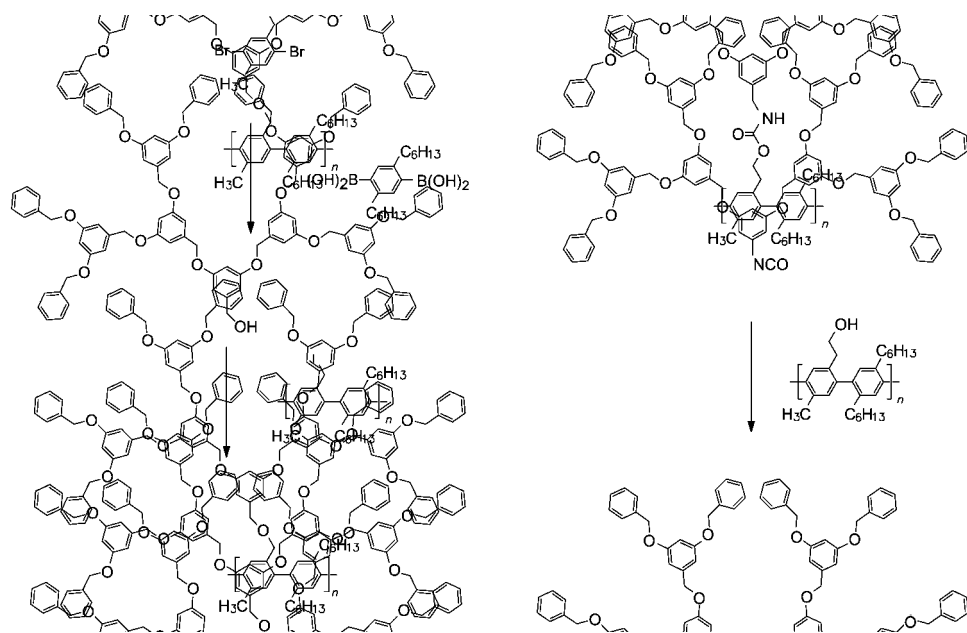


Figure 8. Construction of metallodendrimers by a one-pot reaction.



mers. In this synthetic route, however, only products with low degrees of polymerization could be obtained. The structures prepared this way have the remarkable property that as a consequence of the steric demand of the substituents the polymer chain is forced to take up a stretched form. Furthermore, the macromolecules exist as nanometer-sized rigid cylindrical particles.

Diederich et al. succeeded in preparing oligo(triacetylenes) by polymerization, as well as those substituted with first to third generation Fréchet-dendrons.^[23] They build a kind of insulating layer for the conjugated oligo(triacetylene) without changing the electronic properties of these conductive oligomers. Another preparative method has been presented by Tomalia et al. where they divergently constructed a dendrylic substituent on a linear poly(propyleneimine) with a degree of polymerization of between 100 and 500.^[24] By means of electron transmission microscopy the shape of the polymer chain was observed to depend on the generation of the substituent. The shape changed from a spaghetti-like entangled polymer to slightly elongated nonspherical clusters and rod-like shapes in the fourth generation. The latter line up in bunches parallel to each other, and represent an example of the self-assembly of nanometer-sized particles on a surface.

The aggregation of negatively and positively charged porphyrin dendrimers in solution through electrostatic interactions has been described by Aida et al.^[25] The group noticed an increased transmission at 500 nm on mixing stoichiometric amounts of the two compounds together. Furthermore, fluorescence microscopic pictures showed 10–20 μm large aggregates that crystallized from the solution after some time. In the case where there is an excess of one of the dendrimers exact spectroscopic analyses revealed that not more than four molecules

Figure 9. Variants on the synthesis of dendritically substituted poly(*p*-phenylenes) (dendrimers with a (comb) polymer as the core cell).

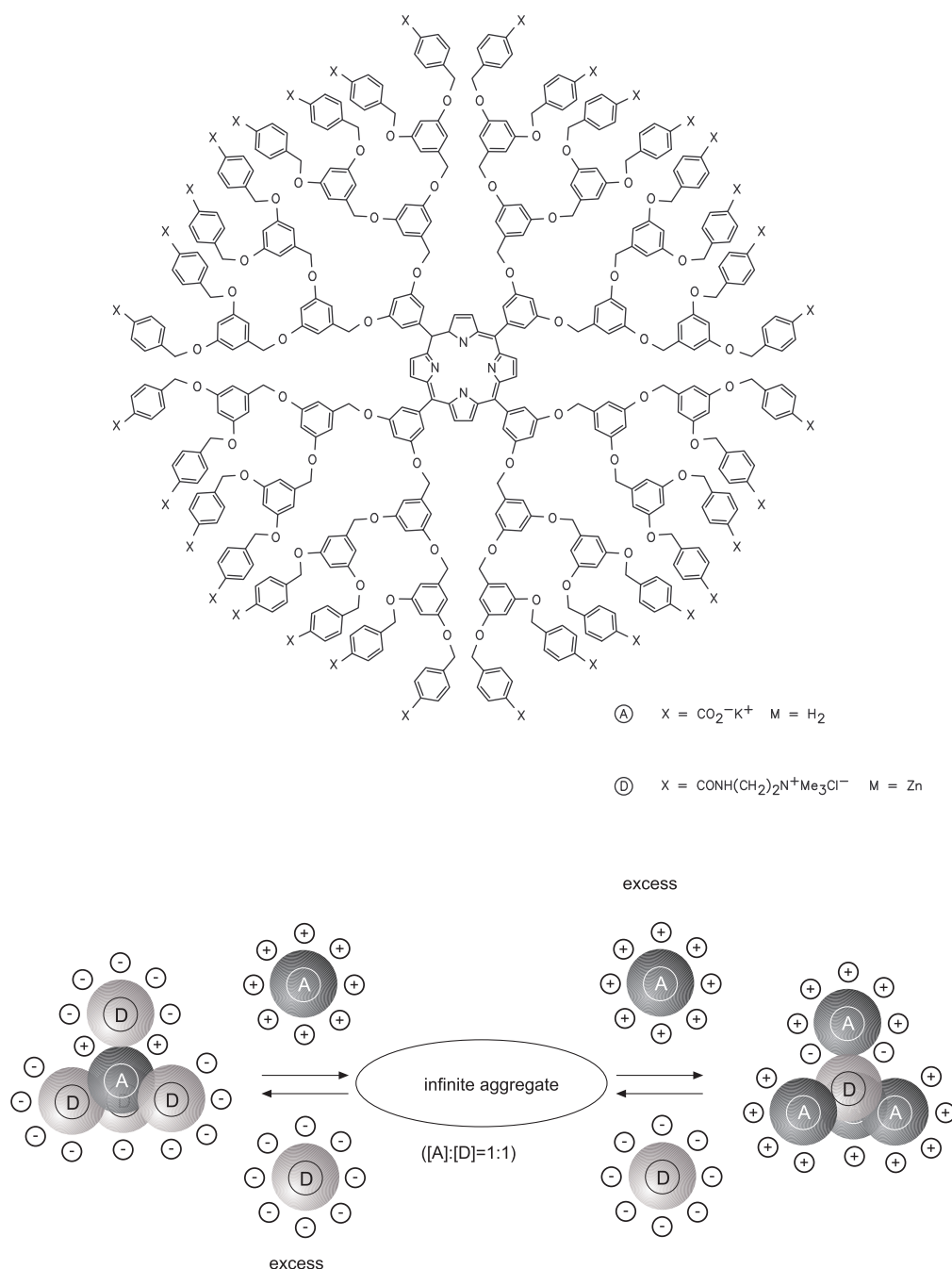


Figure 10. Electrostatic association of functionalized porphyrin dendrimers in solution.

of the excessive dendrimer interact with the other one and transfer energy (Figure 10). The formation of nanometer-sized aggregates, however, only occurs if both of the molecules that transfer energy between each other are present in stoichiometric ratios.

Percec et al. worked on the supramolecular formation of dendrons in the liquid-crystal state.^[26] They described the properties of dendrons with tapered shapes and focal receptor moieties (Figure 11). This molecular architecture was obtained by using gallic acid as a branch juncture and long exterior alkyl chains. After covalently attaching a focal crown ether unit, the self-assembly of first generation dendrons to

form cylindrical architectures was observable from their interaction with alkali metal ions by means of X-ray scattering experiments.^[26a] In the melt these substances show liquid-crystalline behavior, the thermodynamic parameters of which allowed the formation process to be analyzed. By employing different functional groups at the focal position or on the periphery of the dendrons, different supramolecular interactions, such as hydrogen bonds, hydrophobic and fluorophobic effect, or ion complexation by crown ethers, could be compared and their energetic effect on the self-assembly could be evaluated. Based on these results, Percec and co-workers succeeded in polymerizing monomeric units that

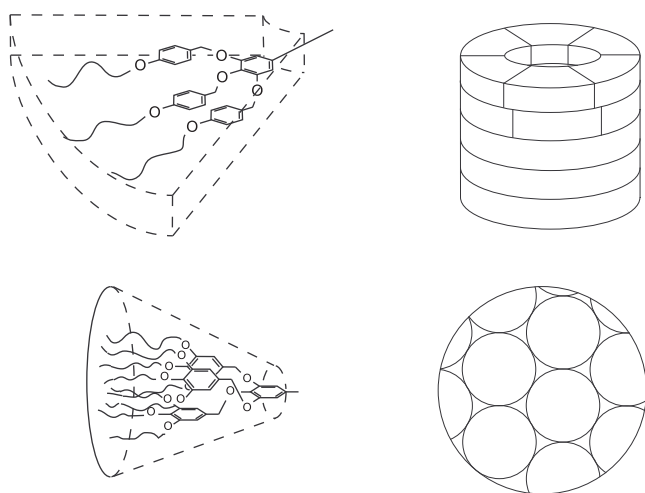


Figure 11. Self-organization of structurally different dendrons in the melt.

were focally attached to analogous dendrons to yield cylindrical structures with liquid-crystalline properties.^[26b] Further construction of these gallic acid dendrons up to the fourth generation and additional studies revealed that in the melt they arrange to form supramolecular dendrimers in a form analogous to spheric micells (Figure 11).^[26c] The formation of a new type of liquid-crystalline phase with cubic symmetry has been observed. The respective number of conical dendrons in these phases could be estimated by electron-density measurements.

Most recently Percec et al.^[26e] have successfully visualized the structures described above by electron microscopic methods. By means of atomic force microscopy,^[26f] they tried to solve the geometry of polymerized, conical second generation dendrons^[26d] and found a correlation between their structure and the degree of polymerization: At a low degree of polymerization a spherical molecule exists while at a higher degree a cylindrical molecule exists. This way the macroscopic architecture can be synthetically controlled.

These intermolecular associations in the liquid-crystalline state and the particular properties resulting from it are also in the focus of other research groups and under intensive investigation.^[27] In addition to the main-chain and side-chain liquid-crystal polymers, dendritic systems represent a third group of liquid-crystalline, high-molecular compounds. In principal, liquid-crystalline properties can be achieved by a form-resistant, dislike skeleton,^[27a–e] mesogenic, flexible units as branch junctures,^[27f] or by functionalizing the dendrimer periphery with mesogenic units.^[27g–i]

4.3. Combinatorial Chemistry

The idea of using dendrimers as a homogeneous soluble carrier for combinatorial libraries has been realized by Kim et al.^[28] to produce a small substance collection, based on Fischer's indole synthesis, with modified PAMAM dendrimers as carrier substances. Therefore they successively employed three *N*-protected aminoacids, three keto carboxylic acids, and three hydrazine derivatives in a split synthesis. As a consequence of the solubility of the compounds the

substances formed could be separated by size exclusion chromatography and resulted in a library of 27 compounds. The more reasonable ratio of carrier material used to substance yield and the possibility of permanently controlling the reaction by spectroscopic methods is advantageous compared with the solid-phase synthesis. An automation of this process is also possible.

A strategy for specifically varying certain properties, such as solubility, viscosity, or reactivity, for desirable applications has been found by Newkome et al.^[29] This group attached a triple branch juncture to poly(propyleneimine) dendrimers and were able to graft on additional functionalities (Figure 12). By using mixtures of this branch juncture and different end groups the outer layer of the dendrimer can be changed by a kind of combinatorial synthesis to result in nanometer-sized particles with numerous different shapes and functionalities. Hence, "screening methods on specific properties" can be applied to discover new materials.

4.4. Surface Coating

The peculiarities of dendritic structures offer new possibilities to manipulate the properties of surfaces by coating them. In the formation of dendrimers at interfacial areas, either gas–water or gas–solid transitions, a change in conformation compared to that in solution has been noticed. Its extent depends on the strength of the respective interaction.^[4d] The stronger the attractive forces, the more flattened the spheric shape becomes and the more it resembles a disc, so that for higher generations of elliptical PAMAM dendrimers an axle ratio of 1:3–6 has been observed.^[4f]

To examine the sensory properties of such surfaces Crooks et al. covalently linked PAMAM dendrimers to the carboxylic groups of a self-assembling monolayer (SAM) of sulfanylundecanoic acid (mercaptoundecanoic acid, MUA) on a gold surface (Figure 13).^[30a] They found on the basis of acoustic wave (SAW) mass balances that dosing this modified surface with volatile organic compounds gave the essential attributes for an ideal chemical sensor. Immediately after the addition, an answer was detected which was reversible and showed a high signal-to-noise ratio. Furthermore, the sensor material guaranteed a differentiation between acids, alcohols, and hydrophobic substances by the signal intensity. In addition it was noted that PAMAM dendrimers can be irreversibly attached to gold surfaces even without covalent binding.^[30b–e] By additional adsorption of hexadecylthiol the dendrimer layer was compressed, thus distorting the molecules to flattened spheroids. Cyclovoltammetric analyses revealed them to act as reversibly switchable gates for redox-active ions, which at pH 6.3 let only $[\text{Fe}(\text{CN})_6]^{3-}$ ions pass, and at pH 11 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ions also to exchange with electrons on the gold surface. A complete, pH-selective distinction between cations and anions has been achieved by a new ultrathin multilayer structure formed from an amphoteric polymer and covalently bound PAMAM dendrimers.^[30c] At low pH values these mixed membranes were permeable exclusively to anions, whereas in high pH-value regions only cations could pass.

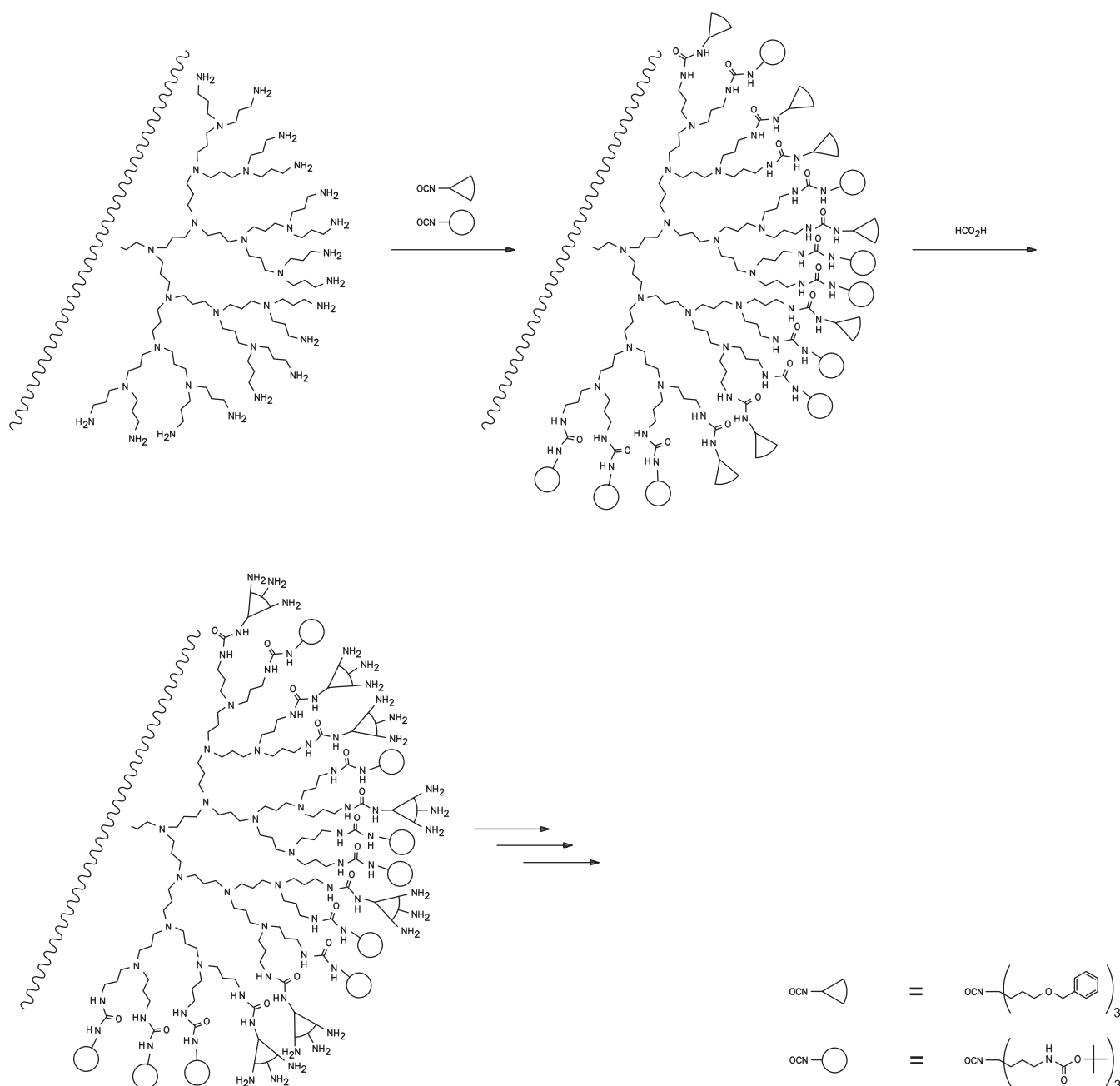


Figure 12. The grafting of additional functionalities on the surface of dendrimers.

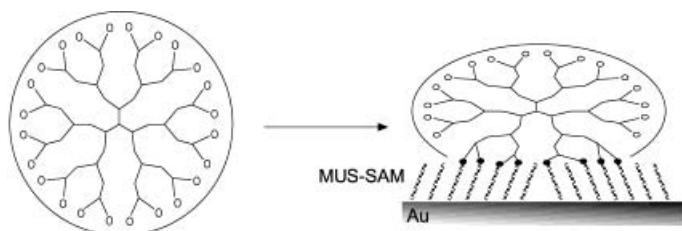


Figure 13. Covalent attachment of dendrimers to a self-assembled monolayer (SAM) of MUA molecules.

4.5. Unconventional Photoactive Systems

Molecular systems can be manipulated rapidly, mildly, and often reversibly by light. The first photo-switchable dendrimer with six azobenzene units in the periphery has been

synthesized in Bonn, and the entire switching of all six groups from the *E* to the *Z* isomer has been proved.^[31] Jiang and Aida,^[32] as well as Junge and McGrath,^[33] successfully fitted in such an azobenzene unit as the initiator core of a dendrimer with aryl ether branch junctures. The former study found a transformation into the *E* form under irradiation with ultraviolet light, although the re-isomerization under irradiation with infrared light proceeds 260 times faster than the thermal reaction at 21 °C under light exclusion. This fact has been ascribed to an antenna effect of all the peripheral aryl moieties transferring the absorbed energy through covalent bonds to the central azobenzene unit. McGrath examined the first two generations of the same system, which are not yet subject to any steric hindrance, and noted analogous photo-switchability (Figure 14).^[33]

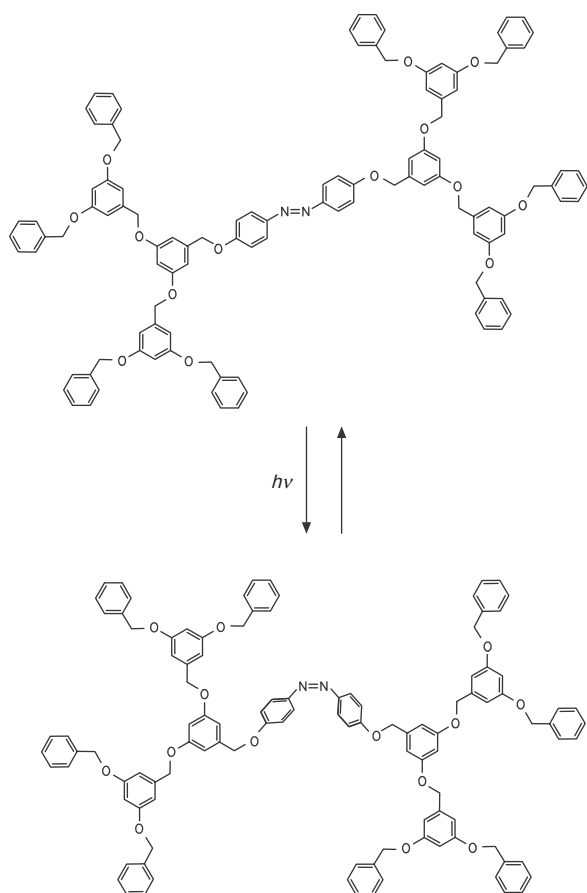


Figure 14. Photo-switchable dendrimer with a central azobenzene unit.

As recently reported by Balzani et al. and our group, no quenching effects occur even with a high number of azobenzene substituents on the periphery of a poly(propyleneimine) dendrimer and the photo-switchable units have the same properties as the monomeric azobenzene.^[34] Moreover, these dendrimers with up to 32 functional groups turned out to be suitable materials for holographic data storage. Subsequent studies, that aimed to solve the host properties of the photo-active molecules, led to the discovery that in the presence of fourth generation dendrimers the fluorescence of eosin molecules is effectively suppressed.^[35] This fact can be put down to the eosin being inserted into the dendrimer skeleton, since the only possible quenchers are the tertiary amino groups located there. Further evidence of this inclusion is offered by the kinetics of the photoisomerization of the azobenzene units for which eosin acts as a sensitizer. The increasing reaction rate confirms that the eosin is occluded in the dendrimer with the *Z* form of the molecule being a better host than the *E* form.

4.6. Redox-Active Dendrimers

Apart from their photochemical peculiarities, the shielding effects of redox-active moieties in the core or their multiplication on the periphery can also be subjected to further research. Whereas earlier works dealt with dendritically substituted porphyrin or bis(terpyridine) complexes, more

recently the redox behavior of dendrimers with ferrocene as the active unit has been closely examined.^[4e] Morán et al. reported in 1996 on ferrocenyl-functionalized poly(propyleneimine) dendrimers with up to 64 active groups, then the highest number known for organometallic substituents on the periphery of a dendrimer.^[36] The ferrocenyl functionalities do not reveal any steric effects despite the high number, and they are reversibly oxidized independently from one another at the same respective potential.^[36a] By the inclusion of single ferrocenyl substituents into the hydrophobic interior of a β -cyclodextrin host molecule, the lipophilic dendrimers have been solubilized in an aqueous solution and by this the redox properties have been changed.^[36b] It turned out that all ferrocenyl units could be complexed completely up to the second generation, whereas this was impossible for higher generations because of steric effects from the large cyclodextrin molecules. Furthermore, the adsorption of the ferrocenyl dendrimers on a platinum electrode surface has been studied.^[36c] Accordingly the molecules in their oxidized form are taken up in multilayers by the electrode at positive potential. On reduction they desorb into solution to leave a monolayer on the electrode. The kinetics of these processes depend on the dendrimer size. The monolayer has been examined by means of atomic force microscopy and the adsorbed dendrimers were noted to be flattened spheres (see Section 4.4). Astruc et al. observed that the ferrocenyl dendrimers can serve as supramolecular redox sensors for inorganic anions.^[37] The standard redox potential changed significantly on the addition of one equivalent of salt per ferrocene unit so that it was then possible to distinguish between four different anions. To complete these analyses Cardona and Kaifer prepared monodendrons with a single ferrocene moiety at the focal position.^[38] These showed a redox behavior that depended on the generation; the rate of electron transfer and the diffusion coefficient decreased with increasing generation numbers.

5. Supramolecular Interactions

The dendrimers hitherto described mainly have to be synthesized by covalently linking bonds step-by-step and as a result of this way of construction structure defects in higher generations are hardly avoidable (see Section 3). In contrast, nature uses the self-assembly of identical protein segments to give large structures that are directed by hydrogen bond formation, such as, for example, the tobacco mosaic virus, which consists of 2130 identical protein segments and a single-stranded RNA containing 6400 bases.^[39] Noncovalent self-assembly combines the advantage of a minimum energetic effort in the synthesis and the possibility to tailor single segments to achieve the largest interaction.

Based on this principal Zimmerman et al. developed molecules in which two isophthalic acid moieties are connected through a rigid spacer.^[40] The carboxyl groups of these compounds can interact intermolecularly through hydrogen bonds and assemble in a series of linear aggregates or in a cyclic hexamer depending on the steric demand of the spacer (Figure 15). By controlling the steric demand by Fréchet-type

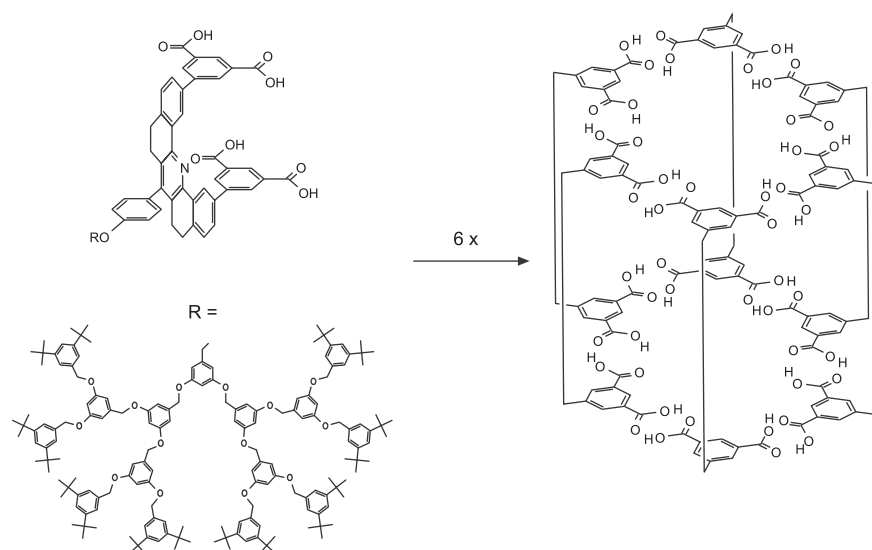


Figure 15. Supramolecular arrangement to form a hexameric dendrimer.

dendritic substituents attached to the spacer, one can influence the equilibrium of different aggregates in such a way that the cyclic hexamer structure is favored. It has been postulated on the basis of model calculations that these defined aggregates take up the shape of a flat disc that is about 9 nm in diameter and about 2 nm thick. For the first time the supramolecular construction of a nanometer-sized dendrimer by self-organization through hydrogen bonds could be demonstrated.

The same group worked on the formation of heterogeneous supramolecular structures from low-molecular guest compounds and dendritic building blocks that contained hydrogen-bonding sites that were complementary to each other.^[41a] As opposed to the aggregation of equal molecules, the energetics and stoichiometry of two components self-assembling can be examined exactly by titration experiments. Again Fréchet-type dendrons of different generations, which were bound to an anthryridine core, served as the dendritic units. This core has a triple hydrogen-bonding acceptor unit whose interaction with a monomeric and a dimeric benzamidine

derivative (double hydrogen bonding donor), respectively, was followed by NMR spectroscopy. In both cases the complexation was detected successfully, and the dimeric benzamidine derivative is really of a 1:2 stoichiometry. Subsequently, a recent work reported on experiments to bind analogous monomer guest molecules to a naphthylidene core (double hydrogen bond acceptor) that carried two dendryl substituents through hydrogen bonds.^[41b] In these studies it was possible to examine and to compare two different dendritic systems. On the one hand the usual Fréchet-type dendrons were coupled to the naphthylidene core, and on the other hand the phenylacetylene dendrons developed by Moore^[41c] were used. Both systems revealed approximately the

same ability to associate guest molecules and an analogous solvent dependence in all the generations analyzed; hence no major differences either in polarity or in the free accessibility of the core unit has been registered. Only the weaker association constant of a more voluminous guest molecule for both skeletons reflects the shielding of the host molecule.

The formation of a didendron described above by means of specific hydrogen bond interactions between monodendrons and a divalent core molecule can also be achieved through the directed formation of a metal complex, as mentioned by Newkome et al.^[42a] This group coupled polyamide dendrons^[42b] through two alkane chains of different length to a terpyridine moiety and arranged them into heterodimers after the successful complexation of a ruthenium(II) ion (Figure 16). The dendritic surrounding of this bis(terpyridine) complex affects the redox potential significantly, with the irreversibility of the redox process increasing with higher generations. Chow et al. synthesized similar dendritic homoleptic complexes based on terpyridine^[43a] by coupling polyether dendrons^[43b]

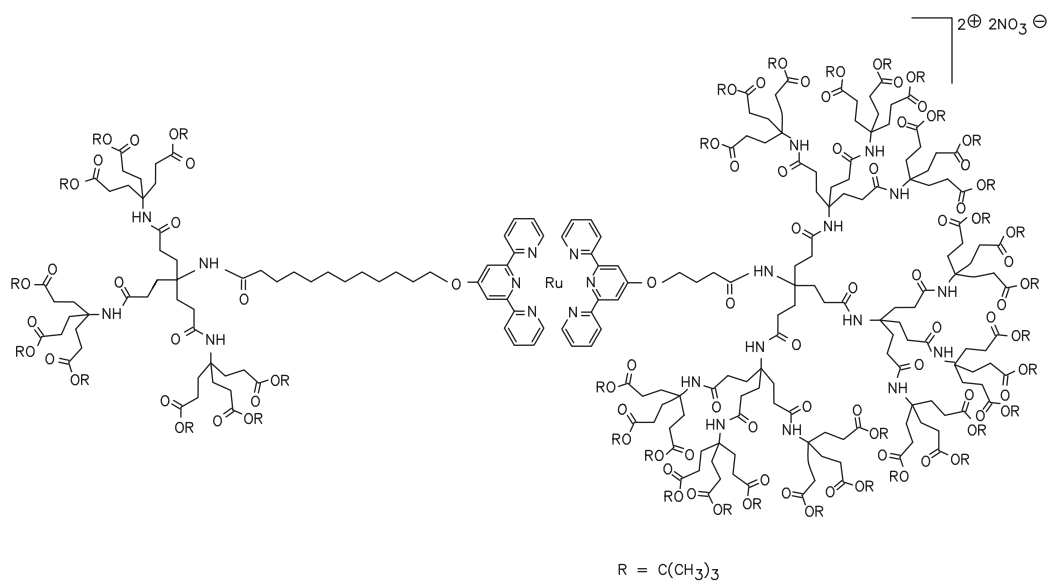


Figure 16. Synthesis of a didendron by directed metal complexation.

bearing a terpyridine core with an iron(II) ion. For these compounds the redox process was likewise irreversible with higher generations, and this has been ascribed to the comparatively large distance between the redox center and the surface of the electrode.

A different kind of self-organization that used dendritic segments was first used by Tzalis and Tor who chose a 1,10-phenanthroline ligand that was supplied with dendryl substituents in positions C3 and C8.^[44] This ligand allows the stability and the spacial arrangement of the supramolecularly connected dendrimer to be directed by the coordination number and coordination geometry of the complexed metal ion. Complexation of a copper(I) ion with a phenanthroline derivative substituted with two first generation polyether dendrons yielded a tetrahedral orientation of two ligands, whereas the interaction of three ligands with an iron(II) ion led to a complex with an octahedral geometry. Such an octahedral self-organization of three ligands by metal complexation has been successfully carried out with higher generation dendryl substituents by Balzani et al. and us.^[45] A 2,2'-bipyridine substituted with first to third generation polyamide dendrons and a ruthenium(II) ion served as the ligand and metal center, respectively. We only succeeded, however, in synthesizing the octahedral ruthenium complex with up to the second generation bipyridine dendrons. For higher generations steric reasons seem to prevent complex formation. In studies of the photophysical properties the second generation supramolecular dendrimer obtained with 54 peripheral ester groups showed an excited luminescence state with a remarkably high lifetime, which can be ascribed to the quenching by atmospheric oxygen as a result of steric effects.

Only recently Kawa and Fréchet reported the association of polyether dendrons up to the fourth generation by coordination to lanthanide ions.^[46] This self-organization differs from the other examples described before by the ionic interaction between the tripositive erbium, terbium, and europium ions and the focal carboxylate functions predominant here (Figure 17). The preparation was carried out by simply exchanging the ligands of lanthanide triacetate by carboxylic acid dendrons. The increase in luminescence activity with higher generation polyether dendrons turned out to be a particular property of these compounds. This is caused by a strong antenna effect of the dendritic ligands and by the extremely effective insulation of each lanthanide ion.

Besides using supramolecular interactions to associate dendrons as described above, Aida et al. studied the steric effect that dendryl substituents exert on supramolecular interactions.^[47a] They determined spectroscopically the binding constants between a zinc-porphyrin derivative substituted with Fréchet-type dendrons up to generation five and an imidazole group equally functionalized. As expected, the values diminished with higher generation of the substituent on either the porphyrin or the imidazole group, and for the highest number of generations studied hardly any association was detectable. This agrees with the long half-lives of analogous porphyrin-imidazole-oxygen complexes. These are protected by the surrounding dendrimer skeleton, which forms a hydrophobic shell and prevents the complexes from being deactivated by dimerization and from being oxidized by

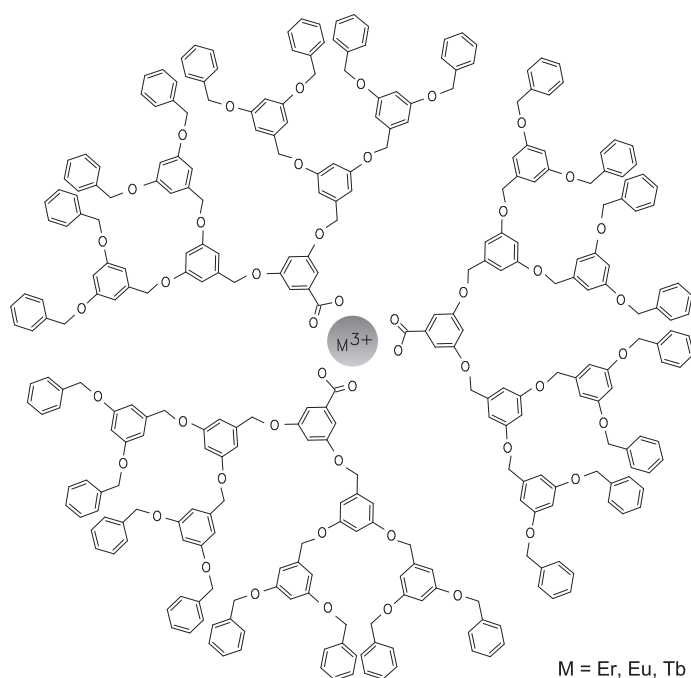


Figure 17. Synthesis of a dendrimer by ionic coordination to a lanthanide ion.

contact with water.^[47b] The use of such an apolar dendritic cover for performing liquid–liquid extraction of water-soluble dyes into organic solvents has been described by Meijer et al.^[48a] They succeeded in proving that poly(propyleneimine) dendrimers substituted with long alkyl chains are able to transfer rose bengal or fluorescein from aqueous solution to an organic layer in high yield. A total re-extraction is possible because of the process being pH dependent. DeSimone et al. successfully employed dendrimers functionalized with perfluoropolyether chains to extract methyl orange into liquid carbon dioxide.^[48b] Both methods are based on the fact that the corresponding dendrimer acts as a unimolecular micelle and represents a supramolecular host molecule for several dye molecules that carries them into the other layer.

6. Dendrimers and Chirality

Even in the early days of dendrimer synthesis compounds that were constructed with lysine as a chiral branch juncture have been mentioned.^[2b, 49] In these studies, however, the chiroptical properties of the compounds were not analyzed, but one expected interesting properties with respect to surface modification, metal-ion complexation, and pharmaceutical application. In the following years numerous works were published that demonstrated the varied ways in which chiral moieties can be introduced into a dendrimer. Mostly substances from the pool of optically active natural compounds were used as chiral moieties, such as amino acids and carbohydrates. Only recently Seebach et al.,^[50a] Peerlings and Meijer,^[50b] and Thomas and Tor^[50c] summarized the work done. Peerlings and Meijer classified chiral dendrimers for the first time as dendrimers with:

- 1) a chiral initiator core,
- 2) a chiral branch juncture,

- 3) chiral end groups,
- 4) two or three of the characteristics listed above,
- 5) constitutionally different segments anchored to a chiral initiator chore,
- 6) a rigidly chiral conformation without possessing any stereogenic centers or chiral moieties, and
- 7) interactions with noncovalently bound chiral ligands.

For dendrimer types 6 and 7 there are no experimental examples yet. In addition, they pose many problems that have to be studied in future projects, for example, the question as to whether a chiral initiator core and achiral building blocks lead to optical activity or whether chiral moieties in a dendrimer facilitates enantioselective host–guest complexation or clathrate formation. Before one can employ dendrimers as chiral chromatography materials, catalysts in asymmetric synthesis, or for similar applications, it should be known how the molecular chirality of the dendritic building blocks and the macroscopic chirality of the compound are related to each other.

Investigations by Meijer et al.^[50b, 51] on chiroptical properties of poly(propyleneimine) dendrimers with Boc-protected amino acids as terminal groups (Boc = *tert*-butoxycarbonyl) revealed that the optical activity of the compounds diminishes with an increasing number of generations until it is hardly detectable in the fifth generation with 64 end groups. This effect is not caused by racemization, concentration, temperature, or solvent influence, but arises from steric effects of the amino acid moieties that lie closely together. Their optical rotation is very sensitive to the local surroundings. If a flexible alkyl chain is introduced between the amino acid moiety and the terminal group of the dendrimer the steric tension at the end groups is reduced and the optical activity is approximately constant from the first to the fifth generation.^[50b] The appearance of an induced circular dichroism by inclusion of certain dye molecules gave a remarkable indication of the presence of chiral information that was locked inside the dendritic box by 64 *N*-Boc-L-phenylalanine moieties.^[51c]

Sharpless et al. observed a specific rotation that increased with the generation number in polyether dendrimers devel-

oped by them with asymmetric 1,2-diol branch units and trimesinic acid as the core molecule (Figure 18).^[52] They discovered that the molar optical rotation is proportional to the number of monomer building blocks. Thus it is generated

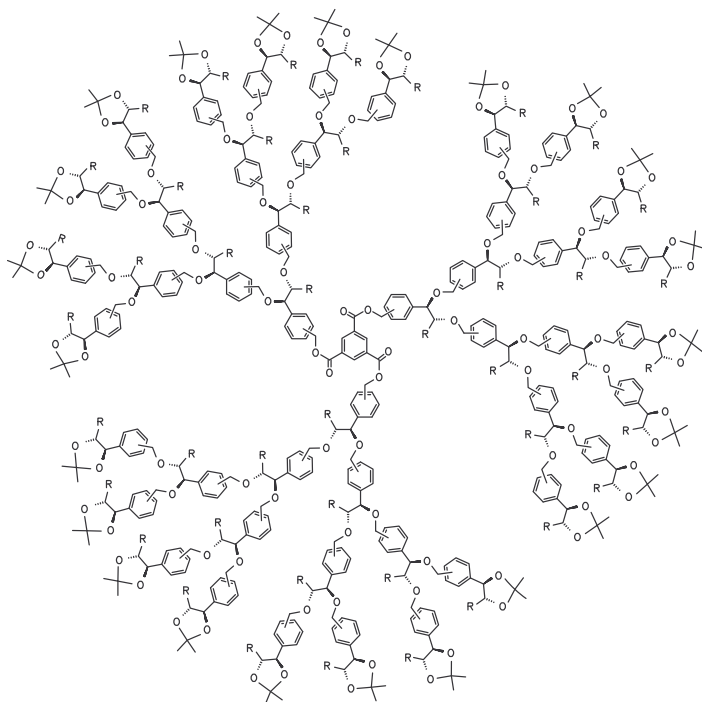


Figure 18. Dendrimers with chiral branch junctures. The optical rotation is higher with increasing generation.

by the chiral moieties and not by a conformational arrangement of the dendrimer skeleton. Analogous results have been obtained by Chow and Mak from dendrimers containing L- or D-threitol as a connector between the phloroglucinol branch juncture units (Figure 19).^[53] They also noted that in the presence of both enantiomers every D-threitol moiety exactly extinguishes the rotational effect of a L-threitol moiety.

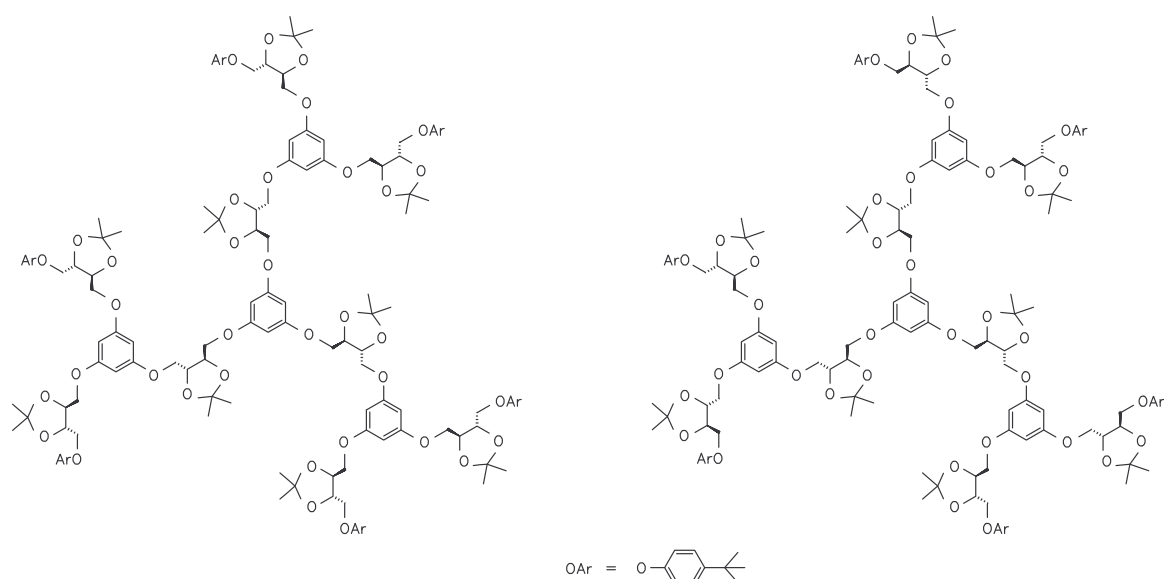


Figure 19. The rotational value can be extinguished by specifically incorporating both enantiomers of a chiral moiety.

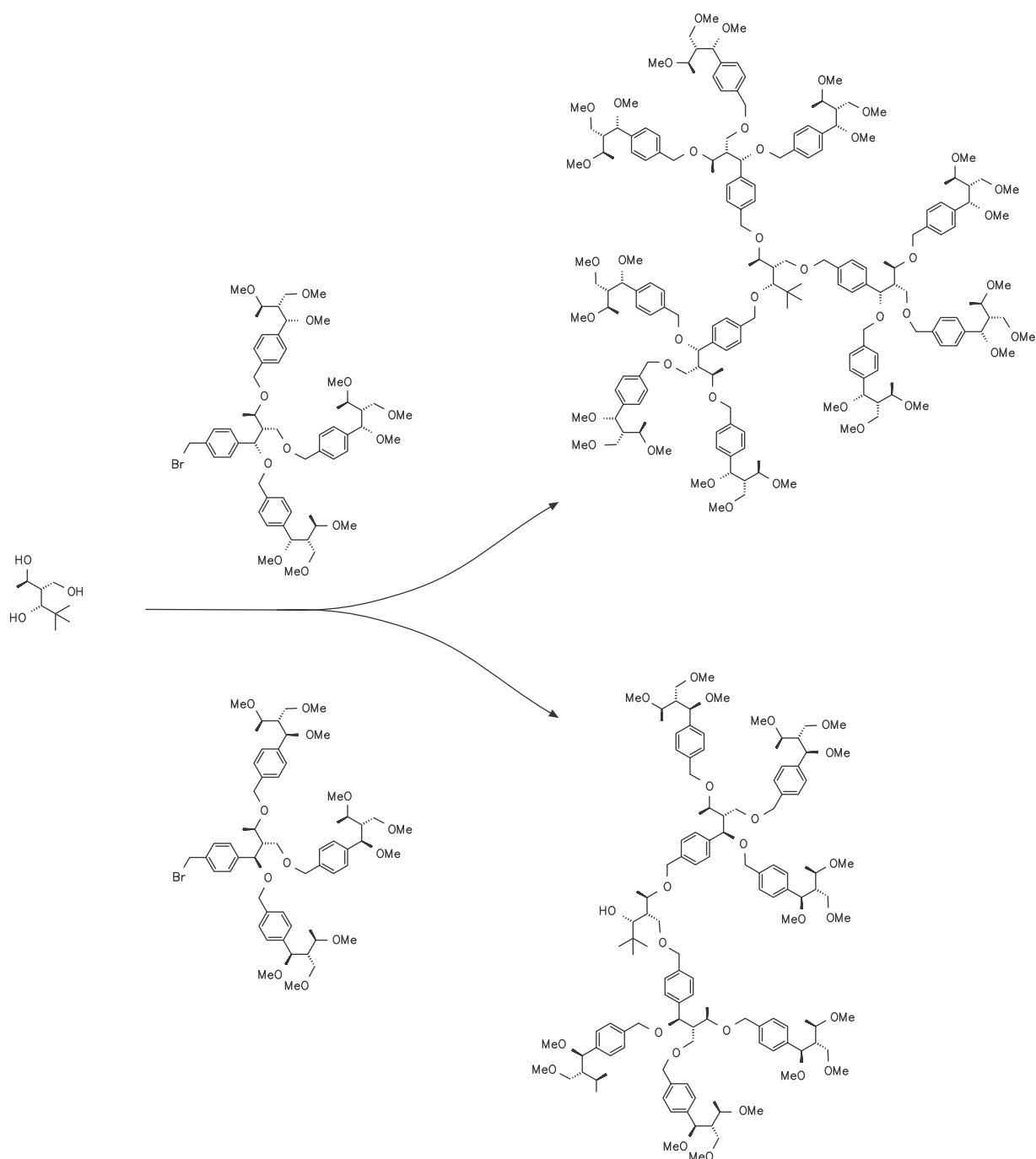


Figure 20. Diastereoselectivity in the synthesis of a chiral dendrimer.

Seebach et al. developed dendrimers based on a chiral tris(hydroxymethyl)methane derivative as the initiator core.^[54] Subsequently they attached Fréchet-type dendrons of zero to second generation directly to the core or separated from it by two different spacers.^[54a] For the direct anchoring, as well as for the use of a comparatively rigid spacer (*p*-xylylene), they observed a decrease in the rotational value with higher dendron generation, whereas an analogous compound with a flexible spacer (*n*-propyl) exhibited only an insignificantly low optical activity for all three generations. In successive works the same core molecule has been reacted with fully chiral, twofold and threefold branched den-

drons.^[54c, d] In both cases the first to third generation dendrons obtained this way show an inversed sense of optical rotation when going from one generation to the next higher one. This fact possibly indicates a conformationally chiral arrangement of the dendrons. In addition, when a threefold branch juncture with the opposite configuration on one of the three stereogenic centers is employed, a case of diastereoselectivity has been noticed (Figure 20). The corresponding second generation dendron could be attached successfully to the chiral triol initiator core only up to the doubly substituted adduct. A threefold reaction to prepare the complete dendrimer did not succeed.

The decreasing optical activity of a chiral initiator core with achiral dendryl substituents has also been studied by Meijer et al. (Figure 21).^[55] Initially they did not use a chiral core for constructing the dendrimer, but they tried to produce chiral information by coupling four dendrons of different generation to pentaerythritol.^[55a] They, however, failed to separate the racemic mixture chromatographically, so it was not possible to examine the optical properties. To avoid this problem the two enantiomers of a new dendrimer were synthesized by using the chiral glycerol derivative (*S*)-(+)-solketal as the core unit.^[55c] This was done by specifically tethering different generation Fréchet-type dendrons. Chiroptical analyses revealed no sign of optical activity for either enantiomer. Hence, these substances can be described as “cryptochiral”.^[50, 56] Obviously, the conformational flexibility and the small difference in the electron density of the dendryl substituents cause the optical activity to vanish. In addition, the geometry of the two enantiomers is also only slightly different. Surprisingly, Meijer and co-workers have successfully prepared an analogous molecule (Figure 21) in a chiral form by the introduction of sterically more demanding branch units (2,6-substitution at the benzene ring).^[55c] A small rotational value was measured for this molecule, and a weak signal appeared at low temperature in the CD spectrum and vanished at 30 °C. This might be a hint that the compound produced has a rigid structure as a consequence of the different branches and despite the low generation number. Because of this the molecule can take up a chiral form, though it is not favored at raised temperatures because of the higher flexibility.

The position of a chiral moiety that influences the chiroptical properties of the dendrimer has been studied by McGrath et al.^[57] First four dendrons that carried their chiral branch units at different positions in the skeleton were prepared through a combination of divergent and convergent synthetic methods (Figure 22).^[57a] In this synthesis compounds were also obtained that possessed a chiral moiety at the focal point of the dendron and with four different generations of Fréchet-dendrons. These did not show any remarkable difference in their molar rotations. Therefore, the size of the dendryl substituent has no distinct influence on the chirality of the individual moiety. In contrast, the molar rotation per chiral moiety rises significantly if this moiety is not located at the focal point, but is instead incorporated shell-like into the skeleton. This, however, is apparently a consequence of the position of the chiral moiety in the skeleton rather than to a conformational change in the molecule caused by it. The analysis of analogous dendrimers constructed only with chiral branch junctures and their comparison with low-molecular model compounds gave further instances of this thesis.^[57b] In fact, changes in the chiroptical properties with increasing generation number have been observed, but they were ascribed to constitutional changes from chiroptical data of model compounds. It was not possible to prove that a disturbance in the conformational equilibrium, so that a certain chiral conformation is preferred, was the cause of the particular optical properties.

All these results not only show that the position and the number of chiral moieties strongly influence the resulting

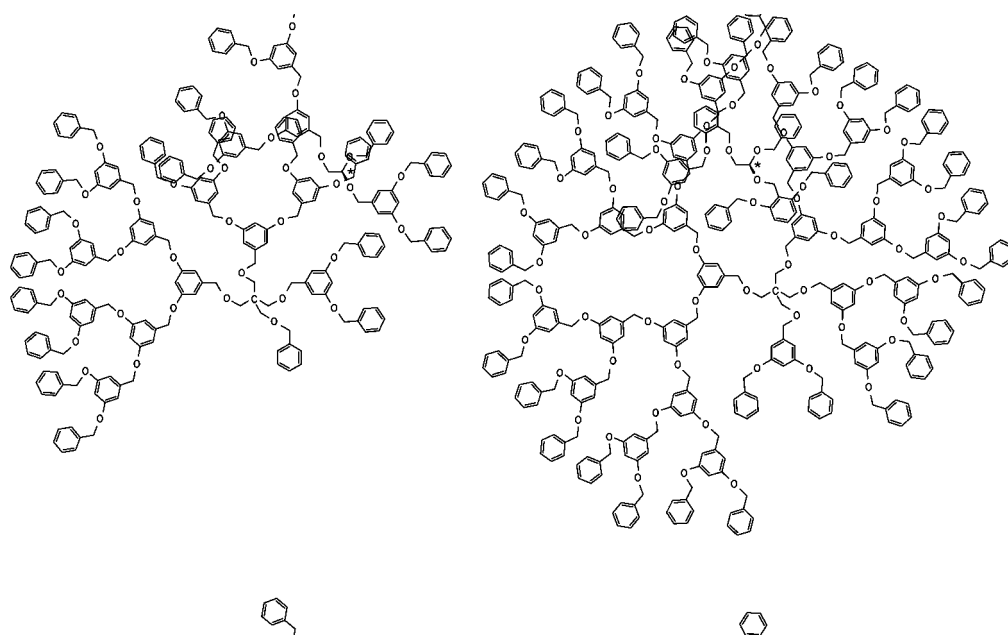


Figure 21. Dendrimers with a chiral core moiety and achiral dendritic substituents.

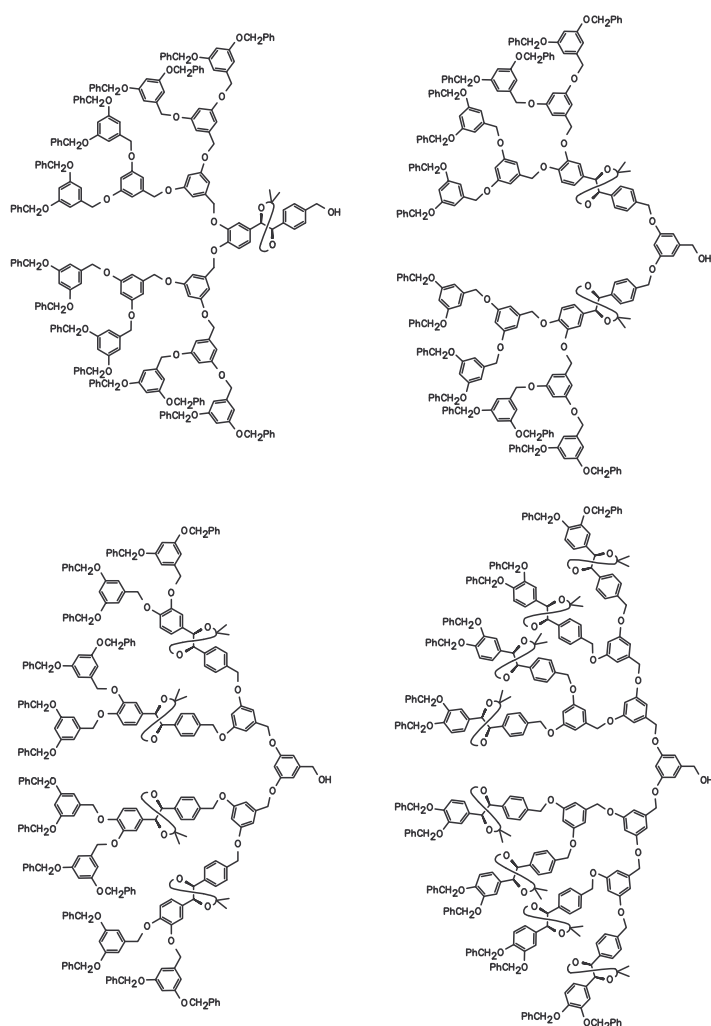


Figure 22. The position of a chiral moiety in the dendrimer skeleton significantly influences the chiroptical properties.

macroscopic optical properties of the dendrimer, but also that in all cases the optical properties are determined by the flexibility of the dendritic segments. In the search for new materials or enantioselective catalysts^[58] great effort has to be made to solve exactly the influence of chiral moieties on the conformational formation of the dendrimer skeleton.^[50]

7. Medicine and Diagnosis

To medicine dendrimers are substances whose possible applications as diagnostic or even therapeutic agents have been discussed and studied.^[59] On the molecular level one is particularly interested in the multiplication of certain functionalities to attain high activity by multiple interaction. Thus, saccharide clusters from natural carbohydrate building blocks can compete in recognition processes on the cell surface and intervene in specific adhesive phenomena to prevent bacterial or viral infection.

Meanwhile dendrimers have repressed polymers as skeleton building blocks for the development of carbohydrate clusters as a consequence of their exactly defined structure. In this field Roy et al. have done pioneering work on the

synthesis of the first lysine dendrons, which were substituted with up to 16 α -sialinic acid moieties.^[60a] They found that these compounds are highly active in inhibiting the hemagglutination of human erythrocytes by influenza viruses. For a better understanding of the multivalence and its role in carbohydrate–protein interactions, glycodendrimers with variable density and conformation of the active moieties have been prepared.^[60b, c] From these substances they noted that in general a larger carbohydrate–protein interaction occurred with an increase in the number of sialinic acid groups. In the same respect, Lindhorst and Kieburg developed an elegant method to react multiamino-functionalized dendrimers with different glycosyl isothiocyanates to form glycoside clusters, the carbohydrate epitopes of which can be varied as desired.^[61] Furthermore, the synthesis of a dendritic carbohydrate cluster with glucose as a central building block recently succeeded.^[62] Stoddart et al. reported on carbohydrate dendrimers with up to 64 peripheral groups, which were constructed convergently, divergently, or by coupling to a pre-prepared dendrimer skeleton.^[63]

Dendrimers have been tested as therapeutics in boron neutron capture therapy.^[64] This therapy represents a method for the treatment of presently incurable forms of cancer. The exceptionally high neutron capture cross-section of the ^{10}B isotope for thermal neutrons is used to produce a radiation energy that is lethal to the surrounding cells. The problem is that a compound as boron-rich as possible is required, which can be selectively coupled to molecules that recognize tumour cells. As well as the earlier studies on the synthesis of statistically boronated polymers, dendrimers with covalently attached boron atoms have been prepared.^[65] The first lysine dendron that specifically couples to proteins and carries 80 terminal boron atoms on the end groups has been developed by Moroder et al. (Figure 23).^[66] They succeeded in substituting the eight peripheral amino groups of the third generation dendron by a carboranyl amino acid and to conjugate the focal thiol group with an antibody fragment. First tests on this compound have given positive results.

Dendrimers have been applied therapeutically in gene therapy as well.^[67] “Vectors” are employed to exchange a defective gene by a functioning one by the transfer of the genes through the cell membrane into the nucleus. Although viruses and liposomes have been mainly used for this, PAMAM-dendrimers have also been tested.^[68] They turned out to be efficient transfection reagents and guaranteed the transfer of genetic material into mammalian cells. By means of activation by thermally generated structure defects a two to three times higher efficiency has been obtained so that the dendrimers do not necessarily have to be structurally perfect for this application. In the meantime a transfection reagent based on these studies is commercially available,^[69] and is applied in the research fields of molecular and cell biology.

The use of dendritic structures for diagnostic purposes is also based on the possibility of multiplying certain functionalities and hence achieving higher sensitivities. Dendritic substances have made crucial advances and have already been tested in preclinical studies, particularly in the field of contrast media for magnetic resonance. The magnetic resonance imaging (MRI) method^[70] serves to picture organs, blood

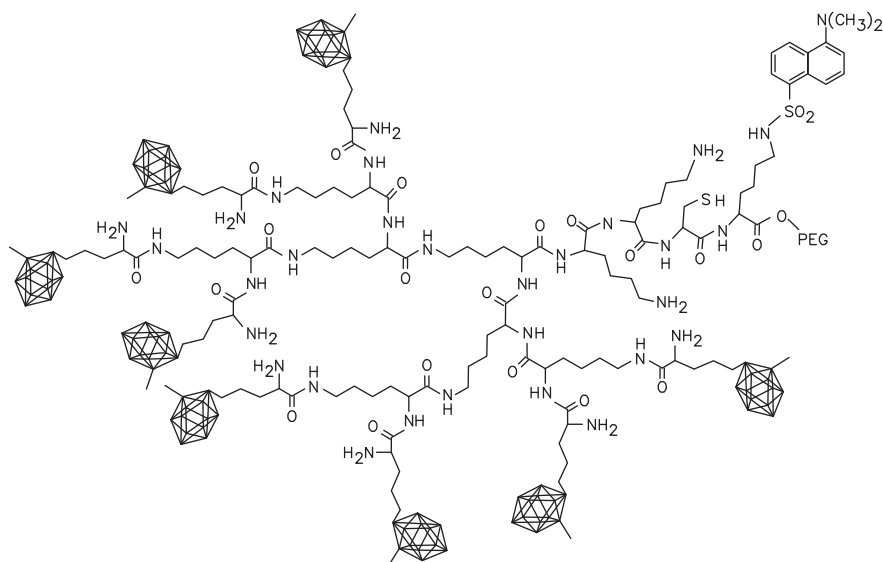


Figure 23. Lysine dendron with 80 boron atoms used in boron neutron capture therapy.

vessels, or tissue inside the human body by generating defined inhomogeneous magnetic fields that allow the nuclear resonance signal of water to be assigned to its place of origin and then to convert it into pictures. The relaxation time of the water protons are significantly shortened by application of the contrast media (paramagnetic metal cations) into the organ that is to be visualized and leads to a picture with a very good signal-to-noise ratio. However, clinically used contrast media, such as Magnevist (gadolinium salt of diethylenetriaminepentaacetic acid, DTPA),^[71] reveal a disadvantage. Immediately after intravenous application they diffuse into the extravascular area as low-molecular compounds. Therefore they are only partially suitable to picture the vessels. To prevent the diffusion higher-molecular weight compounds have been developed in which several gadolinium complexes are covalently bound to albumin,^[72] dextran,^[73] and polylysine^[74]. No preparation is on its way to clinical studies, however, because of bad renal elimination of the compounds. Several groups have turned to dendrimers as basic skeletons in their recent research,^[75] including a team from Schering AG who have developed the substance most suitable to date (Figure 24).^[76] This compound consists of a trimesinic acid central building block, to which second generation lysine dendrons with a total of 24 complexed gadolinium ions are anchored. Animal tests showed that the quantitative renal elimination and the higher intravascular retention time, which ensures an excellent signal-to-noise ratio, turn out to be the outstanding properties of this new contrast medium. The intravenous application of small doses of this dendrimer therefore promises a highly resolved and contrasted visualization of blood vessels (angiography, in particular coronar angiography).

8. Summary and Prospects

This review of topical works in the field of dendrimer chemistry demonstrates the great variety in this research field.

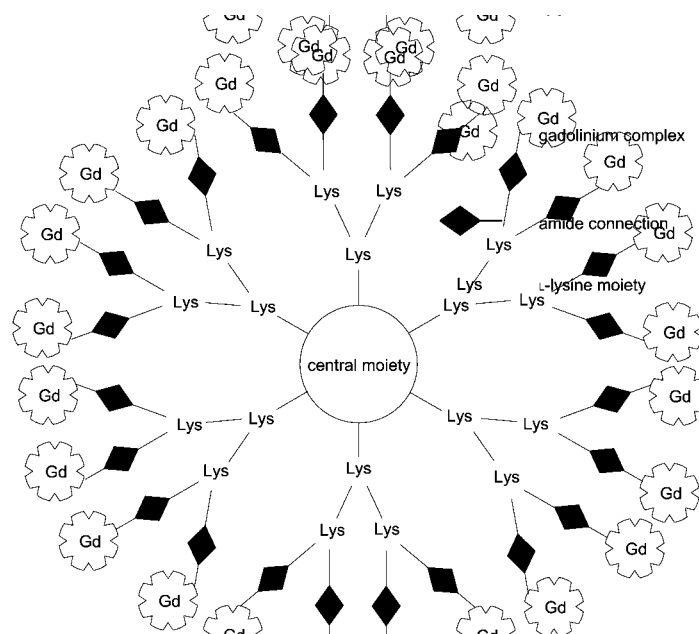


Figure 24. A dendrimer with 24 complexed gadolinium ions used as an MRI contrast agent.

nation of an exactly defined particle structure and a very high surface functionality is crucial, seem to successfully pass this cost–benefit analysis. In particular the works on transfection reagents and contrast media described in Section 7 verify this fact. On the other hand, the basic research on structurally perfect systems can examine more precisely and better

understand new effects and the knowledge obtained can be applied to compounds of lower structural perfection.

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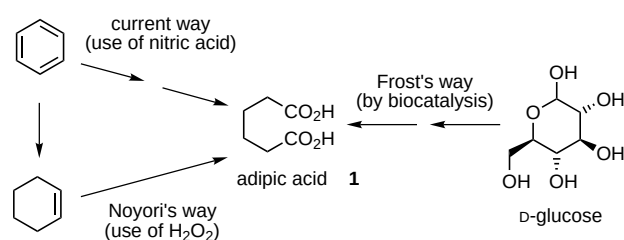
The Search for New Environmentally Friendly Chemical Processes

Carsten Bolm,* Oliver Beckmann, and Olivier A. G. Dabard

The environmentally conscious design and use of chemical processes focuses on minimizing hazards for human health and the environmental risks of chemical products and manufacturing.^[1] Because a high level of environmental protection can be highly cost-intensive, for instance by proper disposal of toxic waste, the development and implementation of such processes may also offer a competitive advantage from an economical point of view. Hence, the efforts to consolidate economic growth and ecological balance, often encompassed in the expression “sustainable development”, are becoming stronger in industry.

An example of a new, environmentally benign synthesis of a well-established intermediate in the chemical industry was recently presented by Noyori and co-workers. They disclosed a “green” route to adipic acid (**1**), the annual production of which runs up to 2.2 million metric tons worldwide.^[2]

The usual industrial synthesis of this compound, which is an intermediate utilized in the production of nylon-6,6, brings about a problematic oxidation step: cyclohexanone and cyclohexanol—both obtained from benzene—are oxidized by nitric acid (Scheme 1). Although efficient technologies to



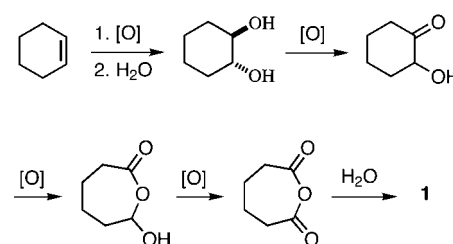
Scheme 1. Currently employed process for the manufacture of adipic acid (**1**) and environmentally friendly alternatives.

reduce the emission of nitrous oxide have been developed and implemented in industrial processes, the use of nitric acid as an oxidant still leads to a significant emission of N_2O , which is believed to play a role in the development of such phenomena as smog and acid rain, and which has been implicated in atmospheric ozone depletion and global warming. It is reckoned that N_2O emission from the nylon-6,6

production accounts for 5 to 8% of the total amount released by man.

In comparison to the traditional manufacture of adipic acid the alternative catalytic route proposed by Noyori et al. reveals several features that represent an improvement in terms of pollution prevention. First, the oxidant is now 30 percent hydrogen peroxide, a well-known “environmentally friendly” oxidant from which the sole by-product is water.^[3] Furthermore, the oxidation reaction is both solvent- and halide-free. An additional advantage of the synthetic route from Noyori et al. is the mild reaction conditions required as opposed to the high temperature and pressure that are involved in the manufacture with nitric acid.

The oxidation by H_2O_2 is made possible through the presence of a catalytic amount of a tungsten oxide along with a phase transfer catalyst. This catalyst system had been applied before in a biphasic manner in the oxidative transformations of olefins and alcohols.^[4] Now, these two previously studied reactions have been combined to give a new one whose mechanistic pathway is suggested to involve the epoxidation of cyclohexene in the first step and, after hydrolytic epoxide opening, two alcohol oxidations and a Baeyer-Villiger rearrangement. Subsequent hydrolysis of the resulting anhydride eventually affords the product (Scheme 2).



Scheme 2. Proposed reaction pathway for the synthesis of adipic acid (**1**) by oxidation of cyclohexene with 30% hydrogen peroxide.

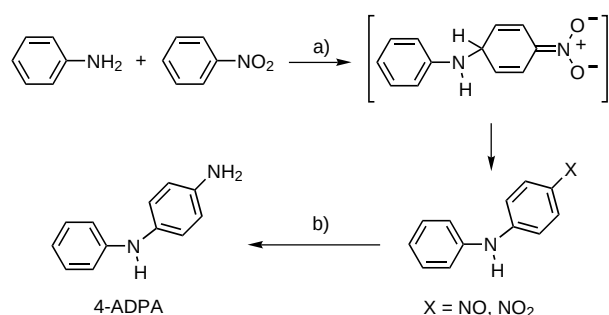
There are other routes to adipic acid (**1**), but none of these or the aforementioned ones eliminates both the problem of nonrenewable petroleum-based feedstocks and the risky use of toxic substances at the same time. A biocatalytic alternative elaborated by Frost and Draths opens up a new perspective: they were able to establish a thoroughly “green” synthesis of adipic acid (**1**) that used an *Escherichia coli* mutant to convert D-glucose into *cis,cis*-muconic acid,^[5] whose subsequent

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hydrogenation affords the desired adipic acid (Scheme 1). The presumably higher cost of this process notwithstanding, the microbial conversion of D-glucose derived from crops might turn out to be a competitive alternative route, for it circumvents the use of environmentally hazardous reagents as well as starting materials that stem from nonrenewable resources. To put it in other words, it avoids ever-rising costs for the handling of chemical waste and for precious nonabundant chemicals. However, scale-up and processing problems, and the need for an appropriate restructuring and adaptation of agricultural production and chemical industry provide obstacles to a switch of processes.

The United States Environmental Protection Agency (EPA), in an ongoing effort to give incentives for surmounting such barriers, presented one of its 1998 *Presidential Green Chemistry Challenge Awards* to Frost and Draths for their microbe-based synthesis. This prize is part of an EPA program that aims to promote the development and implementation of innovative chemical technologies that accomplish pollution prevention.^[6]

Another recipient of an EPA green chemistry award was Flexsys,^[7] which was honored for having found a novel strategy to 4-aminodiphenylamine (4-ADPA). This aromatic amine represents a key intermediate in the production of rubber antioxidants. Flexsys's current process for the preparation of 4-ADPA is based on the chlorination of benzene and requires the storage of large amounts of chlorine gas. None of the used chlorine, however, ultimately ends up in the final product, and significant quantities of an aqueous stream containing high levels of inorganic salts contaminated with organic compounds is generated. The treatment of this waste stream is awkward and expensive. Therefore, a more environmentally compatible access to 4-ADPA was sought. This was eventually achieved by utilizing a novel nucleophilic aromatic substitution reaction,^[8] which consists of a base-promoted direct coupling of aniline and nitrobenzene and avoids the intermediate use of halogenated reagents (Scheme 3). By



Scheme 3. New atom-efficient route to 4-ADPA, a key intermediate in the rubber chemicals family of antioxidants. a) Base; b) catalytic hydrogenation.

means of a nucleophilic attack of the anilide anion on nitrobenzene, a σ complex is formed first, which is then converted into 4-nitrosodiphenylamine and 4-nitrodiphenylamine by intra- and intermolecular oxidations. Catalytic hydrogenation finally affords 4-ADPA. Azobenzene, which is formed as a by-product, can be hydrogenated to aniline and thus recycled into the process. Switching to this alternative

synthetic strategy effects a dramatic reduction of chemical waste and waste water, as demonstrated by Flexsys in a pilot plant operation.^[9]

In the described novel route to 4-ADPA no extra leaving group or external oxidant is required, making this process environmentally benign and more efficient. In the light of the "atom economy" concept introduced by Trost^[10, 11] this synthesis comes closer to an ideal reaction. It uses atoms so efficiently that a higher possible number of atoms out of all the starting materials ends up in the desired product. According to Trost's concept the ideal chemical reaction is a simple and selective addition that does not need any further reagents except in catalytic amounts. Hence, by obeying atom economy in the design of chemical manufacture the amount of feedstocks used and the amount of waste produced may be reduced.

However, following the concept of atom economy and eliminating waste at the source will often be difficult to realize immediately. Apart from the fact that implementing a new process while phasing out an old one takes time, in many cases novel and more efficient synthetic routes are still to be found and developed. Despite these difficulties one can also modify traditional syntheses to meet the needs for more environmentally benign processes. Such an approach to pollution prevention will not completely overcome an established, yet environmentally inefficient synthesis, but it will seek to substitute hazardous chemicals currently used in it, recycle waste streams, or find milder reaction conditions.

For instance, supercritical carbon dioxide might be a suitable replacement for organic solvents since it combines such benefits as nontoxicity, nonflammability, and the elimination of solvent residues and waste.^[12, 13] Furthermore, homogeneously catalyzed reactions conducted in supercritical fluids (SCFs) exhibit increased selectivities as well as accelerated mass transfer and reaction rates as a consequence of the special physical properties such solvents are endowed with, and allow facile separation of reagents, catalysts, and products by selective precipitation.^[14] Beyond its use as a mere solvent, supercritical carbon dioxide can even serve as an abundant source of C_1 building blocks in chemical synthesis, with CO_2 fixation in the form of formic acid and its derivatives being possible by means of transition metal catalysis.^[15] In future its use in chemical processes that are still to be developed could eliminate toxic starting materials, which might, in addition, be derived from nonrenewable resources. Thus, supercritical carbon dioxide represents an attractive tool as to both resource substitution and a more efficient chemical technology.

We are convinced that the responsible care of industry and the increasing political concern about environmental issues will necessitate the use of "green" technologies in the long term: an assessment of the true value of pollution prevention will promote the implementation of emission- and waste-avoiding processes, and enforce the existing environmental partnership between chemical industry, academia, and governmental agencies.^[16]

German version: *Angew. Chem.* **1999**, *111*, 957–959

Stichwörter: arenes • homogeneous catalysis • oxidations • polyoxometalates • waste prevention

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Phosphate and Vanadate in Biological Systems: Chemical Relatives or More?

Winfried Plass*

The biological function of vanadium is well established and has been documented in several review articles.^[1,2] Of particular importance is the ability of vanadium to influence phosphate-metabolizing systems and the fact that vanadium is an inherent part of enzymatic active sites. For both types of vanadoenzymes known today, the vanadium-containing haloperoxidases and the vanadium nitrogenases from the nitrogen-fixing bacteria *Azotobacter*, functional analogues are found in nature which are either more widely spread or more efficient, for example the heme-containing haloperoxidases and the conventional nitrogenases with molybdenum cofactor, respectively. This immediately leads to the question of how these enzyme systems evolved and in particular whether the vanadium-containing enzymes known today are retained functional analogues, which withstood evolutionary forces. New insight concerning these questions may be gained on the

basis of newly found similarities for vanadate and phosphate in biological systems.

The widespread physiological effects of vanadium are mainly attributed to the similarity of vanadate(v) ions and phosphate ions. But there are also important differences between these two anions. At physiological pH values monovanadate is found as doubly protonated $[\text{VO}_2(\text{OH})_2(\text{H}_2\text{O})]^-$ species, whereas phosphate occurs in the monoprotonated form HPO_4^{2-} . This is also important for possible mechanisms of the transport systems for these two anions.^[3-5] In addition vanadium is easily reduced under physiological conditions to yield cationic species. The third difference is given by the pronounced ability of vanadium to adopt higher coordination numbers. The higher coordinative flexibility of vanadium can be deliberately used for the structural characterization of phosphate-metabolizing enzymes.

Recently the crystal structures of several stable enzyme aggregates of phosphatases with vanadate as transition state analogue have been reported. Interesting examples are the protein tyrosine phosphatases,^[6,7] which are involved in signal transduction mechanisms for controlling and regulating intracellular processes (e.g. the insulin receptor system)—in

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this context it is worth noting that vanadate complexes show insulin-mimetic effects.^[8] In these aggregates the vanadium center resides in a trigonal-bipyramidal environment and is linked to the protein through a single axially bound cysteine residue, whereas the oxygen atoms of the vanadate moiety are involved in a hydrogen-bonding network. A similar structure is found for the active site of rat prostatic acid phosphatase with the complexed transition state analogue vanadate^[9] (Figure 1, top). In this case the vanadium is linked to the protein through an axially bound histidine residue.

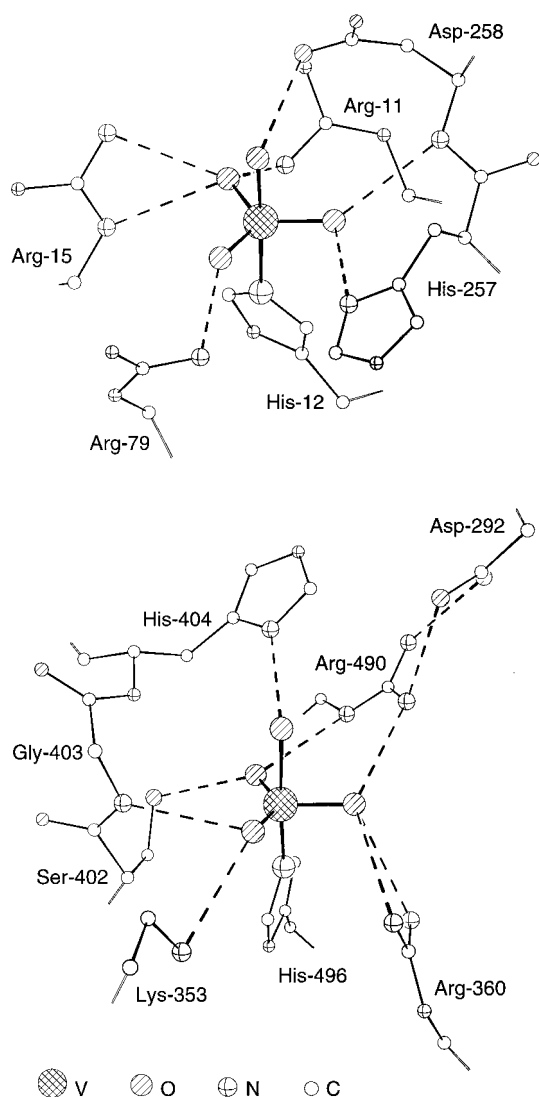


Figure 1. Top: Structure of the active site of the rat prostatic acid phosphatase with complexed vanadate.^[9] Bottom: Structure of the active site of vanadium haloperoxidase from the fungus *Curvularia inaequalis*.^[11] Hydrogen bonds are shown as broken lines.

Striking similarities are observed for the only structurally characterized vanadoenzyme known today, the chloroperoxidase of the fungus *Curvularia inaequalis* (Figure 1, bottom).^[10–12] As in the case of the rat prostatic acid phosphatase the vanadate is directly linked to the protein only through the axially bound histidine residue and is embedded in the protein through an extensive hydrogen-bonding network.

Vanadium-containing haloperoxidases (V-HPO) catalyze the two-electron oxidation of halide ions (X^-) to the corresponding hypohalous acids [Eq. (1)].^[13] HOX may



further react with a broad range of nucleophilic acceptors to form a diversity of halogenated compounds. These haloperoxidases are named after the most electronegative halide they are able to oxidize, and thus a vanadium chloroperoxidase (V-CPO) is able to oxidize chloride, bromide, and iodide ions.

Based on the recently published crystal structures of the native and *apo* forms as well as the peroxide- and azide-bound derivatives of this chloroperoxidase^[10–12] several interesting questions arise: 1) What is the electronic structure of the active site vanadate moiety, that is mono- or dioxovanadium(v) species (VO^{3+} or VO_2^+)? 2) How is the peroxo group or the chloride ion bound to the active site? 3) What is the influence of the protein environment upon the structure of the active site and the mechanism? 4) Does the *apo*-protein of this chloroperoxidase exhibit phosphatase activity as expected based on its structural similarities to phosphatases?

As Wever et al. recently showed, the *apo*-protein of the chloroperoxidase isolated from the fungus *Curvularia inaequalis* indeed exhibits phosphatase activity.^[14] Although it is obvious from the kinetic data that the active site of this V-CPO is not optimized for phosphatase activity, it is nevertheless clear that within the same supramolecular environment it is possible to catalyze two very different reactions such as those of haloperoxidases and phosphatases. Consequently this structural motif should also be observed for the active sites of other V-HPOs and phosphatases. Moreover, this structural similarity should give rise to genetic relationships as well. According to the sequence alignment of relevant enzymes nearly all amino acid residues coordinating vanadate in V-CPO isolated from *Curvularia inaequalis* (Lys353, Arg360, Ser402, Gly403, His404, Arg490, and His496 see Figure 1, bottom) are conserved in the V-HPOs sequenced thus far as well as within three families of acid phosphatases (Figure 2).^[14] Within this active site architecture the fully conserved histidine residues play an important role. His496 covalently links the vanadate to the protein, whereas His404 is essential for catalysis and is proposed to function as an acid–base acceptor group. Together with the observed phosphatase activity of the *apo*-protein of the V-CPO this indicates a very similar architecture of the active sites of these V-HPOs and acid phosphatases.

A dendrogram (Figure 2), based on the sequence alignment of 14 phosphatases and V-HPOs, indicates that these enzymes have divergently evolved from a common ancestor. In combination with the observed stability of V-CPO from *Curvularia inaequalis* to high concentrations of the substrate (H_2O_2) and the product (HOX), which would readily inactivate the heme-containing HPOs, this contradicts the introductory hypothesis that V-HPOs are retained enzymatic systems that have withstood evolutionary forces. This is further evidenced by the optimized functionality of V-CPO from *Curvularia inaequalis*, which is given by its recently proposed putative role as source for the starting material for

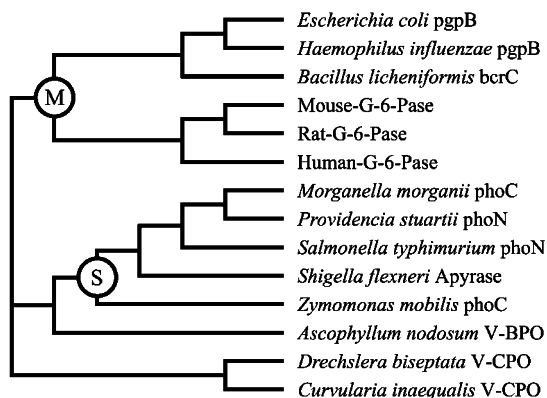


Figure 2. Dendrogram based on the alignment of 14 acid phosphatases and vanadium haloperoxidases (according to reference [14]). The group of membrane-bound phosphatases is marked with an M, and the group of soluble phosphatases is marked with an S. Abbreviations: pgpB, phosphatidyl glycerophosphate phosphatase B; bcrC, gene product from *Bacillus licheniformis*, no function has been assigned yet; G-6-Pase, glucose-6-phosphatase; phoC and phoN, class A bacterial acid phosphatase; Apyrase, ATP-diphosphohydrolase; V-BPO, vanadium bromoperoxidase; V-CPO, vanadium chloroperoxidase.

the production of a chemical weapon, the hydroxyl radical [Eq. (2)]. The hydroxyl radical in turn can be used by the fungus to damage the protective lignocellulose of plant tissues allowing the penetration of the plant cell for nutrient acquisition.^[15]



It is remarkable, however, that for another class of acid phosphatases, that is exemplified by the rat prostatic acid phosphatase (see Figure 1, top), no sequence similarity is found with the enzymes given in the dendrogram of Figure 2. Nevertheless the observed structural similarity of the active site, obvious from Figure 1, indicates that a convergent evolution seems to have occurred with respect to these enzyme systems.

Besides the interesting evolutionary aspects of these enzymes, there are also important implications for both research in the acid phosphatase and in the V-HPO field. A first remarkable example for this—in particular from an anthropocentric point of view—is the structure of mammalian glucose-6-phosphatase (G-6-Pase). This enzyme catalyzes the last step in both gluconeogenesis and glycogenolysis and as such it is the key enzyme in glucose homeostasis. G-6-Pase deficiency is the cause of glycogen storage disease type 1 (von Gierke disease) and is characterized by severe clinical manifestations such as hypoglycemia, kidney enlargement, or growth retardation, which usually cause an early death of the patients.

The recently published model for the membrane topology of human G-6-Pase^[16] is not consistent with the results outlined above and had to be modified accordingly as depicted in Figure 3.^[17] The active site residues of G-6-Pase identified by sequence alignment are all situated on the same side of the membrane and are located within the helices II–V. In this new model the histidine residue His176 is the nucleophile that forms the phosphohistidine enzyme-sub-

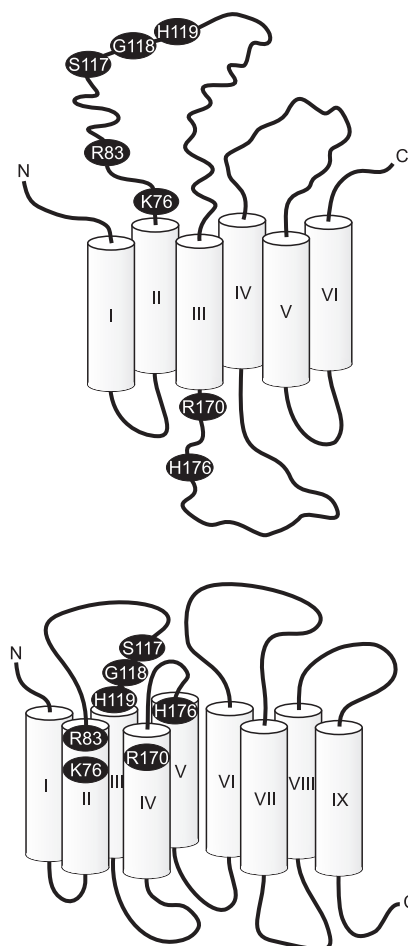


Figure 3. Membrane topology models for glucose-6-phosphatase. Top: Current six transmembrane-helix topology model.^[16] Bottom: Newly proposed nine transmembrane-helix topology model.^[17] Putative G-6-Pase active site residues are depicted as black ovals.

strate intermediate. The phosphate moiety is positioned by interaction of the negatively charged oxygen atoms with the positively charged amino acids Lys 76, Arg 83, and Arg 170. In analogy to the active site of V-CPO the residues Ser 117 and Gly 118 may also donate hydrogen bonds and the histidine group His 119 may provide the proton needed to liberate the glucose moiety.

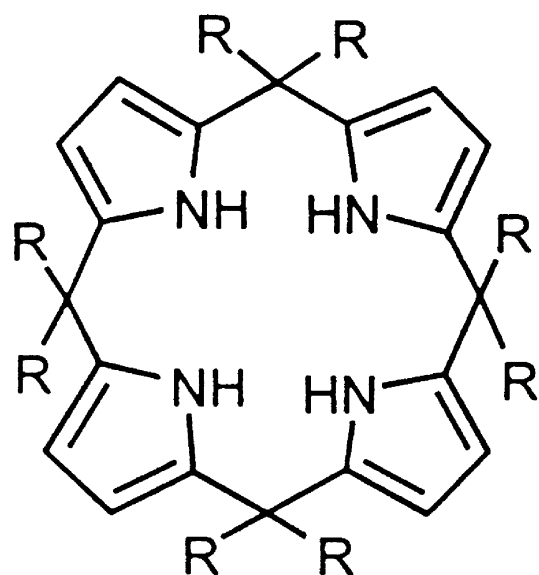
As shown by this first example, the common architecture of the active sites of the vanadium-containing peroxidases and the aligned acid phosphatases has important implications that even reach into seemingly independent fields of research.

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Stichwörter: bioinorganic chemistry • haloperoxidases • phosphatases • phosphorus • vanadium

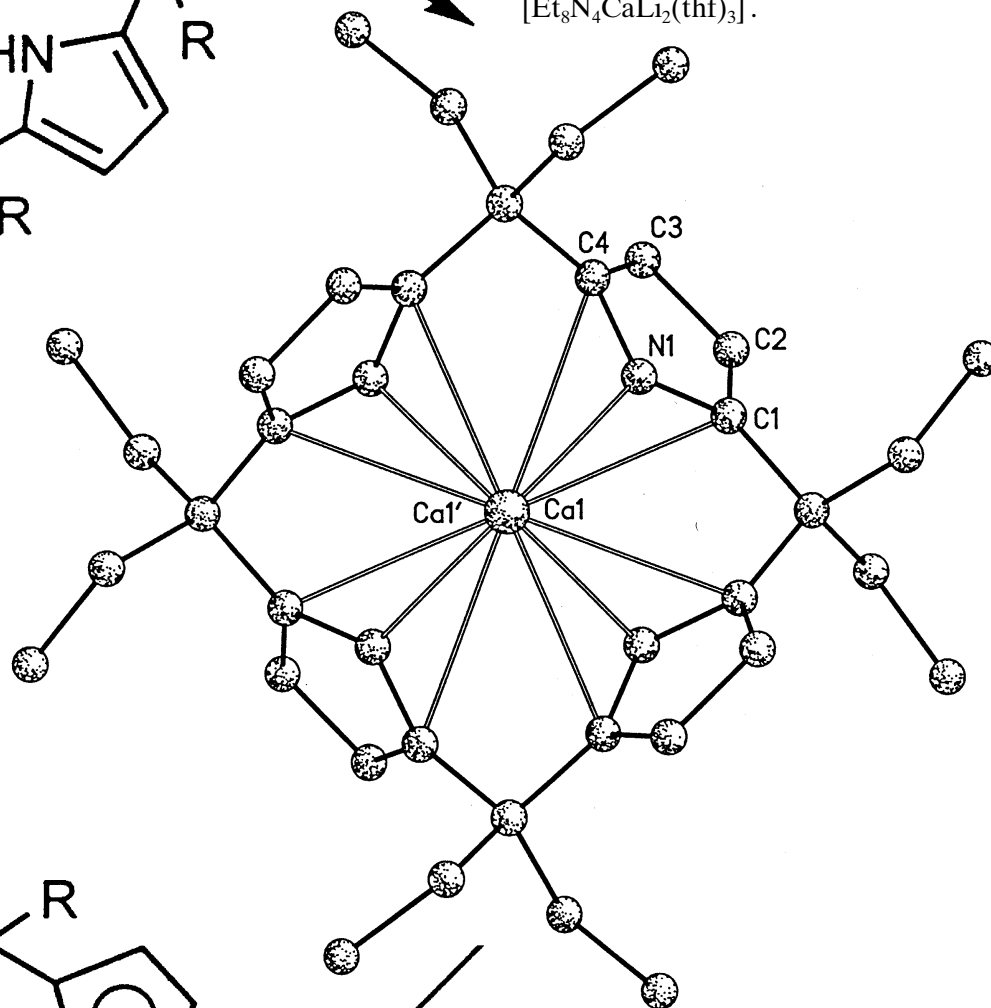
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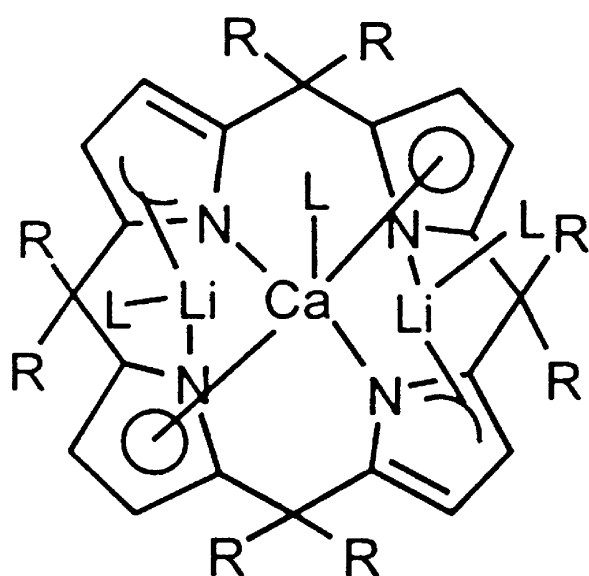


Ca_{act}

The reaction of *meso*-octaethylporphyrinogen ($\text{Et}_8\text{N}_4\text{H}_4$) with calcium metal provided an unprecedented dimetallic complex (see the crystal structure). Further reaction with the lithium–porphyrinogen derivative [$\text{Et}_8\text{N}_4\text{Li}_4(\text{thf})_4$] led to transmetalation and formation of the calcium–lithium–porphyrinogen complex [$\text{Et}_8\text{N}_4\text{CaLi}_2(\text{thf})_3$].



More information is given on the following pages.



[$\text{Et}_8\text{N}_4\text{Li}_4(\text{thf})_4$]

This synthesis emphasizes the role of porphyrinogen as a possible binding cavity for alkali and alkaline earth metal ions and shows the relevance of the solvation of such ions through π interactions with electron-rich aromatic fragments.

The π Complexation of Calcium inside the *meso*-Octaethylporphyrinogen Tetraanion Cavity**

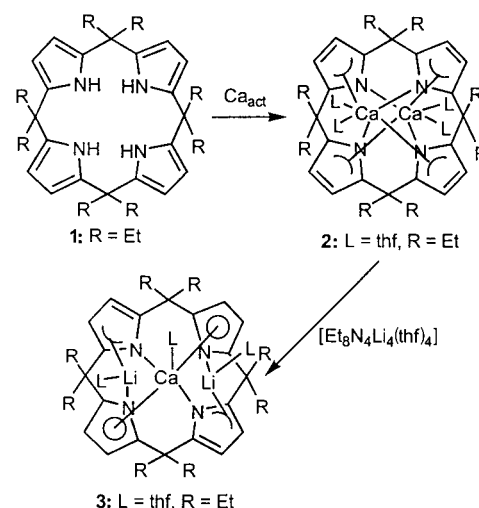
Lucia Bonomo, Olivier Dandin, Euro Solari, Carlo Floriani,* and Rosario Scopelliti

Solvation of alkali and alkaline earth metal cations by a π cavity is a quite remarkable phenomenon in the context of the role played by cation– π interactions in chemistry and biology.^[1–3] Three dimensionally shaped cavities functioning for the π solvation of alkali or alkaline earth metal cations are quite rare. A limited number of examples comes from the transition metal calix[4]arene chemistry,^[4, 5] where the transition metal shapes the cavity, thus encapsulating alkali metal cations, even though the alkali metal cation–oxygen interaction remains by far the strongest one. The macrocyclic tetraanion derived from *meso*-octaalkylporphyrinogen, owing to the presence of *meso* sp^3 -hybridized carbon atoms, is particularly suited to arrange the pyrrolyl anions to function as η^3 or η^5 binding sites.^[6] Such bonding modes allowed the development of novel aspects of organometallic chemistry based on *meso*-octaalkylporphyrinogen as ancillary ligand.^[7] In some of those metal–porphyrinogen complexes the pyrrolyl anions function also as η^1 , η^3 , or η^5 binding sites outside the cavity for lithium or sodium counter cations.^[7]

We report here how all four pyrrolyl anions function together as π binding sites for alkali or alkaline earth metal cations inside the porphyrinogen cavity. The synthetic method we devised, in order to avoid any other ligand for the alkali or alkaline earth metal ion,^[8] makes use of the metal itself for the metalation of porphyrinogen. We addressed our attention to the calcium atom, among other reasons, because of the possible relevance of π solvation of this cation in biological-type systems,^[1] and because of the lack of synthetic and structural information on Ca macrocycles which do not contain oxygen donor atoms.^[8, 9]

The reaction of *meso*-octaethylporphyrinogen (**1**, $\text{Et}_8\text{N}_4\text{H}_4$)^[10] with calcium metal^[11] in the presence of naphthalene led to the synthesis of the unprecedented dimetallic complex **2** ($[\text{Et}_8\text{N}_4\text{Ca}_2(\text{thf})_4]$) containing two Ca^{2+} ions (Scheme 1). The further reaction of **2** with the lithium–porphyrinogen derivative $[\text{Et}_8\text{N}_4\text{Li}_4(\text{thf})_4]$ ^[6a] led to transmetalation with the formation of the calcium–lithium–porphyrinogen complex **3** ($[\text{Et}_8\text{N}_4\text{CaLi}_2(\text{thf})_3]$).

Complexes **2** (Figure 1)^[12] and **3** (Figure 2) have quite similar structural features. In both compounds the porphyrinogen tetraanion has a 1,3 alternate conformation (two N atoms “up”, two N atoms “down”). The two calcium cations are located on opposite sites of the average N_4 plane in **2**



Scheme 1. The synthesis of complexes **2** and **3**. Ca_{act} indicates active calcium.

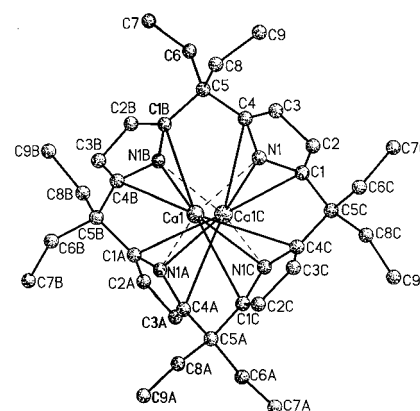


Figure 1. An XP^[13] drawing of complex **2**. Selected bond lengths [Å] and angles [°]: $\text{Ca}-\eta^3(\text{Pyr})$ 2.713(2), $\text{Ca}-\eta^1(\text{Pyr})$ 2.634(2), $\text{Ca1} \cdots \text{Ca1C}$ 3.241(1); $\eta^3(\text{Pyr})-\text{Ca}-\eta^3(\text{Pyr})$ 125.97(7). $\eta^3(\text{Pyr})$ indicates the centroid of the pyrrole ligand. The letters A, B, and C denote the following symmetry operations: $A = -x + 1, -y + \frac{1}{2}, z$; $B = -y + \frac{3}{4}, x - \frac{1}{4}, -z + \frac{3}{4}$; $C = y + \frac{1}{4}, -x + \frac{3}{4}, -z + \frac{3}{4}$. Dashes indicate the η^1 bonding of the two metals. Ca1, N1, N1A are “up”; Ca1C, N1B, N1C are “down”. The thf molecules have been omitted for clarity.

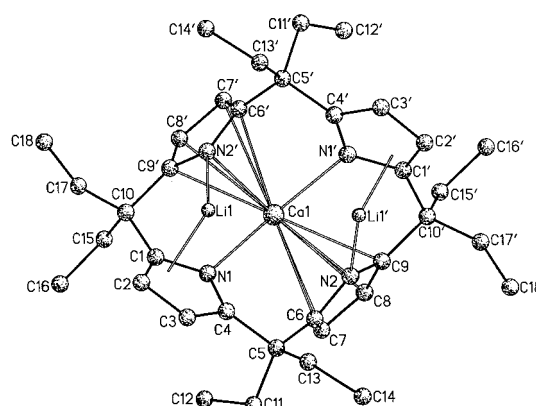


Figure 2. An XP^[13] drawing of complex **3**. Selected bond lengths [Å] and angles [°]: $\text{Ca}-\eta^5(\text{Pyr})_{\text{av}}$ 2.581(2), $\text{Ca}-\eta^1(\text{Pyr})_{\text{av}}$ 2.467(2), $\text{Li}-\eta^3(\text{Pyr})_{\text{av}}$ 2.069(5), $\text{Li}-\eta^1(\text{Pyr})_{\text{av}}$ 2.064(5); $\eta^3(\text{Pyr})_{\text{av}}-\text{Ca}-\eta^3(\text{Pyr})_{\text{av}}$ 165.95(7). $\eta^3(\text{Pyr})_{\text{av}}$ indicates the centroid of the pyrrole ligand. Letters A and B correspond to the following symmetry operations: $A = -x + \frac{1}{2}, y, -z + \frac{1}{2}$, $B = -x + \frac{1}{2}, y, -z + \frac{1}{2}$. The thf molecules have been omitted for clarity.

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(distance $\pm 1.621(1)$ Å), while the calcium cation is opposite to the two lithium cations in complex **3** with respect to the N_4 average plane (out-of-plane distances: $Ca1 \cdots N_4 - 1.256(2)$, $Li \cdots N_4 + 1.635(4)$, $Ca2 \cdots N_4 + 1.242(2)$, $Li \cdots N_4 - 1.593(4)$ Å). In complex **2** each pyrrolyl anion displays $\eta^3:\eta^1$ bonding modes and bridging of the two calcium ions; thus, each calcium ion is η^3 -bonded ($Ca-\eta^3(C1,N1,C4)$ 2.713(2) Å) to a pair of *trans*-pyrroles and is η^1 -bonded ($Ca-N$ 2.634(2) Å) to the other pair. The two calcium ions force the porphyrinogen to form a dinucleating cavity and to make available the maximum number of binding sites inside the cavity, in the case of complex **2**, in the form of four 2-azaallyl fragments. The binucleating cavity also forces the calcium ions to experience a very close proximity ($Ca \cdots Ca$ 3.241(1) versus $Ca \cdots Ca$ 3.947 Å in calcium metal).^[14]

In complex **3**, with less steric constraints, the calcium atom is η^5 -bonded ($Ca1 \cdots Pyr(\text{centroid})_{av}$ 2.581(2) Å; Pyr = pyrrole) to the *trans*-pyrroles, giving rise to a bent cyclopentadienyl-type structure ($Pyr-Ca1-Pyr$ 165.95(7)°),^[15] and η^1 -bonded to the other pair of pyrroles ($Ca1-N_{av}$ 2.467(2) Å), which in turn function as η^3 -binding sites for the two lithium cations ($Li-\eta^3(N1,C1,C2)$ 2.069(5) Å). In both compounds the calcium and lithium atoms complete their coordination sphere with thf molecules. The η^3 and η^5 bonding modes of the pyrrolyl anions have been decided on the basis of the structural parameters and the arbitrary decision that the two $Ca \cdots C$ distances greater than 3.0 Å in complex **2** should be not taken into account as significant interactions. In addition, such parameters are hardly comparable to those of existing structures since they are rather unique. The only comparison could possibly be made with the cyclopentadienyl derivatives,^[14] though the pyrrolyl anion in a macrocyclic structure does not have the same function as the Cp ligand. In the latter case, the $Ca \cdots C$ distances are significantly shorter (in these organocalcium compounds, the average metal-carbon bond lengths range from 2.62(2) to 2.73(3) Å).^[15b,c]

This synthetic and structural report emphasizes the role of porphyrinogen as a possible binding cavity for alkali and alkaline earth metal ions. Furthermore, it shows how relevant the solvation of such ions can be using π interactions with electron-rich aromatic fragments.

Experimental Section

2: A suspension of active calcium^[11] (27.7 mmol) in thf (300 mL) was added to a solution of **1** (7.03 g, 13.0 mmol) in thf (150 mL). The white suspension which suddenly formed was stirred for 2 h, and then the solvent was removed under vacuum. The solid (73%) was recrystallized from thf/*n*-hexane. Crystals used for the X-ray analysis^[12] contain an additional molecule of thf (**2**·thf). ¹H NMR ([D₆]benzene, 200 MHz, 298 K): δ = 6.19 (s, 8H; C₄H₂N), 3.45 (m, 8H; thf), 2.23 (m, 8H; CH₂), 1.99 (m, 8H; CH₂), 1.23 (m, 8H; thf), 0.92 (t, J = 7.32 Hz, 24H; CH₃); elemental analysis calcd for **2** (C₅₂H₈₀Ca₂N₄O₄): C 68.98, H 8.91, N 6.19; found: C 68.44, H 8.82, N 6.11.

3: A solution of [Et₃N₄Li₄(thf)₄]^[6a] (3.22 g, 3.8 mmol) and **2** (3.44 g, 3.8 mmol) in thf (300 mL) was heated under reflux for 24 h. The solution was evaporated to dryness, and the white solid (85%) recrystallized from thf/*n*-hexane. Crystals for the X-ray analysis contain only three thf molecules. ¹H NMR ([D₆]benzene, 200 MHz, 298 K): δ = 6.26 (s, 8H; C₄H₂N), 3.44 (brs, 8H; thf), 2.13 (q, J = 7.32 Hz, 8H; CH₂), 2.04 (q, J = 7.32 Hz, 8H; CH₂), 1.31 (brs, 8H; thf), 1.05 (t, J = 7.32 Hz, 12H; CH₃), 0.98

(t, J = 7.32 Hz, 12H; CH₃); elemental analysis calcd for **3**·thf (C₅₂H₈₀CaN₄·Li₂O₄): C 71.04, H 9.17, N 6.90; found: C 71.07, H 9.01, N 6.90.

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Formation of Giant Liposomes from Crystalline Complexes of Monoalkylammonium Surfactants and 4-Hydroxybiphenyl

Norihiro Yamada,* Mayumi Iijima, Kulthida Vongbupnimit, Keiichi Noguchi, and Kenji Okuyama

Single-chain alkylammonium halides are well-known cationic surfactants that form micelle structures in water. A micelle is a small globular aggregate containing low numbers of surfactants which does not transform into other larger aggregates, such as a liposome, under normal conditions. On the other hand, a low-viscosity micelle solution sometimes transforms into a highly viscous solution when a certain aromatic compound, for example the salicylate ion, is added.^[1] Transmission electron microscope (TEM) pictures reveal that the viscous solutions contain rodlike structures.^[2] Recently, several investigators^[3–5] and some of us^[6] reported that ammonium surfactants form crystalline complexes with various aromatic compounds. These results are suggestive of the formation of an aggregate larger than a micelle from a binary mixture of an ammonium surfactant and an aromatic compound. We thus studied the aggregation behavior of several crystalline complexes in water, and found that the complexes formed giant liposomes on the supermicron scale. The giant liposomes exhibited a dynamic morphological transformation in a manner similar to that observed for aqueous biolipids^[7] and for aqueous dialkylammonium salts which form bilayer structures.^[8]

Dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB), cetyltrimethylammonium chloride (CTAC), and cetyltrimethylammonium bromide (CTAB) were used in the study as typical micelle-forming surfactants. These surfactants dissolved well in water despite the differences in chain length and/or kind of counter anion, giving a foamy and transparent solution with low viscosity. We observed no aggregate in the aqueous solutions (1–400 mM) using an optical microscope equipped with a dark-field condenser; therefore, small globular micelles should have been formed. We then examined binary mixtures of the surfactants and aromatic compounds, namely, biphenyl, 2-naphthol, 4-hydroxybiphenyl, and 4,4'-dihydroxybiphenyl. These arenes can form crystalline complexes with the previously mentioned surfactants when they are ground with an agate mortar and pestle or when the mixture of the aromatic compound and the surfactant is recrystallized from a suitable organic solvent.^[3–6] The crystalline complexes are divided into guest-dependent complexes, whose thermal behavior is very similar to that of the guest crystal,^[6c] and host-dependent complexes, whose thermal behavior is very similar to that of the host crystal.^[6b] All of the crystalline

complexes except for the DTAB/4-hydroxybiphenyl combination form either a guest- or a host-dependent complex (Table 1). The DTAB/4-hydroxybiphenyl combination can form both types of complexes depending on the ratio that is mixed.

Table 1. Crystal types and morphology in aqueous crystalline complexes.^[a]

	DTAX		CTAX	
	X = Cl [–]	X = Br [–]	X = Cl [–]	X = Br [–]
biphenyl	N, no data	N, no data	N, no data	N, h-d (2:1)
4-hydroxybiphenyl	L, g-d (1:1)	L, g-d (1:1)	L, g-d (1:1)	LC, no data
4,4'-dihydroxybiphenyl	C, no data	C, h-d (2:1)	C, h-d ^[b]	C, no data
2-naphthol	L, g-d (1:1)	L, no data	L, no data	LC, no data

[a] The surfactant and the aromatic compound were mixed in a mortar in a 1:1 and a 2:1 molar ratio. The aggregate morphology was unchanged with the mixing ratio, except for the case of DTAB/4-hydroxybiphenyl. This mixture formed a host-dependent complex when the DTAB/4-hydroxybiphenyl ratio was 2:1. At a mixing ratio of 1:1, crystallites were observed together with liposomes (see text). N: no crystallites and no liposomes were observed, L: liposomes were observed, LC: liposomes and crystallites were observed; h-d: host dependent, g-d: guest dependent. [b] CTAC:4,4'-dihydroxybiphenyl:H₂O = 2:1:2.

Three different methods were employed for the observation of morphology (see the Experimental Section). Method I was used in general, whereas the other two methods were used to observe the dissolving process of the complexes or the aromatic compounds in water and the surfactant solution, respectively. The results of the microscopic observations are summarized in Table 1. Figures 1 and 2 show typical microscope pictures of the aqueous DTAC/4-hydroxybiphenyl complex. Liposomes with various shapes were observed (Figure 1a). Large liposomes were seen when the sample was observed according to method II (Figure 1b). The diameter of these liposomes was in the range of 10–50 µm, and the largest liposome has a diameter of greater than 100 µm. The concentration strongly affected the liposome formation. In the case of DTAC/4-hydroxybiphenyl, we observed crystallites instead of liposomes in dilute solutions at concentrations below 10 mM, crystallites and liposomes at 10–20 mM, and only liposomes above 20 mM. The liposomes were maintained without precipitates in the 20-mM solution for more than several months, and, hence, the liposomes were stable at a relatively high concentration. However, it is unusual that high water content favors the crystallite instead of the liposome. Although the phenomenon may have a thermodynamic explanation, it is still under experimental study.

A liposome structure was also observed when 4-hydroxybiphenyl was added to the surfactant solutions (method III). Figures 1c and 2 show the dissolving process of 4-hydroxybiphenyl in the DTAC solution. Myelin figures immediately appeared when the 4-hydroxybiphenyl crystals were saturated with about 30 mM DTAC micelle solution (Figure 1c). The myelin figures grew into long tubular structures (Figure 2a), which transformed into liposomes (Figure 2d) via a rosarylike structure (Figures 2b and 2c). The morphological dynamics is exactly the same as that observed in bilayer membrane systems such as aqueous lecithin^[7] and aqueous dialkylammonium bromide.^[8] The other surfactant/4-hydroxybiphenyl systems and surfactant/2-naphthol also exhibited the same

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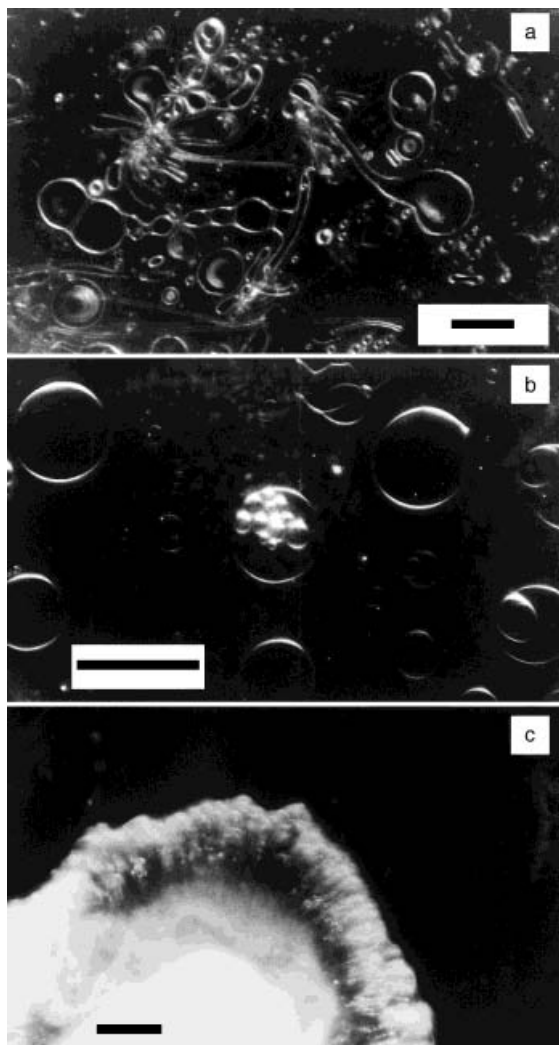


Figure 1. Dark-field light microscope pictures of liposomes formed from the DTAC/4-hydroxybiphenyl complex; scale: 50 μm . a) A 20 mM aqueous solution was used (method I); b) liposomes with a diameter of 10–50 μm were observed at room temperature (method II); c) the crystal of 4-hydroxybiphenyl was swollen, and a myelin figure appeared immediately after mixing (method III).

morphological dynamics. However, the stability of the liposomes depended upon the kind of surfactant because the surfactants with a shorter chain (DTAC and DTAB) maintained a liposome structure without precipitation at 20 mM for more than several months, whereas those with a longer chain (CTAC and CTAB) produced precipitates after incubation for one day at room temperature.

No liposome was observed for the surfactant/biphenyl and the surfactant/4,4'-dihydroxybiphenyl crystalline complexes. Although the former dissolved in water, we observed no aggregate using an optical microscope (method I). The crystals of the biphenyl disappeared within one day upon mixing with a surfactant solution (method III). The surfactant/4,4'-dihydroxybiphenyl crystalline complex did not dissolve in water, and the 4,4'-dihydroxybiphenyl crystals remained intact in the surfactant solution when method III was employed. These results suggest that the chemical structure of the guest molecule is of essential importance.

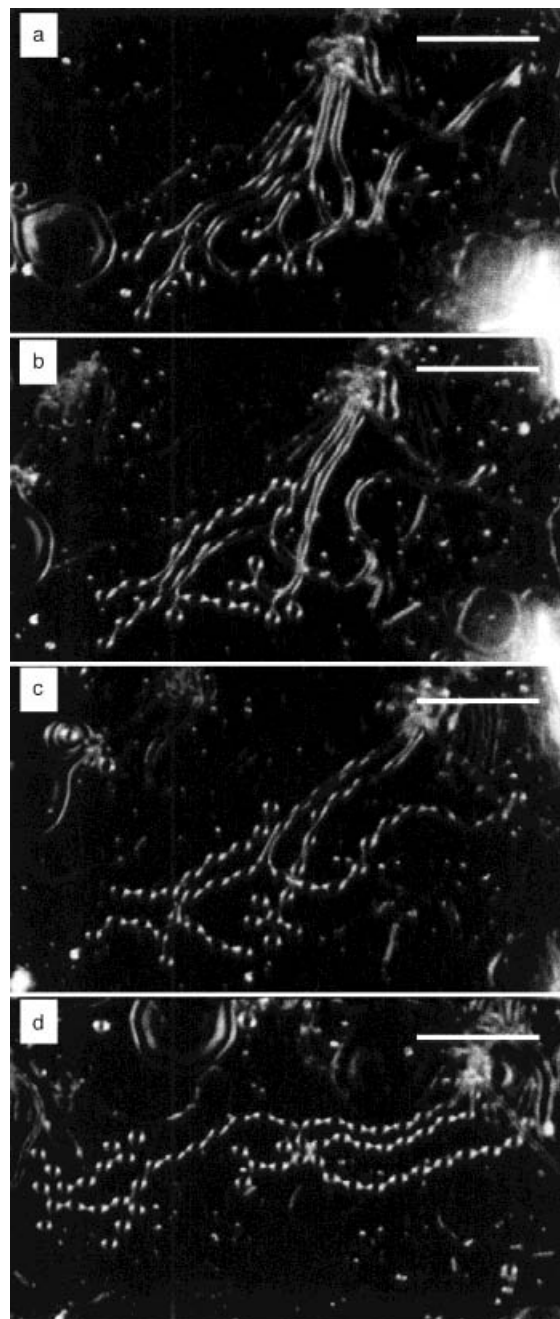


Figure 2. Dynamic morphological transformation of 4-hydroxybiphenyl promoted by the addition of aqueous CTAC solution (ca. 30 mM; method III); scale: 25 μm . a) A flexible tube isolated from the swollen crystal of 4-hydroxybiphenyl (b); c) a rosarylike aggregate transformed from the flexible tube shown in (a); d) small liposomes which appeared from the rosarylike aggregate shown in (c).

The spontaneous liposome formation requires the presence of a phenol group.

Bilayer structures are predominantly formed from double-chain ammonium surfactants, whereas single-chain ammonium amphiphiles form micelle structures.^[9] However, the single-chain ammonium amphiphile can form bilayer vesicles upon mixing with an anionic micelle surfactant,^[10] because the resultant ion pair possesses two hydrophobic chains. In the present case, if a dissociated phenolate group was produced, it could interact with the cationic ammonium head group and

should form a pseudo-double-chain amphiphile similar to the cation–anion pair surfactant, because the hydroxy proton of the 4-hydroxybiphenyl dissociates within the hydrophilic region where the hydroxide anions are condensed. However, liposome formation was observed in 0.1M HCl, in which the dissociation of the phenol group should be prevented. Furthermore, the bromide anion was detected, and the dissociated phenolate was not observed in the single crystal from the aqueous solution of the CTAB/4-hydroxybiphenyl mixture. The crystal structure was entirely the same as that of the crystal recrystallized from organic solvents. Therefore, electrostatic interactions are not the essential factor in the spontaneous liposome formation.

The liposome formation is strongly related to the structure of the crystalline complexes, because a liposome structure was formed only from the guest-dependent complexes. The structural difference between the host- and guest-dependent complexes is in the packing arrangement of aromatic molecules (Figure 3). In the guest-dependent complexes, the long

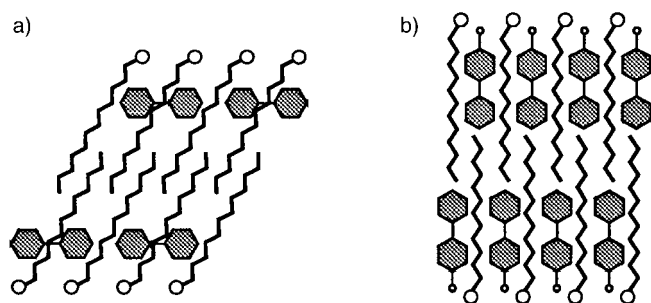


Figure 3. Schematic illustration of the packing arrangement of aromatic compound in crystals of host-dependent (a) and guest-dependent complexes (b).

axis of an aromatic molecule is parallel to that of the surfactant molecules, and, hence, the hydrophobic biphenyl part is buried in the hydrophobic region in the surfactants. In contrast, the aromatic compounds are largely tilted in the host-dependent complexes, and, hence, the long axis of the aromatic compounds is almost perpendicular to that of the surfactant molecules. In this case, the hydrophobic biphenyl part binds to the hydrophilic region of the surfactant and diminishes the hydrophilicity of the surfactants. According to the previous studies on an aqueous bilayer structure, the molecular orientation is almost the same as that in the crystal of the component.^[11] In the present case, the local structure within the giant liposome should also be similar to that in the single crystal of a complex. Therefore, a parallel rather than a perpendicular alignment would be preferred to form a liposome structure, because the perpendicular alignment should reduce the hydrophilicity of the aggregate. In fact, DTAB/4-hydroxybiphenyl formed a liposome when the mixing ratio was 1:1, whereas it produced precipitates when the ratio was 2:1 (Table 1).

Experimental Section

Materials: All of the materials used in the study are commercially available from Wako Pure Chemical Ind., Ltd (Osaka, Japan). The crystalline complexes were prepared by recrystallization of an equimolar mixture of a

surfactant and an aromatic compound from acetone/ethyl acetate (1/1). The single crystal of the complex was obtained in the same manner as described in ref. [6b, c].

Microscopic observations: Three different methods were employed for the observation. Method I: The crystalline complex was dispersed in water by sonication (1–20 mm) and then observed. Method II: A very small amount of the crystalline complexes was sandwiched between a slide glass and a cover glass. The space between the glasses was filled with water and the complex then observed. Method III: A very small amount of the aromatic compounds was sandwiched between a slide glass and a cover glass. The space between the glasses was filled with aqueous surfactant solutions (10–30 mm) and the complex then observed. Excess water or solution was blotted by filter paper from the edge of the cover glass. The sample was sealed with colorless manicure to prevent water evaporation. Observations were performed using a dark-field light microscope (OLYMPUS BHF) with a 200-W high-pressure mercury lamp (OSRAM HBO 200W/2) as the light source. We obtained a negative of 132 magnifications on KODAK Tri-X pan film (ISO 400) using a camera module (OLYMPUS PM-10AD; Figure 1) or obtained an image using a digital still camera system (Fuji Photo Film Co., Ltd.; FUJIX HC-300; Figure 2).

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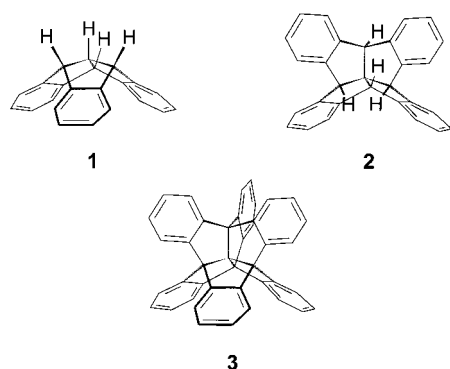
Tribenzotriquinacenes with Sixfold Peripheral Functionalization—Potential Building Blocks for Novel Organic Networks**

Jörg Tellenbröker and Dietmar Kuck*

Dedicated to Professor Kurt Heyns
on the occasion of his 90th birthday

Imaginative further development of polycyclic parent compounds and consequent application of synthetic methodology of organic chemistry has opened up new perspectives on the construction of three-dimensional cage compounds and networks in recent years.^[1] Impressive examples have been established not only in the chemistry of fullerenes^[2] and of fullerene segments such as corannulene and the semifullerenes,^[3] but also with spheriphanes,^[4] cyclophanes,^[5] cyclacenes,^[6] and propellanes.^[7] Likewise, considerable progress has been achieved in the chemistry of two-dimensional networks (graphite cuttings).^[8] In this context polycyclic ring systems with bent, that is, convex–concave or saddle-like molecular surfaces, for example, the $[n]$ circulenes ($n=5$: corannulene,^[9] $n=7$: pleiadannulene^[10]), are of particular interest.

Tribenzotriquinacene **1**^[11] and fenestrindane **2**^[12] represent alicyclic variants of this structural principle and they constitute, on their own, cuttings of the three-dimensional “molecular lattice” of centrohexaindane **3**.^[13, 14] Structural



expansion of the hatlike **1** and the saddle-like **2** by condensed arene networks can be envisioned to eventually result in novel graphite segments, in which the alicyclic “perturbations” would give rise to directed deformations of the otherwise planar surfaces. This is demonstrated by a hypothetical example for the case of **1** shown in Figure 1.

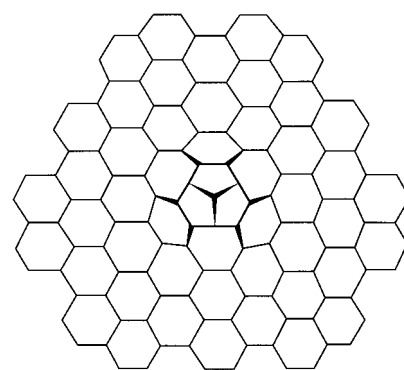
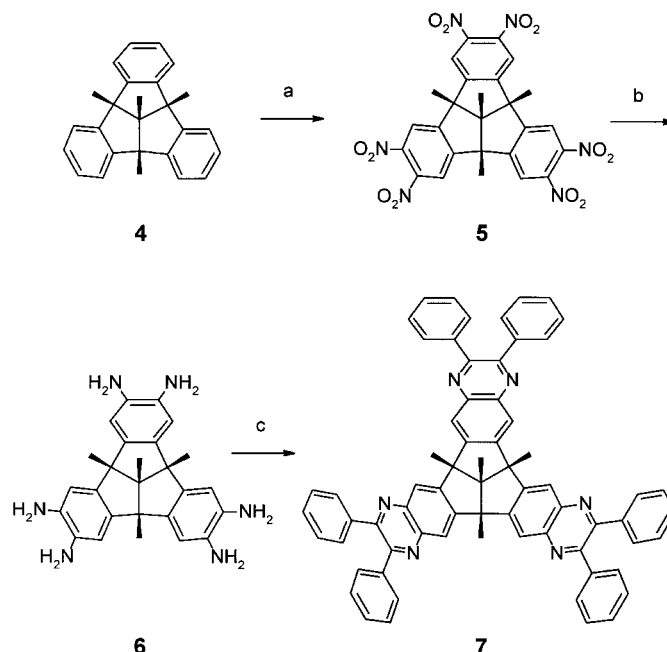


Figure 1. A hypothetical triquinacene bearing a completely closed arene periphery (schematic).

In this work we present the first results of our current investigation on the systematic functionalization of the six peripheral arene positions of tribenzotriquinacene, which may also be of interest for other fields of modern organic chemistry.^[15] For these studies we mainly employed 1,4,7,10-tetramethyltribenzotriquinacene **4**^[16] since this model substrate is chemically inert not only at the central but also at the three benzhydrylic bridgehead positions, which are very reactive in the parent hydrocarbon **1**. We expected the six *ortho* positions at the benzene nuclei to also be unreactive for steric reasons. In fact, we found that nitration of **4** in 100 % HNO₃ and 98 % H₂SO₄ (5/7) gives the 2,3,6,7,10,11-hexanitro derivative **5** in 90 % yield (Scheme 1, Table 1). This clearly



Scheme 1. a) HNO₃ (100 %), H₂SO₄ (98 %) (5/7), 0→20 °C, 6 h, yield 90 %; b) H₂, Pd/C, EtOH, 4 bar, 20 °C, 24 h; c) (PhCO)₂, EtOH/AcOH/H₂O (10/4/1), 120 °C, 4 h, yield 43 %.

corroborates the high efficiency of steric shielding at the *ortho* positions of the centropolyindanes against electrophilic attack.^[17]

Reduction of the hexanitro compound **5** is carried out most favorably by catalytic hydrogenolysis over palladium on charcoal in ethanol.^[18] Notably, the threefold *ortho*-phenyl-

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Table 1. Analytical and spectroscopic data of selected compounds.^[28]

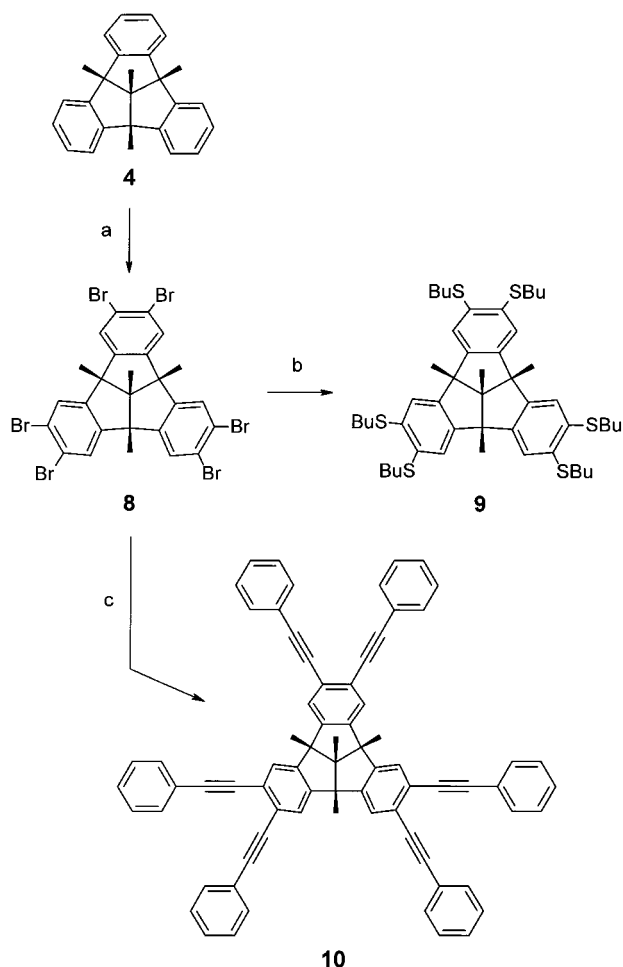
5 (4b,8b,12b,12d-Tetramethyl-2,3,6,7,10,11-hexanitro-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): amorphous colorless solid (from CHCl₃), m.p. > 360 °C; MS (70 eV): <i>m/z</i> (%): 606 (7) [<i>M</i>⁺], 591 (100), 546 (16), 499 (5), 407 (4), 315 (5); ¹H NMR (500 MHz, [D₆]DMSO): δ = 8.76 (s, 6H), 1.80 (s, 9H), 1.38 (s, 3H); ¹³C[¹H] NMR (125 MHz, [D₆]DMSO): δ = 152.6 (s), 143.0 (s), 121.4 (d), 71.2 (s), 63.5 (s), 23.9 (q), 15.0 (q); IR (KBr): $\tilde{\nu}$ = 3049, 2974, 1593, 1545, 1478, 1453, 1440, 1398, 1365, 1143, 916, 862, 853, 756, 742 cm⁻¹. 7 (5b,11b,17b,18b-Tetramethyl-2,3,8,9,14,15-hexaphenyl-5b,11b,17b,18b-tetrahydropyrazino[2'',3'':5',6']indeno[1',2',3':3,4]quinoxalino[6',7':5,6]pentaleno[1,2-<i>g</i>]quinoxaline): long, colorless needles (from CHCl₃), m.p. > 360 °C; LSI-MS (3-nitrobenzyl alcohol (NBA)): <i>m/z</i> (%): 949 (100) [<i>M</i>⁺], 933 (31); ¹H NMR (500 MHz, CDCl₃): δ = 8.37 (s, 6H), 7.46 (d, ³<i>J</i> = 7.9 Hz, 12H), 7.29 (m, 18H), 2.02 (s, 9H), 1.59 (s, 3H); ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 152.8 (s), 152.5 (s), 141.3 (s), 138.9 (s), 129.8 (d), 128.7 (d), 128.2 (d), 123.0 (d), 70.8 (s), 62.8 (s), 27.2 (q), 16.5 (q); IR (KBr): $\tilde{\nu}$ = 3061, 2970, 1551, 1466, 1438, 1403, 1392, 1342, 1261, 1188, 1076, 1056, 1023, 971, 893, 766 cm⁻¹; UV/Vis (CH₂Cl₂, <i>c</i> = 5.43 × 10⁻⁶ mol L⁻¹): λ_{max} (lg ε) = 257 (5.13), 368 (4.79) nm. 8 (4b,8b,12b,12d-Tetramethyl-2,3,6,7,10,11-hexabromo-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): amorphous colorless solid (from CHCl₃), m.p. > 360 °C; MS (70 eV): <i>m/z</i> (%): 810 (41) [<i>M</i>⁺], 795 (100), 730 (6), 715 (18), 635 (7); ¹H NMR (500 MHz, CDCl₃): δ = 7.49 (s, 6H), 1.55 (s, 9H), 1.28 (s, 3H); ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 148.6 (s), 128.0 (d), 124.3 (s), 71.2 (s), 62.0 (s), 25.5 (q), 16.0 (q); IR (KBr): $\tilde{\nu}$ = 3060, 3001, 2978, 2962, 2955, 2927, 2899, 2870, 1477, 1469, 1461, 1446, 1391, 1377, 1365, 1349, 1272, 1248, 1204, 1179, 1121, 1104, 1083, 1034, 887, 874, 772, 763, 684, 640 cm⁻¹. 9 (4b,8b,12b,12d-Tetramethyl-2,3,6,7,10,11-hexa(<i>n</i>-butylthio)-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): glassy colorless oil (from CHCl₃); MS (70 eV): <i>m/z</i> (%): 864 (100) [<i>M</i>⁺], 849 (23), 810 (16), 776 (59), 761 (17); ¹H NMR (500 MHz, CDCl₃): δ = 7.21 (s, 6H), 2.86 (oct., ³<i>J</i> = 7.3 Hz, 12H), 1.63 (sext., ³<i>J</i> = 7.6 Hz, 12H), 1.60 (s, 9H), 1.46 (sext., ³<i>J</i> = 7.5 Hz, 12H), 1.30 (s, 3H), 0.92 (t, ³<i>J</i> = 7.3 Hz, 18H); ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 146.8 (s), 136.8 (s), 123.6 (d), 70.7 (s), 62.2 (s), 33.4 (t), 30.9 (t), 25.6 (q), 22.1 (t), 16.0 (q), 13.7 (q); IR (KBr): $\tilde{\nu}$ = 3015, 2996, 2982, 2966, 2948, 2932, 2919, 2894, 2867, 2854, 2840, 2816, 1587, 1474, 1468, 1460, 1452, 1433, 1402, 1387, 1373, 1355, 1259, 1217, 1187, 1121, 1103, 1084, 1040, 905, 889, 865, 846, 778, 733 cm⁻¹. 10 (4b,8b,12b,12d-Tetramethyl-2,3,6,7,10,11-hexa(phenylethynyl)-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): amorphous brownish solid (from CHCl₃), m.p. > 360 °C; MS (DEI, 70 eV): <i>m/z</i> (%): 936 (100) [<i>M</i>⁺], 921 (94) [<i>M</i>⁺ – CH₃], 468 (10) [<i>M</i>²⁺], 461 (20) [<i>M</i>²⁺ – CH₃], 178 (45); ¹H NMR (500 MHz, CDCl₃): δ = 7.58 (m, 18H), 7.31 (m, 18H), 1.68 (s, 9H), 1.35 (s, 3H); ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 148.4 (s), 131.7 (d), 128.3 (d), 126.4 (d), 125.7 (s), 123.3 (s), 93.3 (s), 88.6 (s), 70.5 (s), 62.6 (s), 25.7 (q), 16.0 (q); IR (KBr): $\tilde{\nu}$ = 3058, 2966, 2925, 1596, 1492, 1473, 1452, 1442, 1405, 1392, 1081, 1068, 1025, 903, 749, 687 cm⁻¹; UV/Vis (CH₂Cl₂, <i>c</i> = 4.38 × 10⁻⁶ mol L⁻¹): λ_{max} (lg ε) = 288 (5.22) nm.	11 (4b,8b,12b,12d-Tetramethyl-2,3,6,7,10,11-hexaiodo-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): amorphous colorless solid (from CHCl₃), m.p. > 360 °C; MS (DEI, 70 eV): <i>m/z</i> (%): 1092 (76) [<i>M</i>⁺], 1077 (100), 966 (5), 965 (4), 951 (8), 950 (4), 823 (22), 696 (2), 681 (4); ¹H NMR (500 MHz, CDCl₃): δ = 7.72 (s, 6H), 1.51 (s, 9H), 1.24 (s, 3H); ¹³C[¹H] NMR (125 MHz, C₂D₂Cl₄): δ = 149.5 (s), 133.6 (d), 107.1 (s), 70.7 (s), 61.5 (s), 25.1 (q), 15.7 (q); IR (KBr): $\tilde{\nu}$ = 2967, 2926, 1474, 1453, 1440, 1392, 1375, 1335, 1267, 1251, 1090, 1030, 891, 860 cm⁻¹. 12 (4b,8b,12b,12d-Tetramethyl-2,3,6,7,10,11-hexaphenyl-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): colorless needles (from CHCl₃), m.p. > 360 °C; MS (70 eV): <i>m/z</i> (%): 792 (69) [<i>M</i>⁺], 777 (100), 716 (16), 701 (27), 389 (18); ¹H NMR (500 MHz, CDCl₃): δ = 7.47 (s, 6H), 7.18 (m, 18H), 7.08 (d, ³<i>J</i> = 7.6 Hz, 12H), 1.86 (s, 9H), 1.54 (s, 3H); ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 148.2 (s), 141.8 (s), 140.6 (s), 130.0 (d), 127.7 (d), 126.3 (d), 125.0 (d), 71.3 (s), 62.7 (s), 26.1 (q), 16.3 (q); IR (KBr): $\tilde{\nu}$ = 3063, 3029, 2968, 1600, 1493, 1477, 1446, 1393, 1229, 1073, 1023, 897, 887, 781, 765, 697, 621 cm⁻¹; UV/Vis (CH₂Cl₂, <i>c</i> = 5.23 × 10⁻⁶ mol L⁻¹): λ_{max} (lg ε) = 248 (4.94) nm. 13 (5b,15b,25b,30c-Tetramethyl-5b,15b,25b,30c-tetrahydro-benzo[<i>f</i>]benzo[9',10']phenanthro[2',3':5,6]phenanthro[9'',10'':5',6']indeno[1',2',3':3,4]-pentaleno[1,2-<i>b</i>]phenanthrene): amorphous colorless solid (from CHCl₃), m.p. > 360 °C; MS (70 eV): <i>m/z</i> (%): 786 (5) [<i>M</i>⁺], 771 (5); ¹H NMR (500 MHz, CDCl₃): δ = 8.84 (s, 6H), 8.78 (d, ³<i>J</i> = 8.2 Hz, 6H), 8.55 (d, ³<i>J</i> = 8.1 Hz, 6H), 7.69 (t, ³<i>J</i> = 7.6 Hz, 6H), 7.59 (t, ³<i>J</i> = 7.4 Hz, 6H), 2.07 (s, 9H), 1.61 (s, 3H); ¹³C[¹H] NMR (125 MHz, C₂D₂Cl₄): δ = 148.9 (s), 129.8 (s), 129.4 (s), 129.2 (s), 127.2 (d), 127.0 (d), 123.2 (d), 122.9 (d), 117.2 (d), 71.4 (s), 62.8 (s), 26.8 (q), 16.3 (q); IR (KBr): $\tilde{\nu}$ = 3077, 2966, 2922, 2854, 1493, 1447, 1434, 1261, 1092, 1049, 1030, 885, 753, 720, 710 cm⁻¹; UV/Vis (CH₂Cl₂, <i>c</i> = 4.07 × 10⁻⁶ mol L⁻¹): λ_{max} (lg ε) = 262 (5.33), 312 (4.43), 332 (3.99), 348 (3.89) nm. 15 (12d-Methyl-4b,8b,12b-trihydroxy-2,3,6,7,10,11-hexaiodo-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): amorphous colorless solid (from CHCl₃), m.p. 314 °C (decomp); MS (DEI, 70 eV): <i>m/z</i> (%): 1098 (19) [<i>M</i>⁺], 1081 (3), 1049 (3), 972 (4), 128 (100); ¹H NMR (500 MHz, [D₆]DMSO): δ = 8.18 (s, 6H), 6.07 (s, 12H), 1.08 (s, 3H); ¹³C[¹H] NMR (125 MHz, [D₆]DMSO): δ = 146.6 (s), 134.7 (d), 109.4 (s), 88.1 (s), 77.2 (s), 12.1 (q); IR (KBr): $\tilde{\nu}$ = 3533, 3422, 3375, 3365, 2987, 2977, 2934, 1790, 1618, 1577, 1442, 1335, 1237, 1183, 1078, 1034, 970, 927, 886, 755, 726, 681, 618 cm⁻¹. 16 (12d-Methyl-4b,8b,12b-tribromo-2,3,6,7,10,11-hexaiodo-4b,8b,12b,12d-tetrahydrodibenzo[2,3:4,5]pentaleno[1,6-<i>ab</i>]indene): amorphous colorless solid (from CHCl₃), m.p. 254 °C (decomp); MS (APCI (chemical ionization at atmospheric pressure)): <i>m/z</i> (%): 1206 (100) [<i>M</i>⁺ – Br], 1158 (23), 1128 (22), 1047 (12); ¹H NMR (500 MHz, C₂D₂Cl₄): δ = 8.08 (s, 6H), 2.17 (s, 3H); ¹³C[¹H] NMR (125 MHz, C₂D₂Cl₄): δ = 143.7 (s), 136.0 (d), 111.4 (s), 79.5 (s), 75.6 (s), 36.5 (q); IR (KBr): $\tilde{\nu}$ = 3070, 2990, 2958, 2947, 2941, 2929, 1573, 1562, 1453, 1441, 1433, 1425, 1420, 1378, 1347, 1341, 1267, 1240, 1214, 1208, 1083, 1061, 921, 867, 856, 846, 838, 823, 815, 755, 699, 674 cm⁻¹.

enediamine **6** formed in this way is extremely sensitive towards oxidation; therefore, it has to be subjected to subsequent conversions in solution after separation of the inorganic components from the reaction mixture. In contrast to other 1,2-diketones, hexaaminotribenzotriquinacene **6** easily reacts with benzil to give the threefold quinoxaline **7**^[19] which, in view of the sensitivity of the hexamine, is isolated in relatively good yield.

Bromination of the six peripheral positions of **4** is also performed under suitable conditions (Scheme 2, Table 1).^[20] Best results are obtained by working with a slight excess (10 %) of bromine in the presence of catalytic amounts of iron and iodine. Hexabromotribenzotriquinacene **8** is isolated in 93 % yield and affords a valuable basis for the synthesis of a

number of other sixfold functionalized congeners or C–C coupled derivatives. For example, the sixfold thioether **9** and the threefold *ortho*-ditolane **10** are accessible from **8** in very good yields.^[21, 22] The pronounced vaulting of the convex–concave molecular structure of **10**, which is strongly enlarged by the six appending phenylethynyl units, is illustrated in Figure 2.

Iodination of **4** with potassium iodide/periodic acid^[23] gives the hexaiodo derivative **11** (Scheme 3, Table 1), in analogy to bromination. This sixfold functionalized tribenzotriquinacene is considered an important key compound for the construction of more highly condensed benzenoid arenes, the ring systems of which are fixed at the central triquinacene unit in an almost perfectly orthogonal orientation to each other. Thus, for example, C–C coupling with phenylboronic acid under Suzuki



Scheme 2. a) Br_2 , Fe (cat.), I_2 (cat.), CCl_4 , 60°C , 24 h, yield 93%; b) CuSnBu , pyridine, quinoline, 170°C , 24 h, yield 94%; c) phenylacetylene, NEt_3 , $[(\text{Ph}_3\text{P})_2\text{PdCl}_2]$, CuI, Ph_3P , 120°C , 48 h, yield 87%.

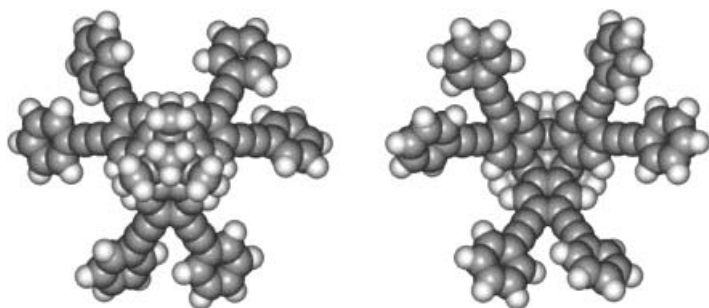
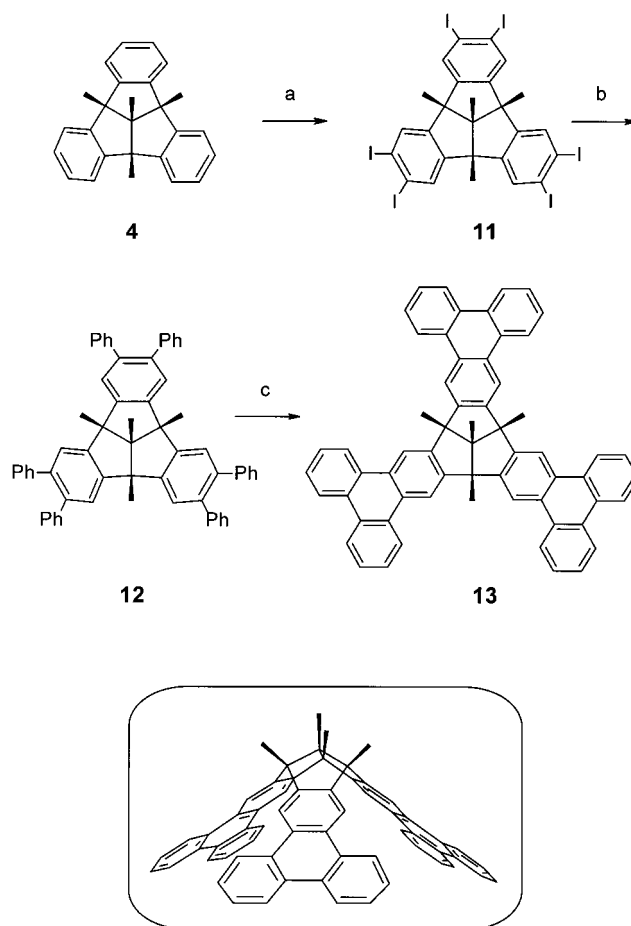


Figure 2. Molecular shape of hexatolane **10** (from MM + calculations); viewed onto the convex (left) and the concave surface (right).

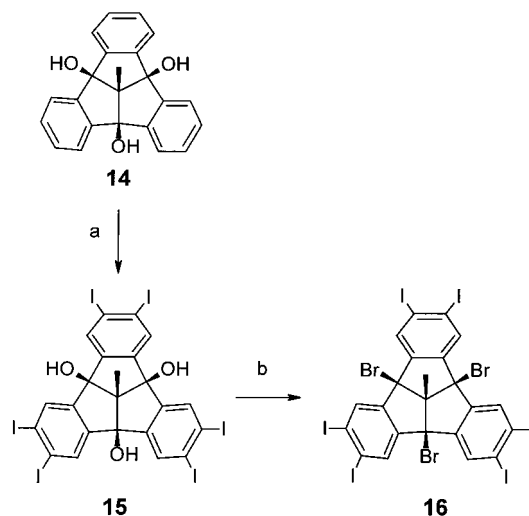
conditions^[24] gives the hexaphenyltribenzotriquinacene **12** in excellent yield (Scheme 3), and subsequent photocyclodehydrogenation under Mallory conditions^[25] yields the tris(triphenylene) **13**.

These examples demonstrate that, besides their versatile conversions at the bridgehead positions,^[11a, 14, 16, 26] tribenzotriquinacenes may also be used as building blocks for various synthetic reactions that lead to complex ring systems. We are currently investigating the synthetic access to higher analogues of **12** and **13**. Moreover, the combined functionalization at both the bridgehead and the peripheral positions is



Scheme 3. a) KI, H_5IO_6 , conc. H_2SO_4 , $0 \rightarrow 20^\circ\text{C}$, 12 h, yield 76%; b) $\text{PhB}(\text{OH})_2$, $[\text{Pd}(\text{dba})_2]$, KOH, PPh_3 , $\text{PhNO}_2/\text{H}_2\text{O}$, 100°C , 24 h, yield 90%; c) I_2 , PhH, $h\nu$, 20°C , 18 h, yield 47%. A spatial representation of **13** is given in the box. dba = dibenzylideneacetone.

possible in particular cases. For example, the tetramethyl compound **4** can be replaced by the easily accessible trihydroxytribenzotriquinacene **14**,^[16a, 27] which is converted sequentially into the corresponding trihydroxyhexaiodotribenzotriquinacene **15** and the tribromohexaiodo derivative **16** (Scheme 4, Table 1). These compounds represent the first



Scheme 4. a) KI, H_5IO_6 , conc. H_2SO_4 , $0 \rightarrow 20^\circ\text{C}$, 12 h, yield 63%; b) THF, PBr_3 , $0 \rightarrow 20^\circ\text{C}$, 12 h, yield 55%.

ninefold functionalized tribenzotriquinacenes and offer interesting possibilities for further synthetic reactions.

On the basis of these results a large variety of novel convex–concave polycyclic hydrocarbon frameworks will become available. In addition, the peripheral functionalization also offers a synthetic access to complex ligands bearing three, possibly cooperative, ligand spheres, such as threefold crown ethers and cryptands, as well as to dendrimers based on the tribenzotriquinacene core.^[15] Moreover, the strategy of synthesis presented here may be successfully transferred to fenestrindane **2** and, at least in part, to centrohexaindane **3**, to incorporate benzoannelated fenestranes as unusual bulding blocks into extended carbocyclic frameworks or into the core of the polyfunctionalized host molecules.

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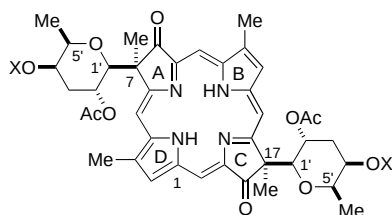
Keywords: carbocycles • polycycles • triquinacene

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- [28] The new compounds **5**, **8**, **9**, and **11** gave correct elemental analytical data and/or exact mass measurements. Compound **16** exhibits a tendency to include solvents.

Total Synthesis of the Proposed Structure of (+)-Tolporphin A *O,O*-Diacetate**

Thomas G. Minehan and Yoshito Kishi*

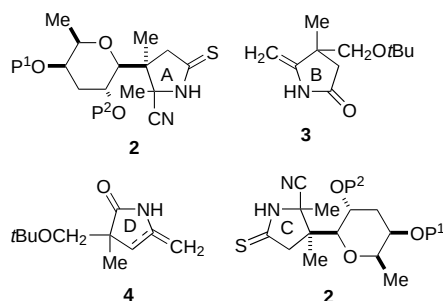
In 1992 Moore and co-workers reported that the lipophilic extract of the cyanophyte microalga *Tolypothrix nodosa* reverses multidrug resistance (MDR)^[1] in a vinblastine-resistant subline of a human ovarian adenocarcinoma line^[2a] and that most of this activity was accounted for by an unusual porphyrin, tolporphin A. Based on extensive spectroscopic studies, the structure of tolporphin A was suggested to be **1a**.^[2a] Subsequently, eight additional tolporphins B–I were



1a (X=H) : "tolporphin A"
1b (X=Ac) : "tolporphin-A-*O,O*-diacetate"

also isolated and found to possess varying degrees of anti-MDR activity.^[2b] Structurally, tolporphin A, the representative member of this class of natural products, consists of an unsymmetrical dioxobacteriochlorin core with quaternary centers at C7 and C17 containing β -linked C-glycosides.

Intrigued by their unique structures and biological activity, we undertook synthetic studies on the tolporphins. Specifically, we were interested in assembling the tolporphin skeleton from the monocyclic precursors **2**–**4**. An obvious

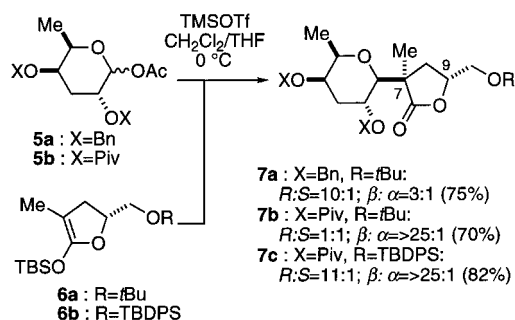


advantage of this approach is that the ring-C precursor is identical to the ring-A precursor (i.e., **2**). The synthesis of the chromophore model^[3] demonstrated the feasibility of this approach: The sulfide contraction/iminoester-cyclization method of Eschenmoser and co-workers^[4] was adopted to assemble an octahydporphyrin precursor, which was then subjected to a double-retroaldol/oxidation sequence to form

the chromophore of the tolporphins. Here we report an extension of this route to the successful total synthesis of the proposed structure of (+)-tolporphin A *O,O*-diacetate (**1b**), and demonstrate that a structural revision is required for this class of natural products.

Recognizing that the tolporphins contain C-glycoside substructures, we planned to synthesize the ring-A precursor **2** from an appropriate carbohydrate such as 3,6-dideoxy-D-xylose. In this connection, one needs to pay attention to two structural characteristics of **2**: the β -C-glycosidic bond and the quaternary carbon atom adjacent to the anomeric center. We have recently demonstrated that sterically hindered β -C-glycosides can be synthesized by Lewis acid promoted addition of silyl ketene acetals to carbohydrate 1-*O*-acetates.^[5] The remaining question is then how to control the stereochemistry at the quaternary carbon atom in the C-glycosidation. It was conceivable that placement of an additional chiral center in the nucleophile would influence the facial selectivity of the reaction. We were specifically interested in silyl ketene acetals containing a protected hydroxymethyl group at C9 for two reasons: 1) Optimization of the stereoselectivity in the C-glycosidation could be achieved by tuning the steric bulk of the protecting group, and 2) the hydroxymethyl group could be removed after the glycosidation by an oxidative diol cleavage. Under the assumption that the carbohydrate adds preferentially to the lactone from the face opposite to the chiral directing group, it was anticipated that an (*R*)-silyl ketene acetal such as **6**^[6] (see Scheme 1) would give rise to a product C-glycoside with the correct configuration at C7.^[7]

Reaction of bis(benzyl ether) **5a** with *tert*-butyl-substituted **6a** in the presence of TMSOTf in CH₂Cl₂/THF (10/1) at 0 °C gave a product mixture with an *R*:*S* selectivity of 10:1 at the quaternary center, but with a β : α selectivity of only 3:1 at the anomeric position (Scheme 1).^[8] It has been previously shown



Scheme 1. Reaction of **5** and **6** to provide **7**. Bn = benzyl, Piv = pivaloyl, TBDPS = *tert*-butyldiphenylsilyl, TBS = *tert*-butylsilyl, TMSOTf = trimethylsilyl trifluoromethanesulfonyl.

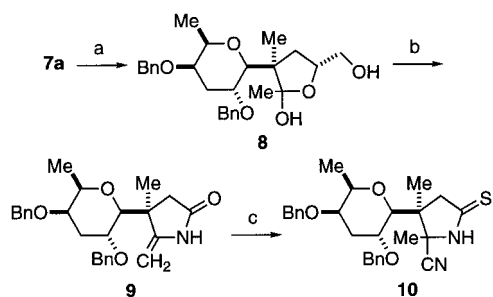
that β selectivity can be dramatically enhanced by placement of an acyl protecting group such as acetate at C2 of the carbohydrate; however, yields by this method often suffer because of by-product formation arising from glycosidation at the carbonyl carbon atom of the acyl group.^[5] We hoped that with a quaternary center at the α carbon atom of the ester protecting group, the undesired side reaction might be suppressed for steric reasons.^[9] This possibility was tested

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with the 2,4-bis-*O*-pivalate **5b**; glycosidation of **5b** with **6a** gave β -substituted C-glycosides in high yield, but with no diastereoselectivity at the quaternary center. Gratifyingly, however, glycosidation of **5b** with TBDPS-protected **6b** produced C-glycoside **7c** in 82% yield, with virtually exclusive β selectivity and an *R:S* selectivity of 11:1 at C7.

Considering the compatibility of protecting groups with the reagents and reaction conditions encountered in the remaining synthetic steps, we identified the optimum protecting groups of the carbohydrate moiety to be benzyl ethers (i.e., $P^1 = P^2 = \text{Bn}$ in **2**). However, attempts to switch the pivalate ester groups of **7c** to the corresponding benzyl ether moieties met with limited success.^[10] Thus, in spite of the poorer β selectivity in the C-glycosidation, material through-put was most effectively secured with C-glycoside **7a** (Scheme 2). The

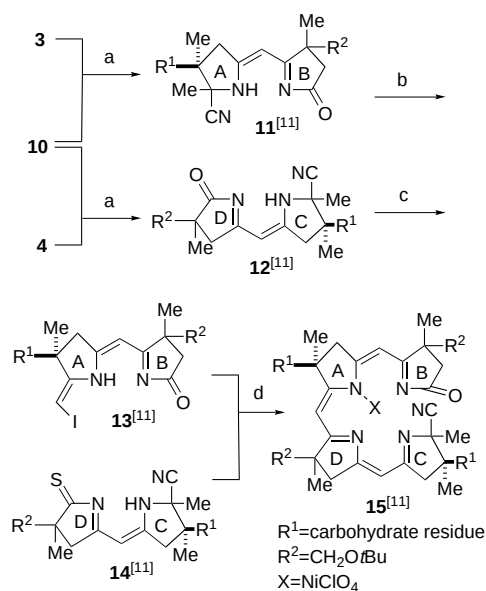


Scheme 2. Synthesis of thiolactam **10**. a) 1. Trifluoroacetic acid (TFA), CH_2Cl_2 , room temperature (RT); 2. MeLi, THF, -78°C (92%); b) 1. $\text{Pb}(\text{OAc})_4$, CH_2Cl_2 , NaHCO_3 , RT; 2. NaClO_2 , NaH_2PO_4 , 2-methyl-2-butene, *t*BuOH, H_2O , RT; 3. EtO_2CCl , Et_3N , THF, RT; NH_3 , RT; 4. xylenes, reflux (70% from **8**); c) 1. KCN, MeOH, 60°C (70%); 2. Lawesson's reagent, toluene, 80°C (98%).

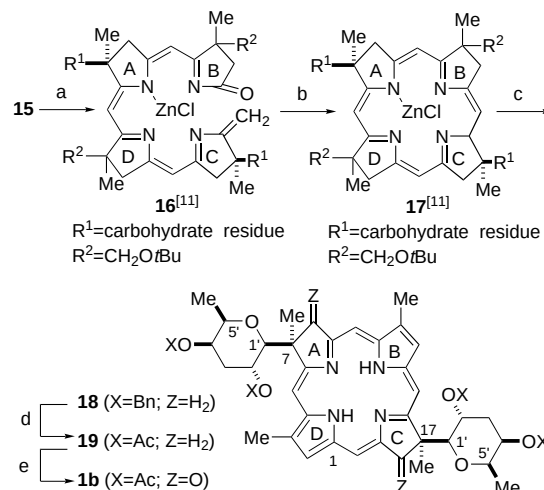
hydroxymethyl group used for control of the stereochemistry at C7 was easily removed by treatment of ketol alcohol **8** with $\text{Pb}(\text{OAc})_4$. Subsequent oxidation, amide formation, and dehydration delivered enamide **9**. The acylenamine group in **9** was protected as its HCN adduct and thionated with Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide) to furnish thiolactam **10**.

The precorphin–metal complex **15**^[11, 12] was efficiently assembled from **3**,^[3] **4**,^[3] and **10** by sequential Eschenmoser reactions as in the model series (Scheme 3).

Transformation of **15** to exo-eneamide–zinc complex **16** was accomplished smoothly by demetalation followed by cyanide elimination and remetalation in one pot (Scheme 4). This air-, moisture-, and light-sensitive intermediate was subjected immediately to the iminoester cyclization conditions optimized for the chromophore model;^[3] treatment of **16** with excess (10 equiv) Et_3OBF_4 or Et_3OPF_6 in the presence of Hünig base (ethyl-diisopropylamine; 5 equiv) in CH_2Cl_2 at room temperature for two days, with addition of methanol (1.7 equiv) after the first day, indeed gave the desired cyclized corphin **17**^[13] in approximately 20% yield. The extreme sensitivity of the reaction mixture to both air and moisture made it difficult to obtain the product reproducibly; the yield varied from 20% down to 0%. However, replacement of the Meerwein salt with MeOTf led to significant improvements in yields (50–60%) and reproducibility.^[14] This experiment



Scheme 3. Synthesis of **15**. a) 1. *N*-Iodosuccinimide (NIS), *t*BuOK, *t*BuOH, C_6H_6 , RT; 2. $(\text{EtO})_3\text{P}$, xylenes, 125°C (65% over two steps); b) 1. *t*BuOK, *t*BuOH, 85°C ; 2. I_2 , K_2CO_3 , CH_2Cl_2 , 0°C (80% over two steps); c) Lawesson's reagent, toluene, 80°C (89%); d) 1. diazabicyclo[5.4.0]undec-7-ene (DBU, 4 equiv), CH_3CN , RT; 2. $\text{Ni}(\text{ClO}_4)_2$, PPh_3 , CH_3CN , RT (50% over two steps).
 $\text{R}^1 = \text{carbohydrate residue}$
 $\text{R}^2 = \text{CH}_2\text{O}^i\text{Bu}$
 $\text{X} = \text{NiClO}_4$



Scheme 4. Synthesis of **1b**. a) 1. KCN, MeOH, RT; 2. *t*BuOK, *t*BuOH, RT; $\text{Zn}(\text{ClO}_4)_2$, MeOH, RT; b) MeOTf (6.8 equiv), pentamethylpiperidine (4.6 equiv), CH_2Cl_2 , RT, 20 h; MeOH (1.75 equiv), RT, 20 h (50% from **15**); c) TFA, anisole, dimedone, RT; MeOH, RT; *t*BuOK, *t*BuOH; 20% HCl, RT (55%); d) 1. ZnCl_2 , EtSH, CH_2Cl_2 , RT; 2. Ac_2O , pyridine, RT (98%). e) $\text{CrO}_3 \cdot \text{dimethyl pyrazole}$ / $\text{CH}_2\text{Cl}_2/0^\circ\text{C}$ (30–40%).

demonstrates that MeOTf^[15] not only effectively methylates the lactam oxygen atom, but is also compatible with the other functional groups present.

Conversion of corphin **17** into tetrahydroporphyrin **18** took place in a moderate overall yield of 30%. This one-pot, three-step transformation involved *tert*-butyl ether deprotection, double-retroaldol reaction/autooxidation, and demetalation. Spectroscopic analysis of the crude reaction mixture suggested that formaldehyde generated in the retroaldol process had been trapped by the chlorin, leading to some of the observed by-products. Thus, it was speculated that a formaldehyde

scavenger might improve both the yields and reproducibility of the reaction; indeed, addition of excess dimedone improved the yield to 55 % with excellent reproducibility.^[16] The benzyl ethers in **18** were removed under Fujita conditions^[17] to yield a tetraol, which was directly acetylated to give tetraacetate **19**. The structure of **19** was established by spectroscopic data (high-resolution mass spectrometry (HR-MS), UV, ¹H NMR); in particular, ¹H–¹H COSY and ¹H–¹H NOESY experiments unambiguously established the proton assignments and connectivities.^[18]

The final double allylic oxidation^[3] to form dioxobacteriochlorin **1b** was carried out in a stepwise manner.^[19] Titration of a solution of **19** in CH₂Cl₂ with a 0.05 M solution of CrO₃·dimethylpyrazole^[20] in CH₂Cl₂ showed clean formation of an approximately 1:1 mixture of A- and C-ring monoketones (thin-layer chromatography and ¹H NMR spectroscopy); however, continued titration resulted in complete decomposition of the substrates. Curiously, when the monoketones were isolated, purified, and resubjected to the same oxidation conditions, the desired **1b** was isolated in 30–40 % overall yield from **19**. The structure of **1b** was fully supported by spectroscopic data (HR-MS, UV, ¹H NMR). However, upon comparison of ¹H NMR spectra, it became evident that the synthetic material was different from the authentic sample prepared from natural (+)-tolyporphin A.^[21, 22] In the following communication,^[18] we present evidence that (+)-tolyporphin A is most likely described by the structure in which the configurations at both quaternary centers C7 and C17 are opposite to those in the originally proposed structure **1a**.

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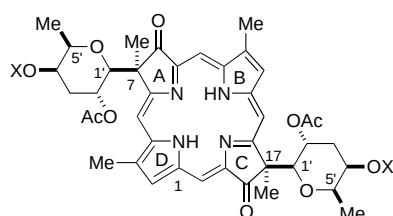
Keywords: glycosides • natural products • porphyrinoids • total synthesis

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- [9] Glycosidation of substrates having less reactive acyl carbonyl groups such as the bis-*O*-benzoate and the bis-*O*-*para*-methoxybenzoate corresponding to **5** was also tested, but the major product in each case was still found to arise from C–C bond formation at the carbonyl carbon atom of the acyl group at C2.
- [10] Base hydrolysis followed by acid work-up yielded three lactones. Treatment with methylolithium or diisobutyl aluminum hydride (DIBAL-H) yielded a ketal or acetal involving the alcohol groups of C9 and C2; attempted hydrolysis of the resultant acetal or ketal failed under a wide range of acidic conditions. Efforts to incorporate “removable” α -quaternary ester protecting groups, for example the α,α -dimethyllevulinoyl group, also met with limited success.
- [11] The product at this stage was a mixture of diastereomers due to the chiral centers at C3, C6, C12, and/or C16.
- [12] Because of its extreme sensitivity to light, purification and manipulation of the cadmium precorphan complex corresponding to **15** presented technical difficulties, resulting in poor overall yields; thus, the nickel-containing substrate was used for further elaboration.
- [13] Eschenmoser and co-workers have suggested^[4] that this cyclization involves three distinct steps: 1) a slow reaction of the lactam carbonyl with the alkylating reagent, 2) a proton-catalyzed isomerization of the double bonds in the precorphan structure, and 3) an electrocyclic ring closure. The addition of methanol serves to release a controlled amount of “catalytic protons” into the reaction medium.
- [14] The MeOTf method was also more effective for the precorphan cyclization in the synthesis of the chromophore model: 55 % (MeOTf) versus 40 % (Et₃OBf₄).^[3]
- [15] For general reactivity of MeOTf, see *Encyclopedia of Reagents for Organic Synthesis* (Ed.: L. A. Paquette), Wiley, New York, **1995**, pp. 3617 ff, and references therein.
- [16] The addition of dimedone was also shown to be more effective for the double retroaldol reaction in the synthesis of the chromophore model: 60 % (with dimedone) versus 30–40 % (without dimedone).^[3]
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- [21] We gratefully thank Dr. Michèle Prinsep, University of Waikato, New Zealand, for kindly providing a sample of natural tolpyorphin A. Acetylation of tolpyorphin A is known to form tolpyorphin A *O,O*-diacetate.^[2a]
- [22] To confirm this result, **1b** was further converted (K₂CO₃, MeOH, RT, 70 %) into the corresponding tetraol and compared with (+)-tolpyorphin D prepared from natural tolpyorphin A. Upon comparison of ¹H NMR spectra, the synthetic material was once again found not to match the authentic sample.

Revised Structure of Tolyporphin A**

Thomas G. Minehan, Laura Cook-Blumberg, Yoshito Kishi,* Michèle R. Prinsep, and Richard E. Moore

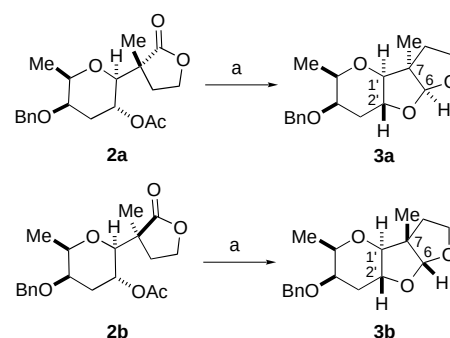
As reported in the preceding communication,^[1] we have completed the total synthesis of **1b**, the structure proposed for (+)-tolyporphin A *O,O*-diacetate, but have found the synthetic compound to be different from the *O,O*-diacetate prepared from natural (+)-tolyporphin A.^[2] The structure of the natural product was originally proposed to be **1a** on the



1a (X=H): "tolyporphin A"
1b (X=Ac): "tolyporphin A *O,O*-diacetate"

basis of ¹H–¹³C HMBC and ¹H–¹H ROESY experiments.^[3] In particular, the configuration at the quaternary centers C7 and C17 was assigned on the basis of NOEs observed between the C1' protons on the carbohydrate residues and the C5 and C15 *meso* porphyrin protons and between the C2' acetyl methyl protons on the carbohydrate residues and the C10 and C20 *meso* porphyrin protons.^[4] Here we describe spectroscopic studies on both the synthetic and natural products and propose a revised structure for tolyporphin A, the representative member of the tolyporphin class of natural products.^[5]

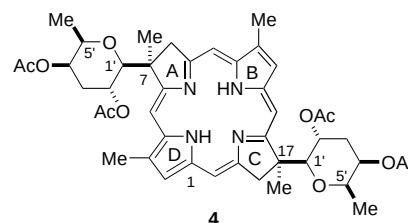
Since rings A and C of the synthetic substance were derived from precursor **2a**, the stereochemical assignment of the quaternary center was first addressed at the monocyclic stage. Both precursor **2a**^[1] and its diastereomer **2b**^[1] were converted into the conformationally rigid tricyclic acetals **3a** and **3b**, respectively (Scheme 1), and subjected to NOE experiments. Irradiation of the C7 quaternary methyl group of **3a** enhanced the signals for both the C6 acetal proton and the C1' axial anomeric proton of the sugar. On the other hand, irradiation of the C7 quaternary methyl group of **3b** enhanced the signals for the C6 acetal proton and the C2' axial proton of the sugar.



Scheme 1. Synthesis of **3a** and **3b**. a) 1. diisobutyl aluminum hydride (DIBAL-H), toluene, –78 °C; 2. pyridinium-*p*-toluenesulfonate (PPTS), DMF, RT.

Based on this experiment, the configurations at the C7 quaternary centers of **2a** and **2b** were assigned as *R* and *S*, respectively, and the diastereomer **2a** was then used to assemble the tetrahydroporphyrin ring.

Considering the synthetic route and the full characterization of all intermediates, we were confident of the structure of synthetic tetrahydroporphyrin tetraacetate **4**. Nonetheless,



thorough spectroscopic studies were conducted to confirm this structure. In particular, a ¹H–¹H NOESY^[6] experiment in C₆D₆ allowed us to unambiguously confirm the connectivity of the porphyrin skeleton (Table 1, Figure 1). As we had

Table 1. Data on NOEs for tetrahydroporphyrin tetraacetate **4**.

H2 ↔ CH ₃ (C3)	H8 ↔ H10	H15 ↔ CH ₃ (C17)
CH ₃ (C3) ↔ H5	H10 ↔ CH ₃ (C12)	CH ₃ (C17) ↔ H18
H5 ↔ CH ₃ (C7)	CH ₃ (C12) ↔ H13	H18 ↔ H20
CH ₃ (C7) ↔ H8	H13 ↔ H15	H20 ↔ H2

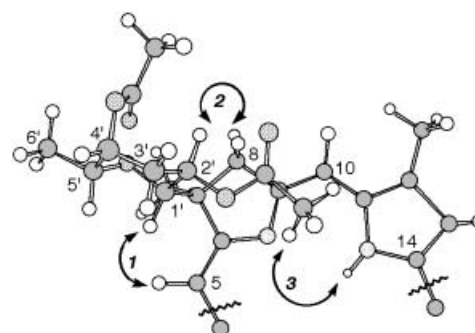


Figure 1. The partial structure of **4**. The NOEs are represented by two-ended arrows: **1**: H1' ↔ H5, **2**: H2' ↔ H8, **3**: H2'Ac ↔ HN.

expected, **4** exhibited a continuous circle of NOEs around the porphyrin ring (Table 1). In addition, strong cross-peaks representing NOEs from the C5 and C15 *meso* protons to

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the carbohydrate C1' protons, as well as NOEs from the C8 and C18 protons to the carbohydrate C2' protons, were observed; NOEs were also seen between the protons of the C2' acetyl methyl groups of the carbohydrate moieties and the porphyrin NH protons (Figure 1). These NOEs confirmed the configuration at the quaternary centers C7 and C17 and also suggested restricted rotation around the C-glycosidic bonds.

The tetrahydroporphyrin tetraacetate **4** was then converted into synthetic *O,O*-diacetate **1b**. The structure of **1b** was fully consistent with its spectroscopic data, including the ^1H - ^1H NOESY data collected in CD_3COCD_3 (Table 2). Because the

Table 2. Data on NOEs for synthetic tolporphin A *O,O*-diacetate (**1b**).

H20 $\leftrightarrow$ H2	H10 $\leftrightarrow$ CH ₃ (C12)
H2 $\leftrightarrow$ CH ₃ (C3)	CH ₃ (C12) $\leftrightarrow$ H13
CH ₃ (C3) $\leftrightarrow$ H5	H13 $\leftrightarrow$ H15
H5 $\leftrightarrow$ CH ₃ (C7)	H15 $\leftrightarrow$ CH ₃ (C17)

C8 and C18 methylene groups were oxidized to the corresponding ketones, the complete NOE circle observed for **4** was discontinued at C8 and C18 in **1b**, resulting in the two NOE sequences listed in Table 2. The other significant difference in NOEs observed for **1b** and **4** was the NOE pattern detected between the carbohydrate moieties and the porphyrin ring; the C5 and C15 meso protons of **1b** exhibited NOEs to both the carbohydrate C1' and C2' protons, whereas the same *meso* protons of **4** exhibited NOEs only to the C1' protons. These observations suggest that, unlike **4**, **1b** exists as a mixture of rotamers (**1b-A** and **1b-B**, Figure 2) with rotation

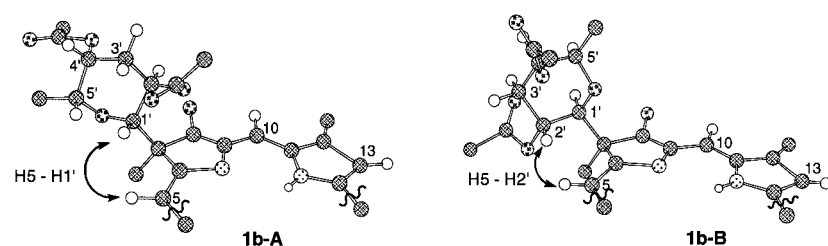


Figure 2. Partial structures of **1b-A** and **1b-B**. The NOEs are represented by two-ended arrows.

around the C-glycosidic bonds due to a lower energy barrier. The difference in the conformational flexibility between **1b** and **4** could be attributed to the difference in the steric effects between the C8 and C18 methylene groups in **4** and the C8 and C18 ketone moieties in **1b**. No NOEs from the carbohydrate C2' acetate groups to the porphyrin ring were observed.

Based on the evidence presented, there is no doubt concerning the structure of synthetic **1b**. However, the ^1H NMR spectrum (Figure 3)^[7] of this product does not match that of the *O,O*-diacetate prepared from natural (+)-tolporphin A.^[2] We therefore set out to elucidate the structure of (+)-tolporphin A.

We collected the ^1H - ^1H NOESY data for natural (+)-tolporphin A *O,O*-diacetate in CD_3COCD_3 and compared that with the data for the synthetic compound. The connectivity of the porphyrin skeleton (Table 3) was found to be identical to the connectivity of the synthetic material. As in the synthetic product, natural tolporphin A *O,O*-diacetate

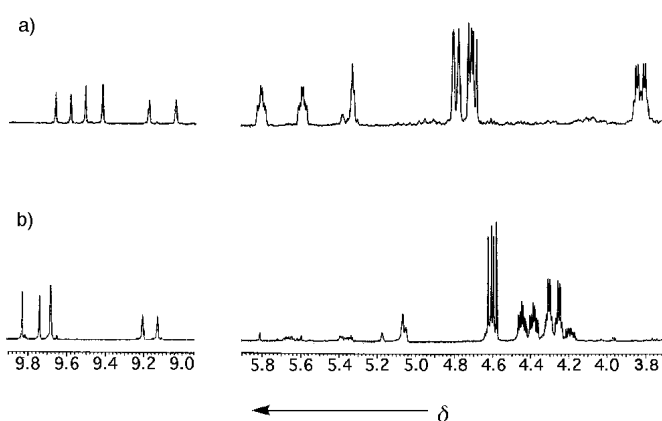


Figure 3. ^1H NMR spectra (600 MHz, CD_3COCD_3) of a) synthetic and b) natural tolporphin A *O,O*-diactates. The spectra cover the regions of carbohydrate ($\delta = 3.7$ – 5.9) and porphyrin ring protons ($\delta = 8.9$ – 9.9).

Table 3. Data on NOEs for natural tolporphin A *O,O*-diacetate.

H20 $\leftrightarrow$ H2	H10 $\leftrightarrow$ CH ₃ (C12)
H2 $\leftrightarrow$ CH ₃ (C3)	CH ₃ (C12) $\leftrightarrow$ H13
CH ₃ (C3) $\leftrightarrow$ H5	H13 $\leftrightarrow$ H15
H5 $\leftrightarrow$ CH ₃ (C7)	H15 $\leftrightarrow$ CH ₃ (C17)

exhibited NOEs between both the C1' and C2' protons on the carbohydrate groups and the C5 and C15 *meso* porphyrin protons. Again, this suggests rotational freedom around the C-glycosidic bonds. These results led us to conclude that the synthetic and natural products must differ only in the configuration at their quaternary centers, and thus there are

four possibilities for the structure of natural (+)-tolporphin A (Figure 4). Structure **A** represents our synthetic product, which does not match the natural product. Under the assumption that rings A and C are derived from the same biosynthetic precursor through the same biosynthetic pathway, it is unlikely that structures **B** or **C** represent the natural product, since the absolute configurations of their C7 and C17 quaternary

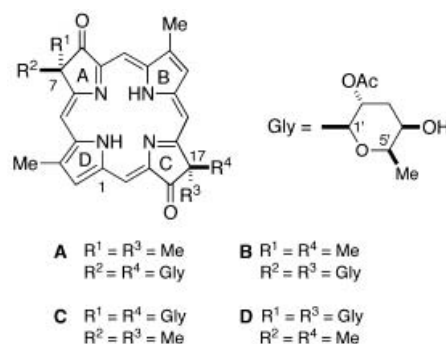


Figure 4. Four possible structures for tolporphin A, the representative member of the tolporphin class of natural products.

centers are opposite. Therefore, we propose that the structure of (+)-tolporphin A is most likely represented by structure **D**. This revised structure is consistent with all the spectroscopic data observed for the natural product and can be

unambiguously established by the synthesis of **D** starting with the monocyclic precursor **2b**.

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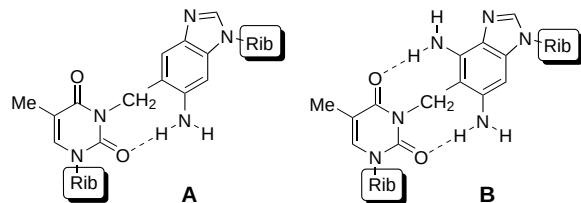
Keywords: natural products • NMR spectroscopy • porphyrinoids • structure elucidation

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[2] Tolyporphin A *O,O*-diacetate was prepared (Ac₂O, pyridine, RT) from natural (+)-tolyporphin A.^[3]
[3] M. R. Prinsep, F. R. Caplan, R. E. Moore, G. M. L. Patterson, C. D. Smith, *J. Am. Chem. Soc.* **1992**, *114*, 385.
[4] The numbering according to the proposed structure **1a** is adopted here.
[5] Eight additional tolyporphins have been isolated from the cyanophyte microalga: M. R. Prinsep, G. M. L. Patterson, L. K. Larsen, C. D. Smith, *Tetrahedron* **1995**, *51*, 10523.
[6] The ¹H–¹H NOESY and ¹H–¹H ROESY data were identical for each of the individual compounds studied. We chose to focus on the ¹H–¹H NOESY data because it displayed a better signal-to-noise ratio.
[7] The ¹H NMR spectra for the synthetic and natural tolyporphin A *O,O*-diacetates were shown to be concentration-independent in C₆D₆.

Covalently Cross-Linked Watson–Crick Base Pair Models**

Xiaoxin Qiao and Yoshito Kishi*

The concept of covalently linked cross sections with molecular architecture similar to Watson–Crick hydrogen-bonded base pairs was introduced by Leonard in the mid-1980s.^[1] Since then, several types of covalently linked systems have been developed and used for a variety of purposes.^[2] In our view, base pairs linked with a methylene bridge such as **A** and **B** may add some new aspects to the

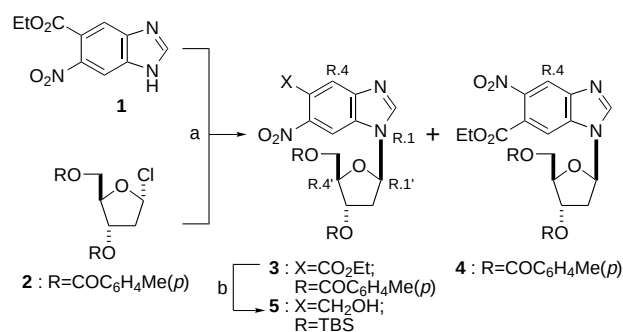


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chemistry of covalently linked nucleosides/nucleotides. These include: 1) only Watson–Crick and reversed Watson–Crick base pairings are possible for these models,^[15] and 2) conformational flexibility is expected to be retained along the bridging methylene bonds, in addition to their expected increased chemical stability. Evidently, there is concern about such models; introduction of the CH₂ bridge would not allow the two bases to be in the same plane, and consequently models such as **A** and **B** might not properly mimic the Watson–Crick base pairs. However, molecular mechanics calculations have suggested that the deformation caused by the introduction of the methylene bridge to a double-stranded oligomer may be insignificant.^[3] Herein we report a general synthetic route to the type **A** base pairs and the elucidation of their structures in both solid and solution states, thereby demonstrating that they indeed possess (some of) the structural characteristics of Watson–Crick hydrogen-bonded base pairs.

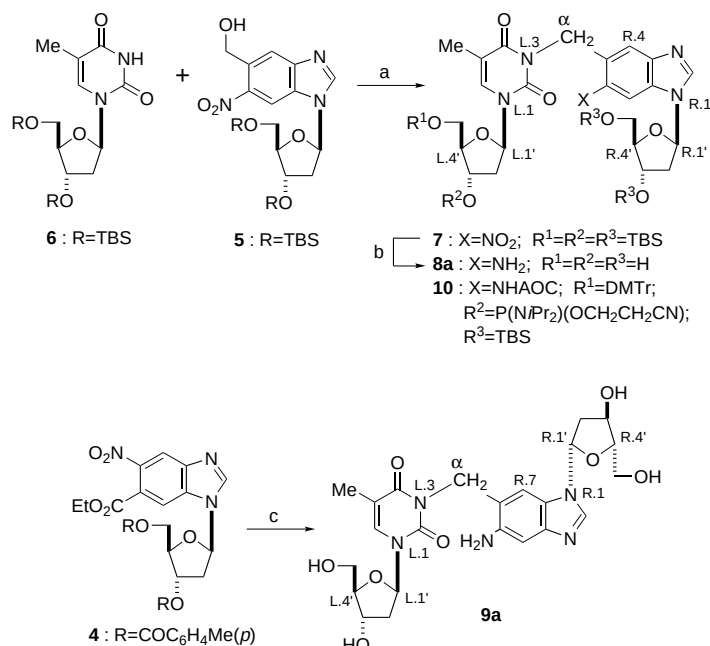
Ethyl 6-nitro-5-benzimidazole carboxylate (**1**; m.p. 154–157 °C (EtOH)) was readily prepared from commercially available 5-benzimidazole carboxylic acid in two steps (1. EtOH/cat. H₂SO₄/reflux and 2. fuming HNO₃/H₂SO₄/50 °C) in 85 % overall yield. Under phase-transfer conditions,^[4] **1** was coupled with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-erythro-pentose chloride (**2**)^[5] to give a 1:1 mixture of N1- and N3-glycosides **3** and **4**, which were readily separated by silica gel column chromatography (CH₂Cl₂ → CH₂Cl₂/EtOAc (4/1)) in 95 % combined yield (Scheme 1). A better than 20:1



Scheme 1. Reagents and conditions. a) KOH/[18]crown-6/MeCN/RT; b) 1. K₂CO₃/EtOH/RT; 2. TBS-Cl/imidazole/DMF/RT; 3. NaOH/*t*BuOH/RT; 4. ClCO₂Et/Et₃N/THF/0 °C; 5. NaBH₄/EtOH/0 °C. TBS = *tert*-butyl-dimethylsilyl.

β selectivity was observed for the N1- and N3-glycosides. The structures of **3** and **4** were established by NOESY experiments. In **3**, the R.1'-H exhibited strong crosspeaks with R.4'-H, R.2-H, and the proton *ortho* to the nitro group (R.7-H), respectively. In **4**, the R.1'-H showed strong crosspeaks with R.4'-H, R.2-H, and the proton *ortho* to the carboethoxy group (R.7-H), respectively.^[6]

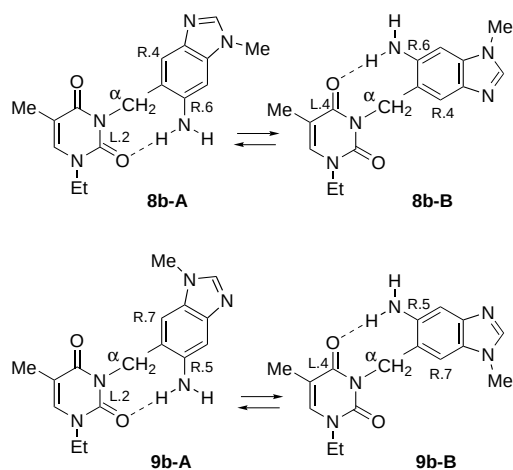
The N1-glycoside **3** was converted to the primary alcohol **5** in five steps in 55 % overall yield (Scheme 1). After mesylation, **5** was coupled with 3',5'-di-*O*-(*tert*-butyldimethyl)silyl-thymidine (**6**)^[7] to give the CH₂-linked product **7** in nearly quantitative yield (Scheme 2). Alternatively, the coupling of **5** with **6** to yield **7** was achieved in one step in 96 % yield under the Mitsunobu conditions.^[8] Reduction of the nitro group of **7**,



Scheme 2. Reagents and conditions. a) 1. Mesylation of **5**: MsCl/Et₃N/CH₂Cl₂/0 °C; 2. K₂CO₃/DMSO/RT or a) DEAD/PPh₃/THF/0 °C → RT; b) 1. SnCl₂/EtOH/RT → 70 °C; 2. TBAF/THF/RT; c) follow the steps shown under b in Scheme 1 and then the steps shown under a and b in this scheme. For the synthetic sequence for **10**, see reference [13]. AOC = allyloxycarbonyl, DEAD = diethylazodicarboxylate, DMTr = 4,4'-dimethoxytriphenylmethyl, Ms = methanesulfonyl, TBAF = tetrabutylammonium fluoride.

followed by *tert*-butyldimethylsilyl (TBS) deprotection, furnished the CH₂-linked **8a** (m.p. 215–216 °C (CF₃CH₂OH/MeOH)) in 65% overall yield from **7**. Using the same sequence of reactions, the CH₂-linked **9a** (m.p. >230 °C (95% EtOH)) was obtained from the N3-glycoside **4**.

Compounds **8a** and **9a** were recrystallized from trifluoroethanol/methanol and 95% methanol, respectively, to give fine needles. However, all efforts to grow a single crystal suitable for X-ray structure analysis met with no success. Therefore, we prepared^[9] the corresponding CH₂-linked base pairs **8b** (m.p. 215–217 °C (EtOAc/EtOH)) and **9b** (m.p. >



230 °C (EtOAc/EtOH)) and then subjected them to X-ray analyses (Figure 1).^[10] The X-ray analyses revealed interesting structural aspects. First, the CH₂-linked base pair **8b** illus-

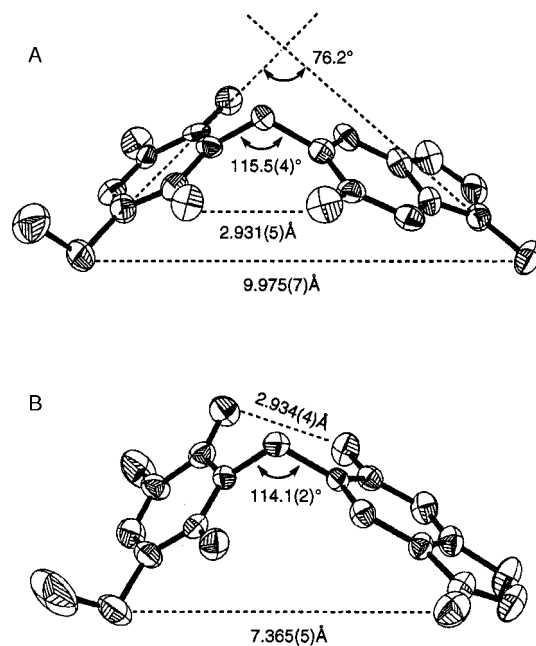


Figure 1. X-Ray structures of **8b** (A) and **9b** (B).

trates an excellent structural similarity to C–G and A–T base pairs; the X-ray structure analysis of **8b** gives the R.1'–L.1' distance of 9.975(7) Å and an angle of 76.2° (see Figure 1), compared with a C1'–C1' distance of 10.60 ± 0.15 Å and an angle of 68 ± 2° for C–G and A–T base pairs.^[11] Superposition of the X-ray structure of **8b** over C–G and A–T base pairs illustrates that the areas that they occupy are nearly identical (Figure 2). Second, a hydrogen bond is

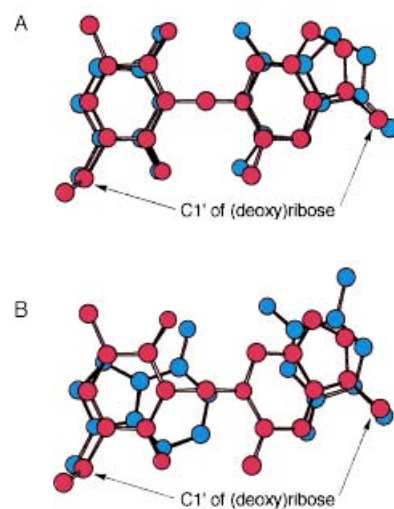


Figure 2. Superposition of the X-ray structure of **8b** (red) over C–G (A; blue) and A–T (B; blue) base pairs.

clearly recognizable between the L.2 C=O and the R.6 N–H group, that is, the distance between the oxygen atom at L.2 and the nitrogen atom at R.6 is 2.931 Å. However, there is no intermolecular hydrogen bond recognizable in the crystal packing of **8b**, that is, there is no Hoogsteen or wobble pairing present even in the solid state. Third, the sp³-bridging displayed

a substantially widened bond angle ($115.5(4)^\circ$). Fourth, contrary to the **8b** series, in the **9b** series the R.5 amino group is hydrogen-bonded with the carbonyl oxygen atom at L.4. Thus, like the **8b** series, both the R.1-Me group and the L.1-Et group remain on the same side of the molecule in the **9b** series. However, the R.1'-L.1' distance of $7.365(5)$ Å and an angle of 43.5° does not allow **9b** to occupy an area similar to that occupied by a C-G or A-T pair. Once again, there is no intermolecular hydrogen bond detected for **9b**.

The rotational freedom along the CH_2 bridge was studied by temperature-dependent ^1H NMR spectroscopy (Figure 3). Most significantly, the proton signals of the CH_2 bridge of **8b** were observed as a sharp singlet at room temperature but split

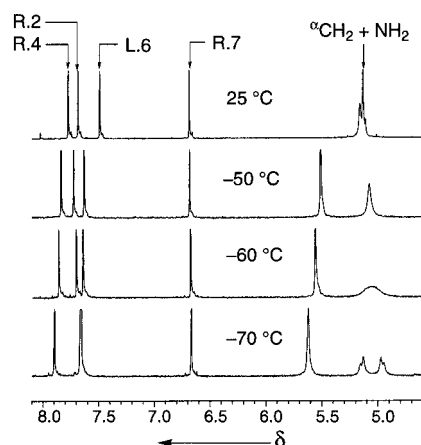
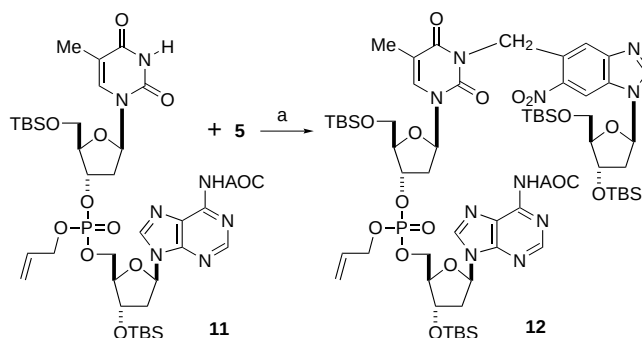


Figure 3. Variable-temperature ^1H NMR spectra (500 MHz, in $[\text{D}_6]\text{acetone}$; $\delta = 4.6\text{--}8.2$ region) of **8b**. The indicated chemical shift assignments represent those at room temperature. However, the chemical shifts of R.2 and R.4 protons are switched with each other at -50°C .

into an AB quartet at about -70°C ; the coalescent temperature of about -60°C suggests the energy barrier for the rotation to be about 10 kcal mol^{-1} .^[12] The trend in chemical shift change observed for the NH_2 protons indicated their involvement in hydrogen-bonding interactions. Although there is no experimental evidence available at present, it is tempting to suggest the preferred solution conformer to be **8b-A**, corresponding to the conformer observed in the solid state. The exact same phenomenon was observed for **9b**, with the energy barrier for the rotation being again about 10 kcal mol^{-1} . Once again, the preferred solution conformer at -70°C probably corresponds to the solid-state conformer **9b-B**. Furthermore, the nucleotides **8a** and **9a** exhibited structural characteristics parallel to **8b** and **9b**, respectively.

The structural characteristics observed for covalently cross-linked base pairs such as **8** and **9** may suggest their interesting and unique potential. It is worth commenting on the flexibility of the current synthetic route. As the base-induced coupling of **5** is effective for the thymidines with $\text{R} = \text{Ac}$, benzoyl (Bz), *tert*-butyldiphenylsilyl (TBDPS), triethylsilyl (TES), tetrahydropyranyl (THP) in **6**, or even with unprotected thymidine ($\text{R} = \text{H}$ in **6**), it should be feasible to synthesize the CH_2 -linked base pairs bearing the left-side 2-deoxyribose moiety protected differentially from the right-side portion. Indeed, the CH_2 -linked base pair **10** (Scheme 2) was obtained

straightforwardly^[13] and should be a useful precursor for preparation of oligonucleotides containing the covalently linked base pair. Alternatively, one could couple an oligonucleotide with an oligonucleotide through methylene bridge formation to obtain a double-stranded oligomer. In order to test the feasibility of this approach, the dinucleotide **11**^[14] was coupled with mesylated **5**, to furnish **12** in excellent yield (Scheme 3).



Scheme 3. Reagents and conditions. a) 1. **5**: $\text{MsCl}/\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2/0^\circ\text{C}$; 2. $\text{K}_2\text{CO}_3/\text{DMSO}/\text{RT}$.

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- The fifth base pair in a self-complementary, double-stranded nanomer $[\text{d}(\text{tgactgact})_2]$ was replaced with the covalently cross-linked base pair **B**, and the resulting nanomer $[\text{d}(\text{tgacXgact})_2]$ ($\text{X}_2 = \text{B}$) was subjected to an energy minimization (MacroModel, force field: Amber), showing that the energy-minimized $[\text{d}(\text{tgacXgact})_2]$ retained B-form without losing any one of 22 Watson–Crick type hydrogen bonds. However, slight deformations were noted for the base pairs and ribose-backbones adjacent to the base pair **B**.
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- Compounds **8b** and **9b** were prepared by functionalization of ethyl 1-methyl-6-nitro-5-benzimidazole carboxylate or ethyl 1-methyl-5-nitro-6-benzimidazole carboxylate (1. $\text{NaOH}/t\text{BuOH}/\text{RT}$; 2. ClCO_2Et /

Et₃N/THF/0 °C; 3. NaBH₄/EtOH/0 °C; 4. MsCl/Et₃N/CH₂Cl₂/0 °C), followed by coupling (K₂CO₃/DMSO/RT) with 1-ethylthymine.

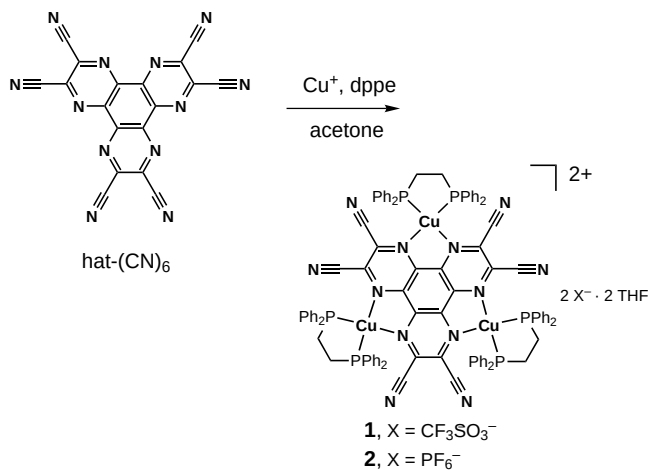
- [10] The L1-Me base pairs corresponding to **8b** and **9b** were also subjected to X-ray structure analyses; their structural characteristics were found to be very similar to those observed for **8b** and **9b**, respectively. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-114659 (**8b**) and CCDC-114660 (**9b**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
- [11] For example, see: *Nucleic Acids in Chemistry and Biology*, 2nd ed. (Eds.: G. M. Blackburn, M. J. Gait), Oxford University Press, Oxford, **1996**, p. 21.
- [12] For reviews on dynamic NMR spectroscopy, see: E. L. Eliel, S. H. Wilen, L. N. Mander, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, p. 502, and references therein.
- [13] This transformation was carried out in six steps: 1. coupling (K₂CO₃/DMSO/RT) of **6** (R = Ac) with mesylated **5**; 2. SnCl₄/EtOH/RT → 70 °C; 3. AOC-OBT/py/RT; 4. NH₃/MeOH/RT; 5. DMTt-Cl/AgNO₃/py/THF/RT; 6. ClP(NiPr₂)(OCH₂CH₂CN)/NEt₃/CH₂Cl₂/RT. AOC = allyloxycarbonyl, BT = benzotriazole.
- [14] The dinucleotide **11** was prepared from 5'-O-(*tert*-butyldimethylsilyl)thymidine 3'-O-(allyl-*N,N*-diisopropylphosphoramidite) and 3'-O-*tert*-butyldimethylsilyl-*N*⁶-allyloxycarbonyl-2'-deoxyadenosine in two steps: 1. 1*H*-tetrazole/MeCN/RT; 2. *t*BuCO₂H/CH₂Cl₂/RT, and then coupled (K₂CO₃/DMSO/RT) with mesylated **5**.
- [15] Note added in proof (February 11, 1999): In the sense of primary hydrogen-bonding base pairing, only Watson–Crick and reversed Watson–Crick base pairings are possible for models **A** and **B**. However, it should be noted that Hoogsteen triplets such as T–AT and T–GC are envisioned for model **B**, but not for model **A**.

A New Anion-Trapping Radical Host, [(Cu-dppe)₃{hat-(CN)₆}]²⁺ **

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Hexaazatriphenylene hexacarbonitrile, hat-(CN)₆, is expected to act as a unique multidentate ligand because the characteristic electron-deficient heterocyclic core has low-lying degenerate π* orbitals. To date several metal complexes

containing hat derivatives have been reported;^[1,2] however, preparation of metal complexes containing the hat-(CN)₆ ligand is extremely difficult because the coordination ability of the aromatic nitrogen atoms in hat-(CN)₆ drastically decreases due to the presence of the electron-withdrawing cyano groups. The one-electron reduction of hat-(CN)₆ efficiently enhances its coordinating ability, and we have been able to demonstrate this by isolating the first transition metal complexes **1** and **2** (dppe = 1,2-bis(diphenylphosphanyl)ethane), which exhibit anion-trapping behavior both in the solid state and in solution (Scheme 1).



Scheme 1. Synthesis of **1** and **2**.

Complexes **1** and **2** were prepared in a one-pot reaction of a copper(I) source ([Cu(CF₃SO₃)₂(benzene)] (**1**) or [Cu(CH₃CN)₄]PF₆ (**2**), hat-(CN)₆, and dppe in acetone. The hat-(CN)₆ ligand is reduced by the copper(I) ion in the solution, affording the corresponding [hat-(CN)₆]^{•-} ion. This anion can also be prepared electrolytically. The crystal structures of **1** and **2** have been determined by X-ray crystallography.^[3]

The cation in **1** has a trinuclear structure in which the unique sixdentate anion radical ligand [hat-(CN)₆]^{•-} coordinates to three Cu-dppe fragments (Figure 1). A similar

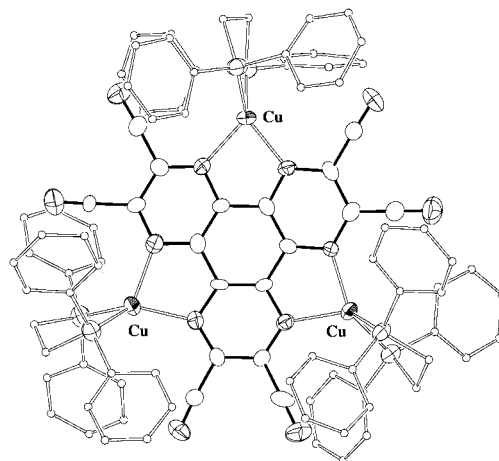


Figure 1. ORTEP view of the cationic moiety of **1** with thermal ellipsoids at 50 % level for Cu, P, C, and N atoms. All the hydrogen atoms are omitted for clarity. Ellipsoids of the carbon atoms in phenyl groups have been arbitrarily reduced for clarity.

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trinuclear structure is found in **2**. Although the molecular structures for **1** and **2** slightly deviate from D_{3h} symmetry, all the copper(I) ions have intrinsically a similar distorted tetrahedral environment with a N_2P_2 chromophore. The ESR spectra for both complexes at 77 K show one similar resonance due to a typical organic radical. The obtained g value of 1.998 is less than that of a free electron (2.002). A similar feature has been observed for metal complexes containing anion radical ligands.^[4] Anisotropic spectra at low temperature display features characteristic of the axial symmetry; the $g_{||}$ and $g_{\perp}$ values estimated from the spectrum at 5.4 K are 1.970 and 2.002, respectively. The UV spectrum of **2** in acetone shows an intense absorption (dark green; 712 nm ($\epsilon = 19600$)) with a shoulder at about 770 nm derived from the radical $[\text{hat}-(\text{CN})_6]^{-\bullet}$. The thin-layer cyclic voltammogram^[5] of **2** in THF shows multiredox couples at $E_{1/2} = +0.47, +0.18, -0.16$, and -0.91 V (vs. SCE), which are assigned to $[\text{hat}-(\text{CN})_6]^{0/[\text{hat}-(\text{CN})_6]^{-}}$, $[\text{hat}-(\text{CN})_6]^{-}/[\text{hat}-(\text{CN})_6]^{2-}$, $[\text{hat}-(\text{CN})_6]^{2-}/[\text{hat}-(\text{CN})_6]^{3-}$, and $[\text{hat}-(\text{CN})_6]^{3-}/[\text{hat}-(\text{CN})_6]^{4-}$, respectively. Thus, the compound $\text{hat}-(\text{CN})_6$ is in the one-electron-reduced $[\text{hat}-(\text{CN})_6]^{-\bullet}$ state at the resting potential (+0.27 V). Notably the coordination of the copper(I) ions induces the positive shift of the redox waves, which results in the formation of a stable radical complex.

The trapping of molecules by synthetic receptors^[1, 6, 7] is one of the most attractive areas of study in the field of host–guest chemistry, and the complexation of anions has recently been recognized and developed as a new area of coordination chemistry.^[1, 7] The anion trapping behavior of the cationic moiety $[(\text{Cu-dppe})_3\{\text{hat}-(\text{CN})_6\}]^{2+}$ in the complexes **1** and **2** is well-illustrated by CPK representations such as that shown for **1** in Figure 2. Six phenyl groups of the three dppe ligands

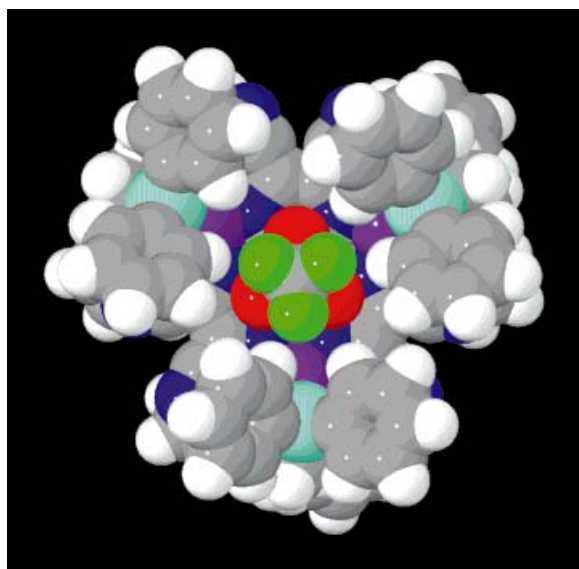


Figure 2. The anion trap structure **1** depicted as a CPK representation (top view). Color code: C: gray, H: white, N: blue, Cu: purple, F: green, O: red, P: sky blue.

create concave cavities on each side of the planar $[\text{hat}-(\text{CN})_6]^{-}$ unit, into which the CF_3SO_3^- ions are effectively trapped. The inclusion occurs on both sides of the cation. Compound **2** has essentially the same anion trapping structure in the solid state.

The anion radical ligand $[\text{hat}-(\text{CN})_6]^{-\bullet}$ in **2** facilitates the evaluation of the host–guest interaction in solution by conventional NMR techniques. The ^{31}P NMR resonance for the PF_6^- ion of $(n\text{Bu}_4\text{N})\text{PF}_6$ and **2** in CDCl_3 at room temperature is given in Figures 3a and 3b, respectively. Figure 3b

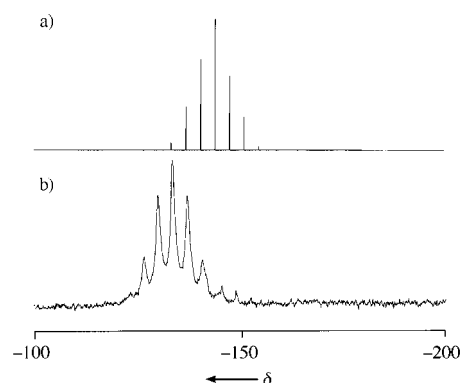


Figure 3. ^{31}P NMR spectra at room temperature for $(n\text{Bu}_4\text{N})\text{PF}_6$ (a) and **2** (b) in CDCl_3 solution.

shows an extremely broad signal at $\delta = -134.1$ (septet $J(^{19}\text{F}, ^{31}\text{P}) = 713 \pm 10$ Hz), which is shifted downfield relative to that for the free PF_6^- ion (Figure 3a). Due to the absence of a direct bonding interaction between the radical center and the counteranion PF_6^- , the temperature-dependence of the chemical shift is simply accounted for by the paramagnetic effect (pseudo-contact term; $\delta_{\text{pseudo-con}}$).^[8] On the basis of Equation (1),^[9] the separation between the $\text{hat}-(\text{CN})_6$ center and the observed nucleus was estimated to be 3.3 Å. The separation is quite similar to the distance (3.9 Å) estimated from the crystal structure analysis, which clearly indicates that the anion sits in a position so close to the $\text{hat}-(\text{CN})_6$ ligand that the electronic-nuclear spin magnetic interaction operates effectively. Thus, the PF_6^- ion is well-trapped in the positively charged concave cavities, even in solution.^[10]

Experimental Section

All operations were carried out under an argon atmosphere by using standard Schlenk techniques. $(n\text{Bu}_4\text{N})\text{BF}_4$ was used as a supporting electrolyte with a saturated calomel electrode (SCE) as the reference electrode. $\text{H}_3\text{PO}_4/\text{D}_2\text{O}$ (85:15) at 23 °C was used as an external shift reference for the ^{31}P NMR spectroscopy.

The compound $\text{hat}-(\text{CN})_6$ was prepared according to literature procedures^[11] from diaminomaleonitrile and hexaoxocyclohexane.

A solution of dppe (1.168 g, 2.93 mmol) in acetone (10 mL) was added to a solution of $[\text{Cu}(\text{CF}_3\text{SO}_3)_2(\text{benzene})]^{[12]}$ (0.740 g, 1.47 mmol) in acetone, and the mixture was then added to a solution of $\text{hat}-(\text{CN})_6$ (0.282 g, 0.73 mmol) in acetone (20 mL). The reaction mixture immediately turned dark green, the color attributed to the formation of $[\text{hat}-(\text{CN})_6]^{-\bullet}$ ion. This indicated that $\text{hat}-(\text{CN})_6$ was reduced by the copper(I) ion. Addition of MeOH (150 mL) to the resultant dark green solution precipitated microcrystals, which were recrystallized from THF/hexane to give dark green crystals (52 %). Elemental analysis calcd for $\text{C}_{98}\text{H}_{72}\text{Cu}_3\text{F}_6\text{N}_{12}\text{O}_6\text{P}_6\text{S}_2$ (%): C 56.91, H 3.51, N 8.13; found: C 55.92, H 3.64, N 7.93.

Compound **2** was prepared in a similar way to compound **1**, except that $[\text{Cu}(\text{CH}_3\text{CN})_4](\text{PF}_6)^{[13]}$ was used as the starting material. All other operations for this species were carried out using the same method as for compound **1**. Elemental analysis calcd for $\text{C}_{96}\text{H}_{72}\text{Cu}_3\text{F}_{12}\text{N}_{12}\text{P}_8$ (%): C 55.97, H 3.52, N 8.16; found: C 55.91, H 3.57, N 7.92.

The complex **2** was also synthesized electrolytically. The compound hat-(CN)₆ was reduced in acetone at −0.3 V (vs. SCE) in a 100 mL electrolysis cell, in which (nBu₄N)PF₆ was used as the supporting electrolyte. The resultant green solution was added to a solution of [Cu(CH₃CN)₄]PF₆ and dppe in acetone. The product corresponded to that obtained from the direct mixing of hat-(CN)₆, [Cu(CH₃CN)₄]PF₆, and dppe.

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- [3] The data collections for **1** and **2** were made on a Rigaku RAXIS-IV imaging plate area detector with graphite monochromatic MoK α radiation. The data for **1** and **2** were collected at a temperature of −30 °C to a maximum 2θ value of 51.4 and 51.3°, respectively. A total of 12 and 14 oscillation images (6.00°) were collected, each being exposed for 8.0 and 18.0 min, respectively. A total of 9858 and 9073 reflections, respectively, were collected. The linear absorption coefficients, μ , for MoK α radiation are 7.4 and 8.0 cm^{−1}, respectively. The data were corrected for Lorentz and polarization effects. Crystal data for **1**: C₁₀₆H₈₈N₁₂Cu₃S₂O₈F₆P₆, M_r =2212.53, orthorhombic, space group $P2_12_12_1$, a =24.545(3), b =26.687(1), c =16.0793(8) Å, V =10532(1) Å³, Z =4, ρ_{calcd} =1.395 g cm^{−3}, T =−30 °C, $2\theta_{\text{max}}$ =51.4°, R =0.073, R_w =0.071. Crystal data for **2**: C₁₀₄H₈₈N₁₂Cu₃F₁₂O₂P₈, M_r =2204.33, monoclinic, space group $C2/c$, a =28.552(2), b =15.777(4), c =25.298(2) Å, β =95.006(7)°, V =11352(1) Å³, Z =4, ρ_{calcd} =1.290 g cm^{−3}, T =−30 °C, $2\theta_{\text{max}}$ =51.3°, R =0.093, R_w =0.135. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-107042 (**1**) and 107044 (**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] The pseudo-contact term; $\delta_{\text{pseudo-con}}$ is represented by Equation (1), where β , k , and S have the usual meaning, while R is a distance between a paramagnetic center and the observed nucleus, and θ is an angle between the molecular principal axis and the R vector. The R

$$\delta_{\text{pseudo-con}} = \beta^2 S(S+1)/9kTR^3(1-3\cos^2\theta)(g_{\parallel}^2 - g_{\perp}^2) \quad (1)$$

value was calculated to be 3.31 Å by using the slope of a linear plot of the observed chemical shift for PF₆[−] against T^{-1} , and the anisotropic g values obtained from the ESR spectrum at 5.4 K.

- [10] Similar trapping behavior (δ = −133.3 at room temperature) for the PF₆[−] ion was observed in benzene, while the anion release occurs in acetone, in which relatively sharp signals centered at δ = −143.6, close to that of free PF₆[−], were observed.
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Noncovalent Assembly of a Fifteen-Component Hydrogen-Bonded Nanostructure

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The design and synthesis of molecules that contain all the necessary information to organize themselves spontaneously into well-defined finite nanostructures provides one of the most important challenges in supramolecular chemistry.^[1, 2] Strong coordinative and much weaker hydrogen bonds are possible interactions that can drive the assembly process. The reversible formation of helicates,^[3, 4] grids,^[5] cages,^[6, 7] metal-lodendrimers,^[8] or hydrogen-bonded rosettes,^[9, 10] capsules,^[11] spheres,^[12] dendrimers,^[13, 14] polymers,^[15] and other architectures^[16, 17] has been realized over the past decade. The major difficulty in this area is to control the assembly process whilst increasing the structural complexity of the assembly.^[18–20]

We have previously reported the formation of assembly **1₃** · (DEB)₆ (DEB = 5,5-diethylbarbituric acid) that is held together by 36 hydrogen bonds.^[21] We are currently investigating the formation of supramolecular libraries of noncovalent assemblies,^[22] with the long-term objective of this work being to develop self-assembled nanostructures with binding properties that mimic those of natural antibodies.^[23] Therefore, we have now investigated the assembly of tetramelamine derivatives **2**, in which two calix[4]arene units are covalently connected through a flexible linker X, with four equivalents of DEB. Variation of the size and chemical nature of the connector unit X creates chemical diversity in the resulting

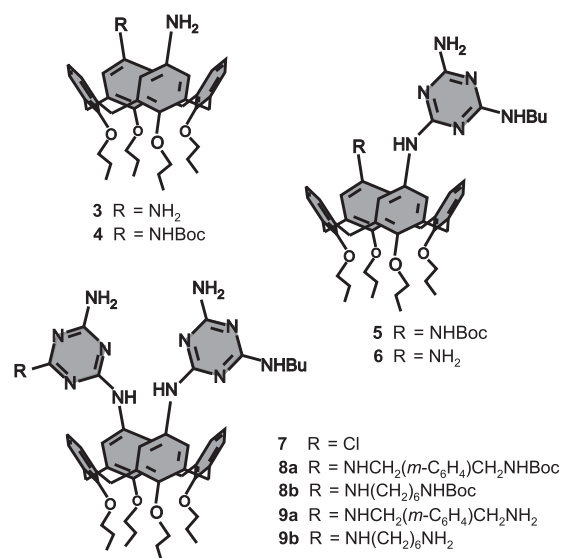
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cavities. Guest binding can be optimized in these cavities by using chemical evolution.^[24]

Herein we report the first generation of such noncovalent assemblies $2_3 \cdot (\text{DEB})_{12}$ that consist of 15 different components and are held together by 72 hydrogen bonds (Figure 1). The assemblies were fully characterized by 1D and 2D ^1H NMR spectroscopy and matrix-assisted laser-desorption time-of-flight (MALDI-TOF) mass spectrometry by using our recently developed Ag^+ -labeling technique.^[25]

The syntheses of the tetramelamine derivatives **2** were completed in ten steps starting from diamine **3**. Mono protection of **3** with *tert*-butylcarbamate (Boc) gives **4**, and subsequent reaction with cyanuric chloride, NH_3 , and *n*-butylamine gives triazine **5**. Removal of the Boc group followed by another reaction with cyanuric chloride and NH_3 affords chlorotriazine **7**, which is converted into either **8a** or **8b** by reaction with mono-Boc protected *m*-xylylenediamine (**8a**) or mono-Boc protected 1,6-hexylenediamine (**8b**). Finally, removal of the Boc groups and reaction with an equimolar amount of **7** gives both tetramelamines **2a** and **2b** in overall yields of 26 % and 32 %, respectively.

The tetramelamines **2a** and **2b** form well-defined assemblies $2_3 \cdot (\text{DEB})_{12}$ in the presence of four equivalents of DEB in CDCl_3 . The ^1H NMR titration of both tetramelamines with DEB proves the 1:4 stoichiometry of the assemblies. At a ratio of $2:\text{DEB} = 1:4$ all the tetramelamine resonances are absent and the spectra exclusively exhibit signals for assemblies $2_3 \cdot (\text{DEB})_{12}$ (Figure 2). Four singlets of equal intensity are observed for assembly $2_3 \cdot (\text{DEB})_{12}$ at $\delta = 14.01$, 13.83, 13.42, and 12.86, which pairwise represent the $\text{C}(\text{O})\text{NHC}(\text{O})$ protons (four different types, total of 24) of the various "floors" of the assembly (see Figure 1). Two singlets at $\delta =$



8.40 and 8.25 correspond to the twelve ArNHAr protons and resonances at $\delta = 7.67$ and 7.32 correspond to the aliphatic and benzylic NH protons, respectively. The addition of excess DEB does not change the ^1H NMR spectrum of the assembly, except for the additional broad signal at $\delta \approx 9$ that corresponds to free DEB (Figure 3).

Tetramelamine **2a** exhibits a characteristic difference in assembly behavior compared to bismelamines **1**.^[26] Whereas assembly of bismelamines **1** with DEB displays positive cooperativity, the formation of assembly $2_3 \cdot (\text{DEB})_{12}$ shows negative cooperativity.^[27] At a ratio of $2_3:\text{DEB} < 1:3$ the ^1H NMR spectrum mainly exhibits broad resonances, which is

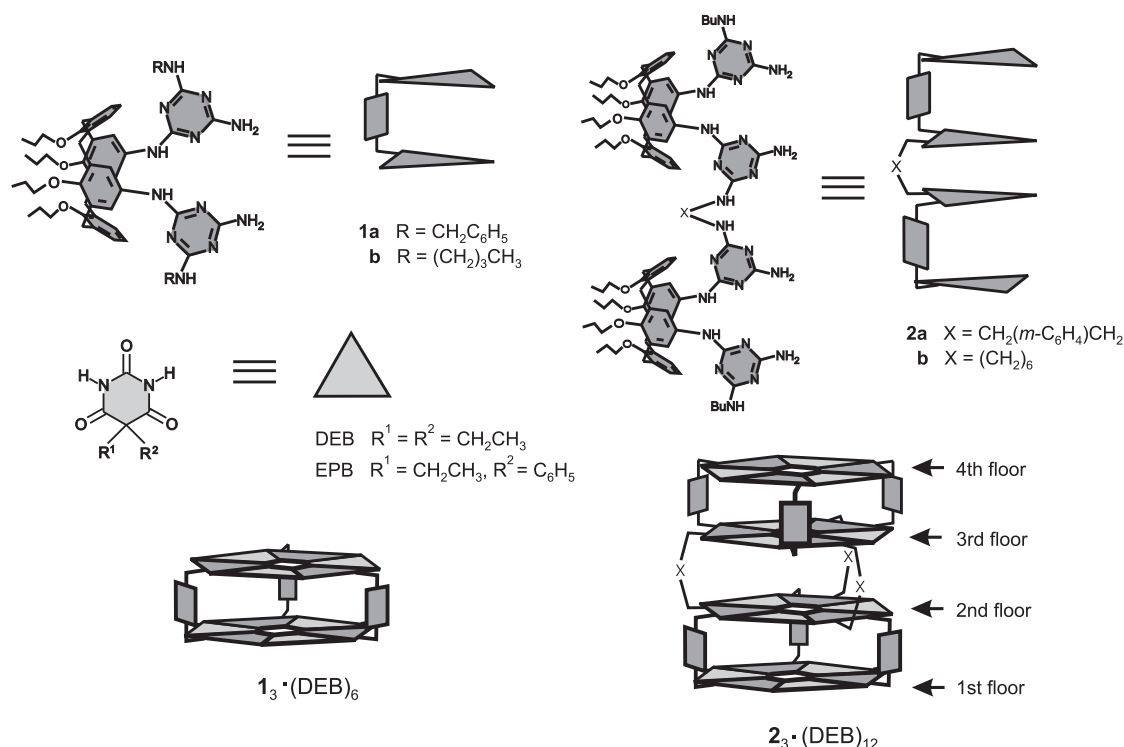


Figure 1. Schematic representation of the aggregates $1_3 \cdot (\text{DEB})_{12}$ and $2_3 \cdot (\text{DEB})_{12}$.

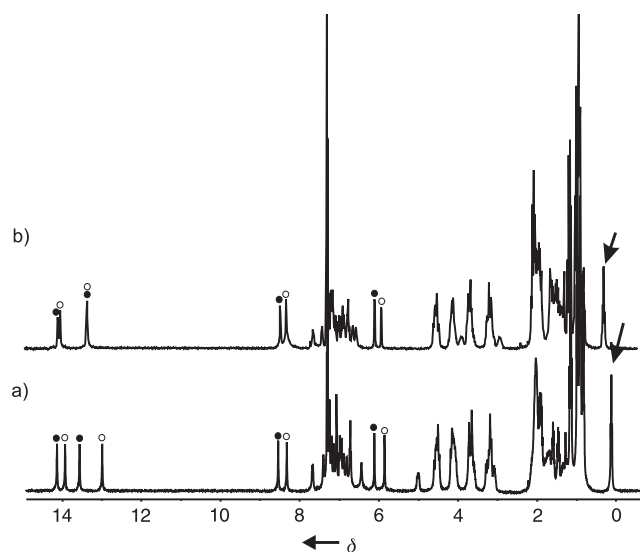


Figure 2. ^1H NMR spectra (400 MHz) of assembly $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ (a) and assembly $2\mathbf{b}_3 \cdot (\text{DEB})_{12}$ (b) in CDCl_3 at room temperature. $\bullet$ indicates first and fourth floor resonances, $\circ$ indicates second and third floor resonances; the arrows on the right mark half of the DEB methyl resonances on the second and third floor.

indicative of the formation of a nondefined assembly (Figure 3). Only at a ratio of $2\mathbf{a}:\text{DEB} > 1:3$ does the assembly $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ start to form. This negative cooperativity leads to a lower stability of $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ than expected from the melting point index $I_{\text{Tm}} = \text{HB}/(N-1)$ for hydrogen-bonded aggregates, where HB = number of particles in the aggregate.^[28] This index predicts an increased stability for assembly $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ (5.1) relative to $1_3 \cdot (\text{DEB})_6$ (4.5). However,

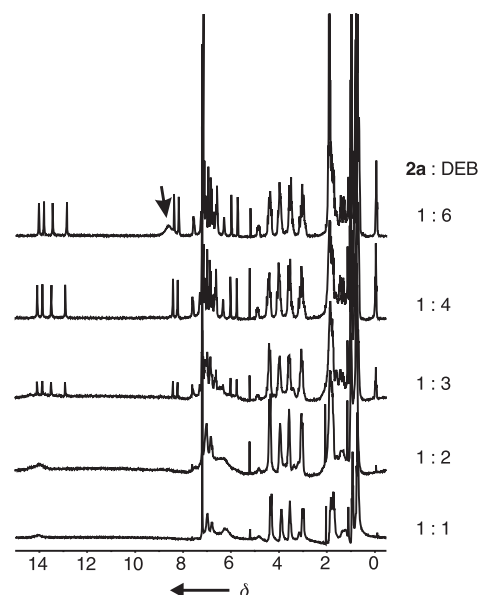


Figure 3. ^1H NMR titration of tetramelamine $2\mathbf{a}$ with DEB in CDCl_3 at room temperature. The arrow in the upper spectrum marks the resonance corresponding to the unbound DEB.

experiments in which assembly $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ was mixed with either bismelamine $1\mathbf{a}$ or $1\mathbf{b}$ clearly show that the stability order is reversed. In both cases only assembly $1_3 \cdot (\text{DEB})_6$ is formed at the expense of $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$. The reduced stability of $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ is most probably related to the inward curvature of the two rosette planes as observed in the X-ray crystal structure of assembly $1_3 \cdot (\text{DEB})_6$ (interplanar distance is 3.2 Å in the center and 3.5 Å at the outside).^[21] Formation of the second double rosette in assembly $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ may therefore be much less favorable because of incomplete filling of the space in between the two double rosettes.

Interestingly, both $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ and $2\mathbf{b}_3 \cdot (\text{DEB})_{12}$ assemble stereoselectively in one of the two possible (sss and ses)

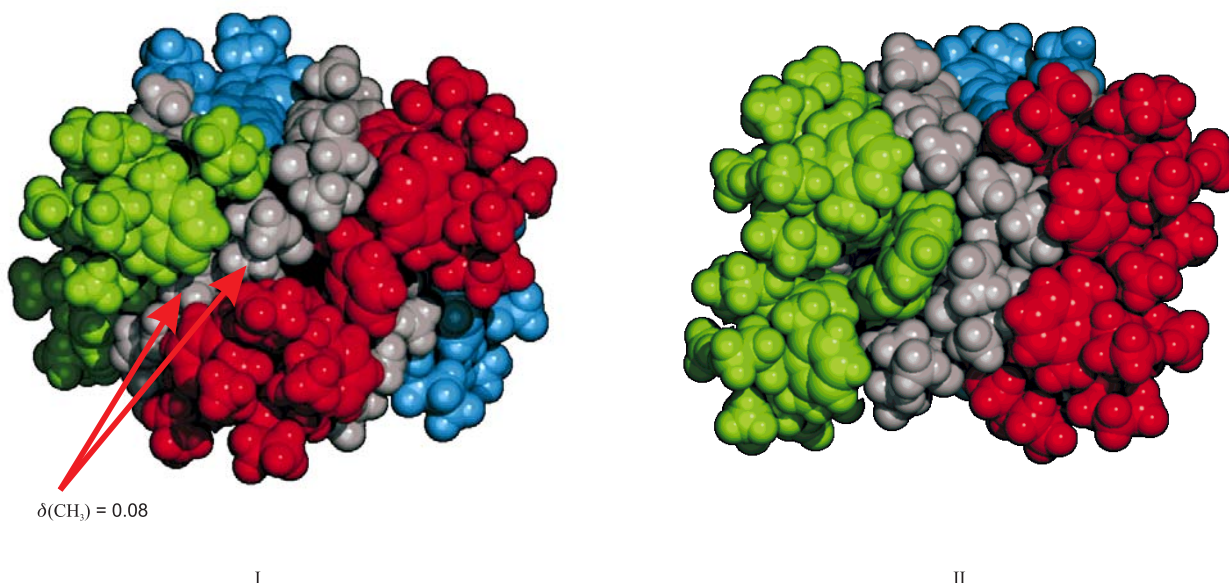


Figure 4. Energy-minimized structures (Quanta/CHARMm 3.3) of the sss (I) and the ses (II) diastereoisomeric forms of $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$.

diastereomeric forms,^[29, 30] as indicated by the presence of one single set of proton signals for both assemblies. Evidence as to which of the two diastereomers is formed comes from the presence of the strongly upfield-shifted resonance at $\delta = 0.08$ ($2\mathbf{a}_3 \cdot (\text{DEB})_{12}$) or $\delta = 0.25$ ($2\mathbf{b}_3 \cdot (\text{DEB})_{12}$), which represents half of the methyl groups of DEB (namely, 18H) that are located in the two middle floors of the assembly. Gas-phase MM calculations (Quanta/CHARMM 3.3)^[31] show that in the sss isomer half of the methyl protons on the second floor point right into the face of one of the calix[4]arene aromatic rings on the third floor (Figure 4), which causes the upfield shift. The same type of interaction is not possible in the corresponding ses isomer as the same aromatic rings are much more remote.

The hydrogen-bonded nanostructures were also characterized by MALDI-TOF mass spectrometry using Ag^+ -labeling.^[25] 5-Ethyl-5-phenylbarbituric acid (EPB) was used for this purpose instead of DEB because of the lack of a binding site for the Ag^+ ion in the assemblies with DEB. Samples prepared by mixing assemblies $2\mathbf{a}_3 \cdot (\text{EPB})_{12}$ and $2\mathbf{b}_3 \cdot (\text{EPB})_{12}$ with 1.5 equivalents of AgCF_3COO in CHCl_3 show intense signals at $m/z = 8585.2$ (calcd for $\text{C}_{468}\text{H}_{546}\text{N}_{96}\text{O}_{60} \cdot {}^{107}\text{Ag}^+ = 8583.1$) and at $m/z = 8525.3$ (calcd for $\text{C}_{462}\text{H}_{558}\text{N}_{96}\text{O}_{60} \cdot {}^{107}\text{Ag}^+ = 8523.1$), respectively, for the corresponding monovalent Ag^+ complexes (Figure 5). Signals corresponding to

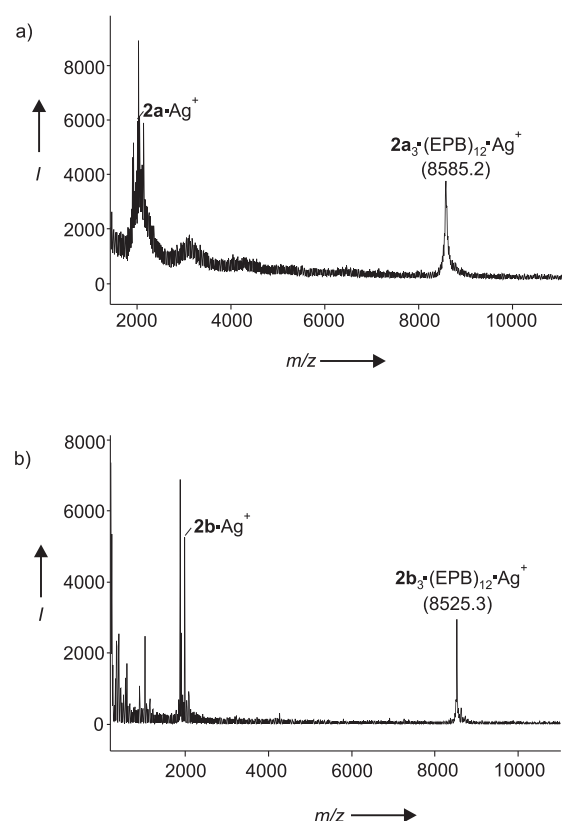


Figure 5. MALDI-TOF mass spectra of the Ag^+ complexes of assembly $2\mathbf{a}_3 \cdot (\text{EPB})_{12}$ (a) and assembly $2\mathbf{b}_3 \cdot (\text{EPB})_{12}$ (b). Matrix = 2,5-dihydroxybenzoic acid.

more highly charged or fragmented Ag -complexed assemblies were not observed.

Both tetramelamines **2** and bismelamines **1** show a high degree of self sorting.^[32] Mixtures of **2a** and **1a**, in a ratio of

1:2, with a slight excess of DEB exclusively show the formation of the homomeric assemblies $2\mathbf{a}_3 \cdot (\text{DEB})_{12}$ and $1\mathbf{a}_3 \cdot (\text{DEB})_6$. Formation of heteromeric assemblies $2\mathbf{a}_2 \cdot 1\mathbf{a}_2 \cdot (\text{DEB})_{12}$ and $2\mathbf{a} \cdot 1\mathbf{a}_4 \cdot (\text{DEB})_{12}$ are not observed. Similar results were obtained for tetramelamine **2b**. Self sorting has been observed before in dynamic covalent libraries of macrocycles^[32] and in metal-coordinated helicates.^[33, 34]

When equal amounts of **2a** and **2b** were mixed with DEB the formation of heteromeric assemblies containing both tetramelamines was observed. This indicates that supramolecular library formation with different tetramelamines is indeed possible. The second generation of assemblies with specific binding sites in the connector unit X, such as metalloporphyrins, is currently under investigation.

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Discovery of Novel Catalysts for Alkene Epoxidation from Metal-Binding Combinatorial Libraries**

Matthew B. Francis and Eric N. Jacobsen*

The quest for practical routes to chiral intermediates has inspired extensive research activity in the field of asymmetric catalysis, which has resulted in the development of numerous useful, highly enantioselective reactions. The vast majority of effective catalysts discovered thus far are metal complexes bearing specific ligands that direct the outcome of the catalytic reaction through control of the steric and electronic properties of the metal center. While progress has been made in elucidating the nature of these interactions, the identification of this finely tuned match between the metal ion and its coordination environment remains difficult and continues to limit the pace of reaction discovery.

Research directed toward the development of new systems for asymmetric catalysis can be divided into two phases. In the initial lead discovery phase effort is directed toward screening a wide variety of metal complexes with the goal of identifying a novel catalyst system for the reaction of interest. This is typically followed by a lead optimization stage wherein a highly enantioselective and reactive system is sought through systematic variation of the ligand components and reaction conditions. In the latter context, combinatorial chemistry has already emerged as a powerful tool: useful chiral catalysts have been obtained through the synthesis and analysis of parallel libraries of structural analogues based on a previously identified design motif.^[1] However, despite the utility of combinatorial chemistry for the efficient investigation of systems that involve numerous interrelated variables, the application of such strategies to catalyst lead discovery remains underexplored. We reported recently the application of metal-binding combinatorial libraries to the identification of coordination complexes.^[2] Herein we describe the successful elaboration of this strategy to the discovery of novel catalyst leads for a reaction of synthetic interest, namely the asymmetric epoxidation of olefins with hydrogen peroxide.

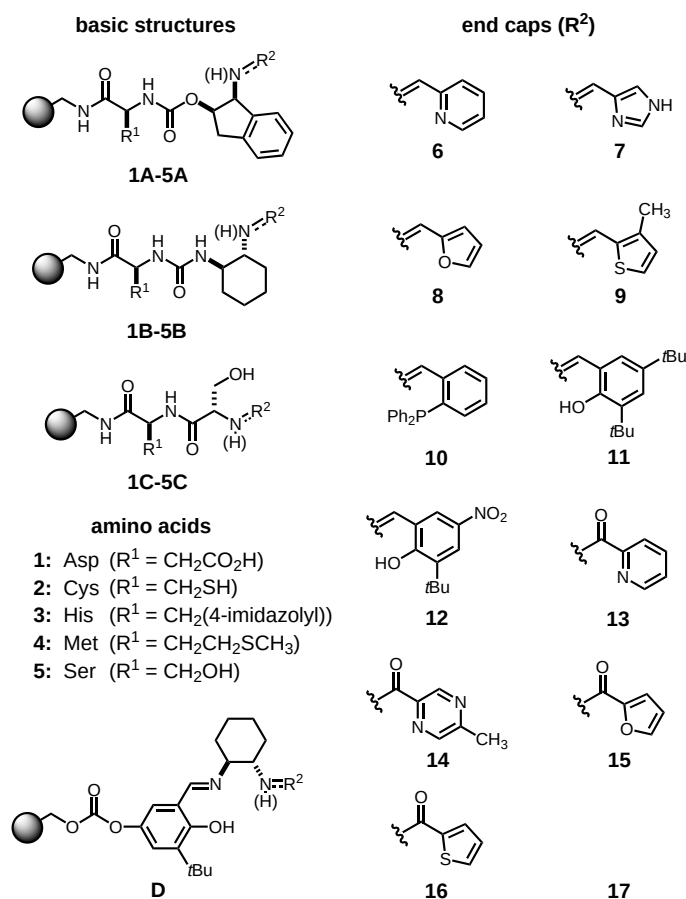
Because of the extreme sensitivity of oxidation systems to the exact coordination environment around the metal center^[3] an ideal library design for the discovery of epoxidation catalysts—and selective catalysts in general—should consist of the widest possible variety of metal ions bound by an assortment of ligands, providing diverse coordination environments. Our initial approach to such a library design is depicted in Scheme 1. The general structural motif involves

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Scheme 1. Composition of a ligand library for transition metals. Sixteen basic core structures were synthesized on a polystyrene support (0.6 mmol g⁻¹ loading, 100–200 mesh particle size), represented by the shaded sphere. A series of 12 end caps were attached, and resulted in the formation of 192 potential ligand structures.

the joining of potential metal-binding moieties by chiral linking groups, and is envisioned to provide a binding pocket for metal-ion guests. Thus, five amino acids that displayed a variety of donor side chains were coupled to aminomethyl polystyrene (100–200 mesh particle size, 0.6 mmol g⁻¹ loading) by standard peptide coupling techniques.^[4] Three linking elements were attached to these building blocks. Derivatives of 1-amino-2-indanol (**1A–5A**) and *trans*-1,2-diaminocyclohexane (**1B–5B**) were chosen to represent conformationally restricted backbones, and the amino acid serine was incorporated to furnish less rigid binding environments (**1C–5C**). In order to include structures similar to known epoxidation catalysts, salicylimine **D** was prepared by coupling 6-*tert*-butyl-4-hydroxysalicylaldehyde to polystyrene substituted with 4-benzyloxymethyl alcohol (Wang resin), followed by condensation with one equivalent of 1,2-diaminocyclohexane. A series of 12 capping agents **6–17**, chosen to incorporate a range of heterocycles, phosphanes, and salicylimines through imine and amide bond formation, were attached to the resulting 16 basic structures. The resulting library of 192 potential ligands was then pooled in preparation for metal ion insertion.

A set of 30 metal ion sources, which differed in metal ion identity, oxidation state, and counterion, was chosen for

incorporation. A pool of the ligand library was exposed to a 0.02 M solution^[5] of each metal ion in THF or THF/MeOH for 1 hour. Facile isolation of the desired complexes was achieved by filtration and thorough rinsing of the beads. After the entire catalyst library was pooled, visual detection of the metal-complex colors combined with the application of qualitative inorganic staining reagents^[2] indicated that approximately 80% of the 5760 possible metal–ligand complexes had been prepared (Figure 1). In all cases, the binding of metal ions to the unfunctionalized polymer support was not observed.

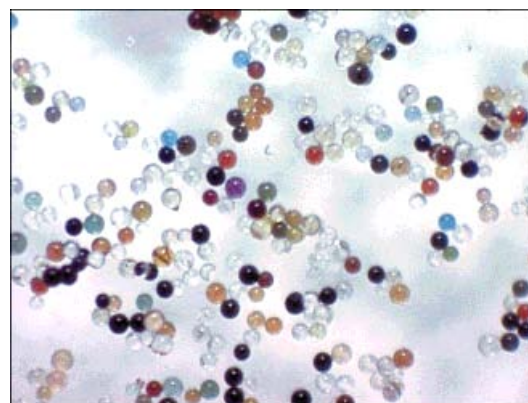


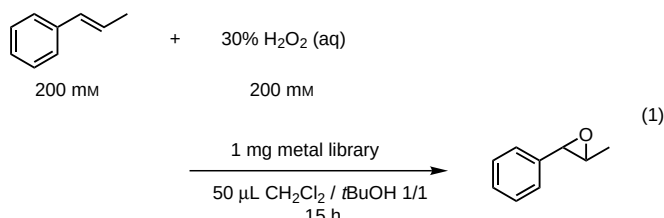
Figure 1. Portions of the ligand library depicted in Scheme 1 were exposed in separate reaction vessels to each of the 30 metal ion sources listed in Figure 2. This microscope photo shows the colors of the potential 5760 metal–ligand complexes after recombination. Each polymer bead is 90 μm in diameter.

The identification of catalysts from large libraries is a challenging endeavor. In addition to issues of rate and turnover number,^[6] the ability to distinguish desired reactivity from nonproductive or unselective pathways is of crucial importance. In the present context, we have chosen to directly assay the products of catalytic reactions by using high-throughput chromatographic techniques. In the case of parallel libraries this allows the full and unambiguous evaluation of each catalyst synthesized, and this approach can be used to accurately screen for virtually any reaction. In addition, while the screening of catalyst mixtures by this technique may not yield reliable information about enantioselectivity, it can be used to rapidly differentiate pools of catalysts that produce the desired product from pools of catalysts that do not. In this way catalyst libraries too large for parallel screening can be reduced to manageable numbers of catalyst candidates in an efficient manner.

Our strategy for the identification of epoxidation catalysts therefore involved a three step process. First, the entire pooled catalyst library was screened for compatible reaction conditions for the epoxidation of the model substrate *trans*-β-methylstyrene (TBMS). This screening led to the identification of aqueous H₂O₂ as a viable oxidant.^[7] Its low cost, lack of deleterious by-products, and ease of handling make hydrogen peroxide a particularly practical oxidant; however, the associated disproportion and homolytic bond cleavage pathways have limited its use with most epoxidation systems.^[8] The optimal solvent system was found to be a 1/1 mixture of CH₂Cl₂ and *tert*-butanol, which was selected for its inertness

toward redox reactions and its ability to dissolve appreciable amounts of aqueous H_2O_2 while still swelling the polystyrene beads.

The second stage in the screening process involved the determination of active metal libraries for the epoxidation of TBMS with aqueous H_2O_2 . For this purpose catalyst sub-libraries were prepared that contained a mixture of all 192 ligands and each individual metal source. Samples of the catalyst pools^[9] were exposed to 50 μL of a TBMS/ H_2O_2 solution (each 0.2 M) in $\text{CH}_2\text{Cl}_2/t\text{BuOH}$ (1/1) for 15 h [Eq. (1)].



Subsequent GC analysis of the reaction solutions revealed that several of the metal libraries demonstrated epoxidation activity, with those prepared with VOSO_4 and FeCl_2 proving the most effective (Figure 2).^[10] A screen of the unbound metal ion sources indicated that VOSO_4 displayed epoxidation activity in the absence of the ligand library; however, the

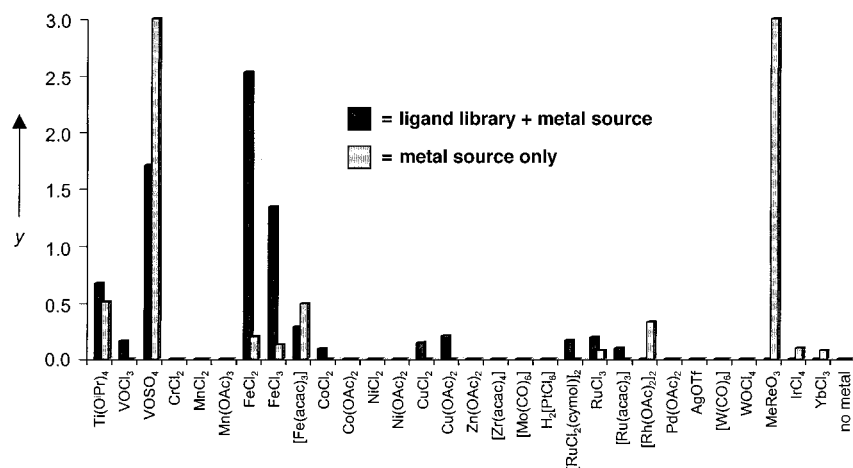


Figure 2. Epoxidation activity as a function of metal ion source under the conditions described in Equation (1). The y axis represents the ratio of detected epoxide product relative to an internal standard (relative yield). The dark bars represent the activity of each metal ion in the presence of the pooled ligand library, and the light gray bars indicate the activity of the metal ion sources in the absence of the ligands.

activity in iron libraries was strongly dependent on the presence of the ligands. It was also observed that the source of iron was important, with complexes prepared with FeCl_2 being roughly twofold more active for epoxidation than FeCl_3 libraries, and much more active than those prepared with $\text{Fe}(\text{acac})_3$ or $\text{Fe}(\text{OAc})_2$ (not shown).

Upon determining that FeCl_2 -derived libraries displayed ligand-promoted epoxidation catalysis, the third step in the screening process was to identify the ligand components necessary for catalytic activity. The ligand library was prepared in 12 batches, each containing a mixture of the 16 basic structures and a different end cap, and the correspond-

ing FeCl_2 complexes were screened under the conditions described above for the epoxidation of TBMS. From the resulting data (Figure 3) it is evident that the end caps have a

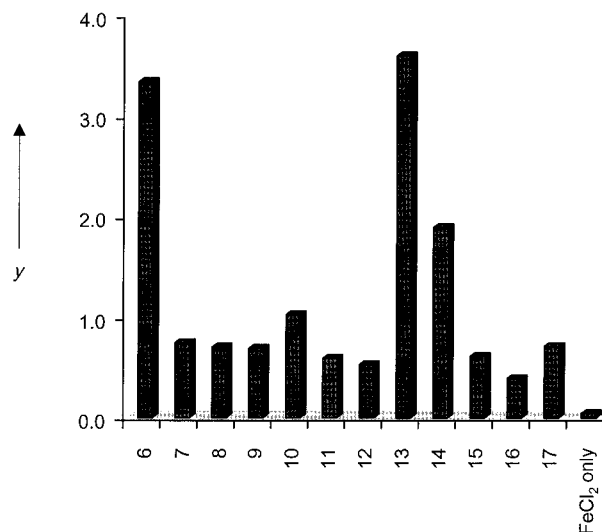


Figure 3. Epoxidation activity for end cap sub-libraries prepared with FeCl_2 as the metal ion source. The y axis represents the yield of epoxide product relative to an internal standard, and corresponds to a mixture of the sixteen basic ligand structures with the indicated end cap.

dramatic effect on epoxidation activity, with pyridine-containing end caps **6** and **13** producing the most active catalyst libraries. Piperazine cap **14** promoted epoxidation to a lesser extent, while all of the other end cap libraries led to very low levels of epoxide formation.

The next logical step in the deconvolution of the catalyst library would be to identify active catalyst structures by evaluating only the sixteen compounds corresponding to the most active end cap pools. However, by overlooking the least active end cap libraries singularly active catalysts present among pools of inactive structures might be missed. We therefore chose to evaluate all of the 192 ligand structures individually^[11] to check the validity of this strategy. Following metal complex formation with FeCl_2 ,

each library member was screened in a parallel fashion for the epoxidation of TBMS with 30% H_2O_2 , and a representation of the epoxide yield data appears in Figure 4. A quick inspection of the data reveals the presence of three ligand structures (**5C-6**, **5C-13**, and **2C-13**) that produce catalysts that are substantially more active than other structures in the library.

A comparison of the corresponding structures (**18–20**) reveals several similarities. First, all of the structures exhibit the expected pyridine-containing end caps **6** and **13**, which indicates that at least in this case a simple ligand deconvolution strategy would not have missed the most active catalysts. Second, the incorporation of serine as a linking

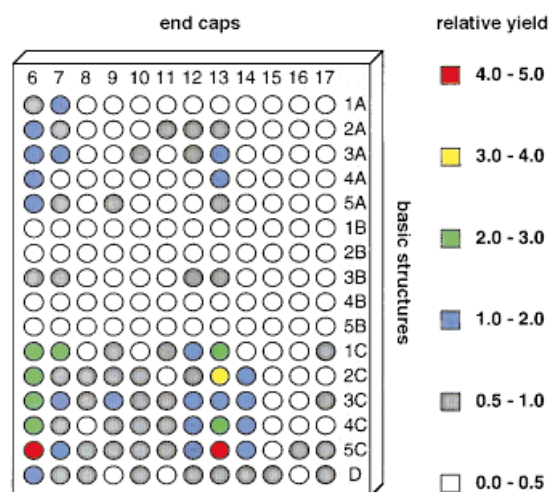
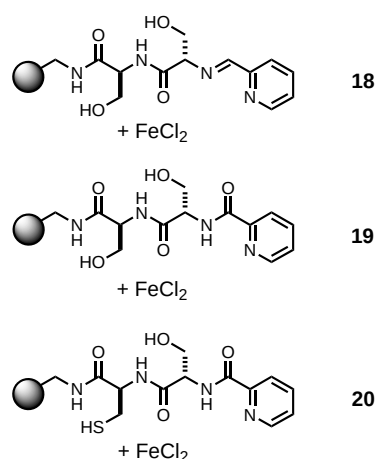


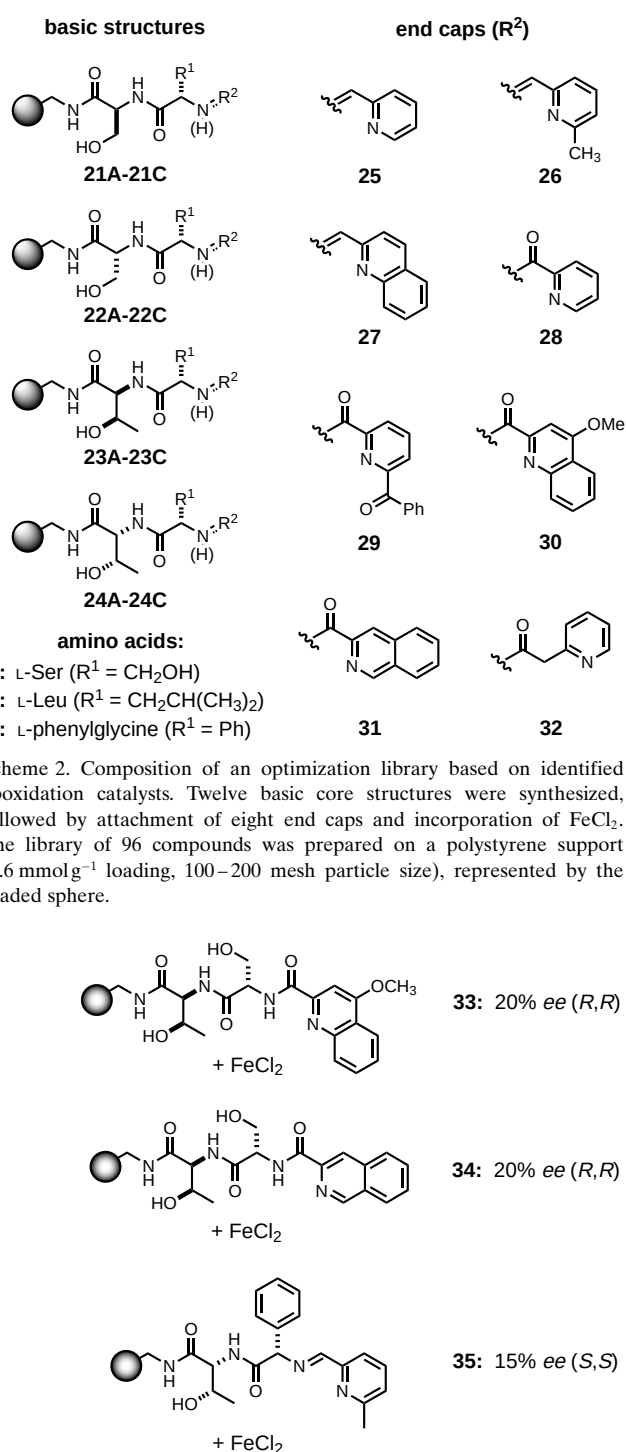
Figure 4. Epoxidation activity in a parallel library of iron complexes. Relative product yields are represented in a color-coded format (right), with the most active (red) combinations reaching 59% conversion. In all cases, the *cis*-epoxide product was not observed. The labeling of the ligands corresponds to Scheme 1.

group (**C**) gives rise to high levels of catalytic activity, while analogous structures with linking groups **A** and **B** are much less active. Finally, the amino acid attached to the solid support has a significant influence on catalytic activity, with serine-containing structures **18** and **19**, and cystine-containing



20 showing product yields twice as high as analogues prepared with other amino acids. The structures of all three ligands were confirmed by resynthesis and electrospray-MS analysis.^[12]

While these complexes represent structurally novel epoxidation catalysts, their value as lead structures would be greatly enhanced if they produced the epoxide products with measurable enantioselectivity. In fact, catalysts **18** and **19** did effect the epoxidation of TBMS in 4 and 7% ee, respectively; however, a better chiral ligand with higher enantioselectivities would clearly be desirable. To this end we have screened the parallel library^[10] depicted in Scheme 2 for the epoxidation of TBMS, which led to the identification of structures **33**–**35** as moderately enantioselective catalysts.^[13] Up to 78% conversion of TBMS can be obtained (with the epoxide as the sole reaction product) by using the optimized reaction conditions of 1.5 equiv of 30% H_2O_2 and 5 mol % of **34**.^[12, 14]



Scheme 2. Composition of an optimization library based on identified epoxidation catalysts. Twelve basic core structures were synthesized, followed by attachment of eight end caps and incorporation of FeCl_2 . The library of 96 compounds was prepared on a polystyrene support (0.6 mmol g^{-1} loading, 100–200 mesh particle size), represented by the shaded sphere.

These experiments serve to illustrate the ability of combinatorial chemistry to discover catalysts that bear no structural resemblance to previously known systems. Through the efficient screening of a library of 5760 ligand–metal complexes three novel, highly efficient catalysts have been discovered for the epoxidation of TBMS with aqueous H_2O_2 . An initial parallel optimization library has produced moderately enantioselective variants, which indicates that the evolution of additional libraries to obtain catalysts that produce synthetically useful enantioselectivities is worthy of pursuit. The identified structures show a synergistic depend-

ence on metal ion, counterion, and all ligand components, and therefore would have been difficult to identify using traditional approaches. Furthermore, the screening method used in these studies should be immediately applicable to the discovery of catalysts for almost any desired reaction, and we are currently evaluating the scope of this strategy in our laboratories.

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- [10] Many other metal–ligand libraries produced a variety of non-epoxide reaction products or catalyzed the nonproductive disproportionation of H₂O₂ to O₂ and water.
- [11] The facile synthesis of parallel ligand libraries was accomplished with an IRORI directed synthesis system.

[12] See supporting information.

[13] As would be expected, nearly all of the 96 FeCl₂ complexes provided good levels of epoxidation activity, which confirmed the validity of **18** and **19** as catalyst lead structures. However, ligands prepared with end cap **32** showed uniformly poor reactivity, which indicates that proper attachment of the pyridine ring to the peptide chain is of crucial importance.

[14] As immobilization of these catalysts to polymeric supports might influence their activity, the evaluation of soluble analogues is underway and will be reported in due course.

Long-Range Electron Transfer through DNA Films**

Shana O. Kelley, Nicole M. Jackson, Michael G. Hill,* and Jacqueline K. Barton*

The possibility of efficient DNA-mediated charge transport has been debated since the discovery of the double helix.^[1, 2] Photoinduced electron transfer between reactants bound to DNA, or between bases contained within the π stack, has led to varied conclusions regarding the nature of DNA as a medium for long-range charge transport.^[3–5] Consistently, however, species well stacked within the helix have exhibited remarkably fast electron transfer over long distances.^[3] Indeed, the integrity of the base stack itself appears necessary for efficient long-range electron transfer, as perturbations caused by intervening mismatches or bulges greatly diminish the yields of DNA-mediated charge transport.^[3, 6]

Electrochemistry has been used extensively to investigate the kinetics of electron transfer through self-assembled monolayers on solid surfaces.^[7] Systems that feature redox-active head groups held at variable distances by aliphatic alkanethiols or conjugated linkers have yielded important information regarding the ability of different media to promote long-range electronic coupling. In an effort to investigate DNA-mediated electron transfer involving ground-state reactants, we have applied these methods to study redox-active intercalators bound at discrete sites within the individual helices of a DNA monolayer on gold.

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We have previously developed techniques for assembling DNA duplexes derivatized at the 5' end with thiol-terminated linkers onto gold surfaces.^[8, 9] Electrochemical assays, radio-active tagging experiments, and atomic force microscopy (AFM) all indicate that the duplexes form densely packed monolayers oriented in an upright position with respect to the gold.^[9, 10] This contrasts the structure of monolayers formed from single-stranded oligonucleotides, where the individual DNA strands may lie flat on the gold surface.^[11]

Duplex-modified electrodes exhibit very high affinities for DNA-binding substrates, and promote efficient electron transfer between the electrode and redox-active intercalators non-covalently bound to the helices.^[8] However, in previous studies the exact location of the intercalators within the duplexes was not fixed, hence a systematic evaluation of the effect of distance on the rate of electron transfer through DNA could not be obtained.

To investigate charge transduction through DNA as a function of distance, we have site-specifically cross-linked a redox-active intercalator, daunomycin (DM),^[12] into the DNA

characterized.^[14b] The site of intercalation was controlled in the duplexes by incorporating a single guanine–cytosine (GC) base step in otherwise adenine–thymine (AT) or inosine–cytosine (IC) sequences; as DM requires the N2 atom of guanine for covalent cross-linking, the intercalator is constrained to these positions. Moving the GC step along the duplex therefore provided a systematic variation in the location of the DM-binding site relative to the thiol-terminated linker. Cross-linking DM to the DNA in solution, and then depositing the labeled duplexes onto gold, afforded a series of films in which the intercalator was linked quantitatively at a known separation from the electrode surface (Figure 2).

The AFM images of films composed of 15-base-pair DM-labeled duplexes were virtually identical to those of the unlabeled analogues,^[9] revealing densely packed monolayers with heights greater than 45 Å at open circuit. Cyclic voltammograms obtained for these electrodes showed the reversible reduction of DM at −0.65 V versus SCE,^[12] and possessed features characteristic of surface-bound species (e.g., linear plots of peak current versus scan rate).^[15] Integration of the electrochemical signals gave surface coverages (*Γ*) of electroactive DM ranging from 60–75 pmol cm^{−2}; these values are in good agreement with the coverages of 15-base-pair duplexes previously measured by ³²P labeling.^[8, 16, 17] Given the 1:1 stoichiometry of cross-linked DM to DNA (confirmed by UV/Vis spectroscopy), these data indicate that all of the bound DM is electrochemically reduced at the modified surfaces; doping the films with increasing percentages of DM-free duplexes resulted in a linear decrease in the electrochemical signals, suggesting that each of the bound intercalators is electrochemically active.

Remarkably, efficient reduction of DM was observed regardless of its position along the 15-base-pair sequence (Figure 3).^[18] Not only were the intensities of the DM signals

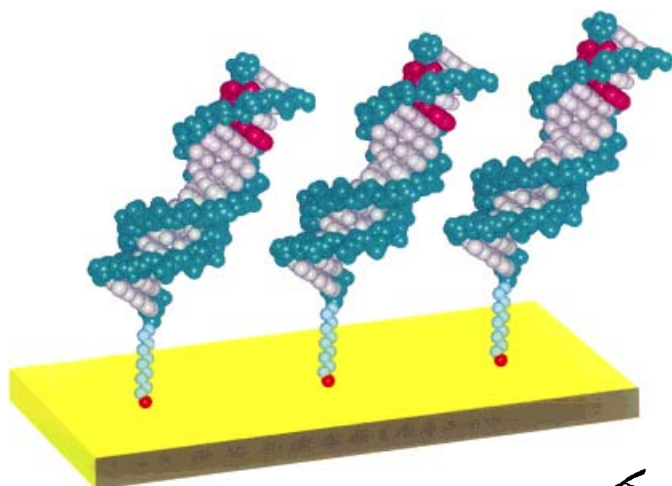


Figure 1. Schematic illustration of a gold surface modified with 15-base-pair thiol-modified duplexes, including the DM–guanine cross-link incorporated into the DNA monolayer. The sizes of all components—DNA base stack (grey), sugar–phosphate backbone (green), DM (magenta), and linker (blue; the terminal thiol is red)—are depicted relative to actual proportions. The helices shown are oriented at an angle of about 70° to the gold surface, which results in a through-space separation between DM (central plane) and the electrode of approximately 50 Å.

films (Figure 1). Daunomycin undergoes a reversible reduction^[13] within the potential window of the monolayers,^[8] and covalent adducts of intercalated DM cross-linked to the 2-amino group of guanine^[14a] have been crystallographically

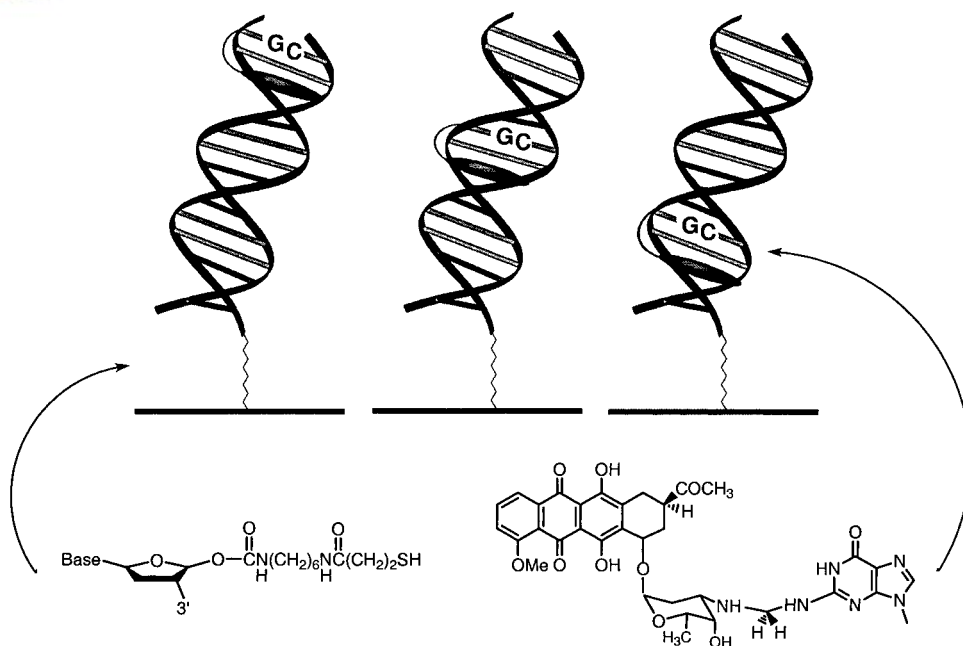


Figure 2. Schematic diagram depicting DNA duplexes used for study of distance-dependent reduction of DM. Also depicted are the DM–guanine cross-link (right) and the thiol-terminated tether which connects the duplex to the electrode surface (left). This tether provides 16 σ bonds between the electrode and the base stack.

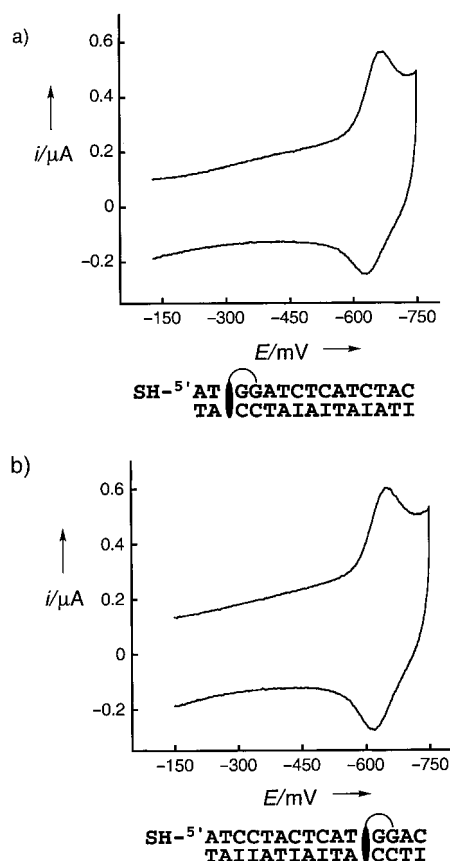


Figure 3. Cyclic voltammograms of gold electrodes modified with the DM-cross-linked thiol-terminated duplexes: a) SH-5'ATGGATCTCATCTAC + inosine complement and b) SH-5'ATCCTACTCATGGAC + inosine complement; where the bold Gs represent the DM cross-linking site. For these duplexes, the surface coverage of electroactive DM, as measured by integrating the currents within these voltammograms, were 0.65×10^{-10} and 0.75×10^{-10} mol cm $^{-2}$. The DM:DNA stoichiometry for these same samples, measured by absorption spectroscopy, were 0.9:1 and 1.1:1, respectively. Thus, the charge does not depend on distance, but does reflect the yield of cross-linking.

the same for each of the duplexes studied, but the characteristic splittings between the cathodic and anodic waves as a function of scan rate were essentially invariant throughout the entire series (Figure 4). Clearly, within the resolution of this experiment, increasing the through-helix DM–gold separation does not substantially affect the rate of electron transfer.

If the electron transfer pathway proceeds through the double helix, these results indicate exceptionally efficient charge transport through DNA and imply that the rate-determining step may be tunneling through the σ -bonded linker. A shallow distance dependence through the π stack would be consistent with the results of photoinduced electron transfer between intercalators and other well-stacked species,^[3] but is in sharp contrast to findings of analogous studies in which the reactants were not or poorly intercalated into the base stack.^[4] These latter studies report values for β (the decay of electronic coupling with distance)^[19] ranging from 0.6–1.4 Å $^{-1}$. It should be noted that β here reflects only an empirical evaluation of the distance dependence.^[20] In our system, a β value of 1.4 Å $^{-1}$ would imply that the rate of electron transfer should drop by more than 15 orders of

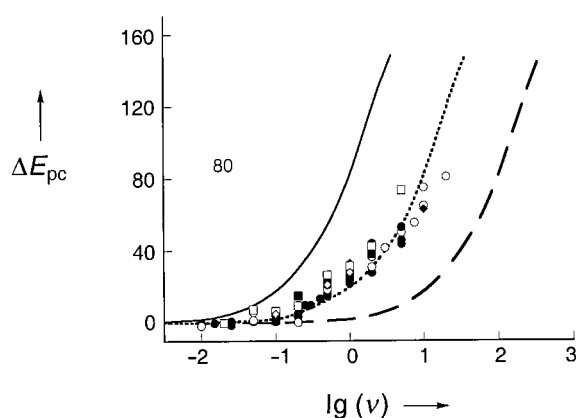


Figure 4. Plot of peak splitting ΔE_{pc} ($\Delta E_{pc} = E_{pc} - E^0$) versus $\lg(v)$ (v = scan rate). Measurements were obtained on the following sets of duplexes: (●) SH-5'ATGGATCTCATCTAC (+ inosine complement), (○) SH-5'ATCCTACTCATGGAC (+ inosine complement), (▲) SH-5'GCATTATATAATTA (+ complement), (□) SH-5'ATATGCTATAATTA (+ complement), (◆) SH-5'ATTATATAGCATTA (+ complement), (■) SH-5'ATTATATAATTGCT (+ complement). For comparison, simulated curves^[34] which correspond to rate constants varying by two orders of magnitude are shown: $k \approx 10^1$ s $^{-1}$ (—), 10^2 s $^{-1}$ (·····), 10^3 s $^{-1}$ (---). IR compensation was employed at scan rates higher than 1 V s $^{-1}$.

magnitude between the two sequences shown in Figure 3. Similarly, if β were 0.6 Å $^{-1}$, these rates would differ by greater than six orders of magnitude. Such large changes are inconsistent with our data; a β value of 0.1 Å $^{-1}$ would yield a difference in the rates for these two assemblies of only one order of magnitude, which may not be detectable by our analysis.

Could an alternative gating mechanism be responsible for the lack of distance dependence observed in these experiments? We considered dynamical processes^[21] that might bring the intercalated DM into direct contact with the metal surface, as well as diffusional mechanisms in which charge might propagate laterally throughout the film from local “hot spots” (e.g., defect sites) where the DM is in close contact with the electrode. To differentiate among these possibilities, we constructed a series of films that featured intervening perturbations in the base stacks of the individual helices, but left the overall structures of the DNA monolayers preserved. A through-helix pathway would result in significantly attenuated rates of electron transfer to the DM, whereas dynamical or diffusional mechanisms would yield rates that are largely unaffected.

To accomplish this, a single site within the 15-base-pair duplex was mutated to produce a CA mismatch between the intercalated DM and the electrode surface. Although CA mismatches are known to cause only local disruptions in the DNA base stack with the bases remaining intrahelical,^[22] the presence of intervening mismatches have been shown to decrease the yield of photoinduced electron transfer through DNA.^[3, 23] Indeed, this one-base change switched off the electrochemical response entirely (Figure 5).^[24] Significantly, sequences in which the positions of DM and the CA mismatch were reversed (such that the mismatch was located above the DM relative to the gold) showed no diminution in the electrochemical response. Moreover, AFM images of the CA-mutated sequences were indistinguishable from those of the

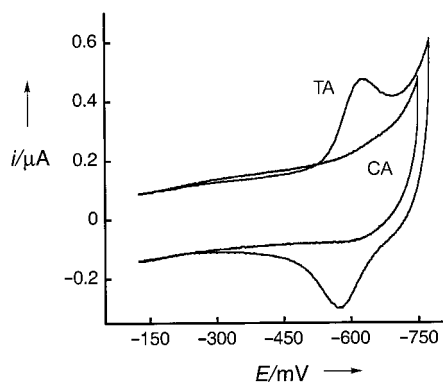


Figure 5. Cyclic voltammetry of gold electrodes modified with the DM-cross-linked thiol-terminated duplexes containing TA and CA base pairs. Sequence: SH-SH-5'ATTATATAATTGCT, where the complement contains either a T or a C opposite from the italicized A residue.

fully base paired analogues, revealing that the bulk structure of the DNA films was not significantly altered by the presence of the mismatch.^[25]

To test for lateral charge propagation through these films, a series of monolayers were doped with increasing fractions of CA-mismatched helices. In all cases, the electrochemical signals decreased linearly with increasing percentages of mutated duplexes. This linear response indicates that electroinactive intercalators (presumably those molecules bound to the mismatched duplexes) are not reduced by the electroactive species, and lateral charge diffusion is evidently quite slow.

The observation of an attenuated electrochemical response in the presence of a point mutation strongly implicates the stacked bases as the pathway for electron transfer. Indeed, the ability to detect such mutations may hold great promise for the development of DNA-based biosensors. Many electrochemical sensors have been developed based on hybridization-dependent responses,^[26] but the sensitivity reported here to single-base mismatches provides an alternative means to detect mutations with genetic sequences. The application of this approach to non-cross-linked systems is currently underway.^[27]

The nature of DNA as an electron transfer medium has been the subject of intense debate,^[3–6, 28] and the extent of charge delocalization within DNA has profound biological implications.^[1] Here, long-range charge transport through DNA-modified films has been demonstrated in a series of structurally characterized assemblies. The recent discovery of conductivity along the base-stacking direction of oriented DNA films,^[29] and now the observation of long-range electron transfer to a ground-state intercalated species, may provide evidence that the DNA base stack exhibits “wirelike” behavior.

Experimental Section

Fabrication of DNA-modified surfaces: Gold electrodes were derivatized as previously reported.^[8] The 15-base-pair oligonucleotides immobilized on a controlled pore glass resin were treated in succession with carbonyldiimidazole and 1,6-diaminohexane (1 g per 10 ml of dioxane, 30 min each) at the 5'-hydroxy terminus before cleavage from the resin.^[30] After deprotection, the free amine was treated with 2-pyridyldithiopropionic acid *N*-succinimide ester to produce a disulfide.^[31] The sequences were

purified by reverse-phase HPLC, converted into free thiols with dithiothreitol, and repurified before hybridization to their complements. Derivatized oligonucleotides were characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and HPLC retention times. Before deposition onto gold surfaces, the presence of free thiol was confirmed using a spectroscopic assay based on dithionitrobenzene.^[32]

Thiol-terminated duplexes (0.1 mM) containing an adjacent pair of guanine residues were hybridized, incubated with 0.2% formaldehyde and 0.2 mM DM in 100 mM phosphate (pH 7) for 1 h, and extracted with phenol to remove excess DM. Subsequently, samples were subjected to gel filtration chromatography and deposited on gold surfaces for 12–24 h in the presence of 100 mM MgCl₂. Before deposition, electrode surfaces were prepared by mechanical polishing with alumina, sonication, and oxidative etching in acid solution. The stoichiometry of the DM-cross-linking reaction was assayed by comparing the absorbance of DNA and the intercalated DM; one DM per duplex was found for all sequences studied.^[33] The surface structures of the DM-labeled films were determined with AFM techniques as previously described.^[9]

Electrochemical measurements: Cyclic voltammetry was carried out on 0.02-cm² polycrystalline gold electrodes with a Bioanalytical Systems (BAS) Model CV-50W electrochemical analyzer at (20 ± 2) °C in 100 mM phosphate buffer (pH 7). A normal three-electrode configuration consisting of a modified gold-disk working electrode (≈0.03 cm²), a saturated calomel reference electrode (SCE, Fisher Scientific), and a platinum wire auxiliary electrode was used. The working compartment of the electrochemical cell was separated from the reference compartment by a modified Luggin capillary. Potentials are reported versus SCE. Heterogeneous rate constants were determined as previously reported.^[8, 34] Measurements were also carried out on modified gold(111) surfaces prepared either by vapor deposition of gold onto mica, or by melting the tip of a gold wire into a small ball (≈2 mm in diameter) with a hydrogen flame. Other than a slight narrowing of the voltammetric peak widths (≈5–10 mV), the signals were very similar for both polycrystalline and Au(111) electrodes.

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- [17] To assess routinely the surface coverage of DM-derivatized DNA on gold, the electrochemical response of $[\text{Fe}(\text{CN})_6]^{4-}$ (2 mM) was monitored. This negatively charged ion is repelled from the surface of the modified electrode by the polyanionic DNA, and exhibits essentially no response when the surface is well covered. While not a direct measure of surface coverage, this technique allows the convenient assay of individual electrodes for adequate modification. As the electrode-modification procedure did not always result in uniform surface coverages for the DM-modified duplexes, only electrodes that appeared well covered using both the integrated current of the DM reduction and the ferrocyanide test were studied.
- [18] Based on molecular modeling, if DM is bound at the end of the duplex closest to the electrode (Figure 3 a), the through-helix DM – electrode separation is greater than 10 Å; if the intercalator is cross-linked to the end of the duplex farthest from the electrode, the DM-electrode separation is greater than 35 Å (Figure 3 b).
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Electron Tunneling in DNA**

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The idea that DNA is an effective medium for long-range electron tunneling is far from new.^[1] Radiation biologists invoked this concept almost 40 years ago to account for the unusually high conductivity of solid DNA.^[2] Later studies found that the conductivity arose from ice particles.^[3] The effect has also been explained in terms of high charge mobility along the outside of the duplex.^[4] Both EPR^[5] and luminescence^[6] results have been interpreted in terms of long-range electron tunneling whereas pulse radiolysis studies^[7] indicate electron tunneling is restricted to less than five base pairs. Other investigators searched in vain for soliton effects.^[8] More

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recently, Barton and colleagues^[9–13] have reported several examples of long-range electron tunneling between intercalated reagents separated by up to 40 Å and proposed that the rate of electron transfer shows a remarkably weak dependence on the number of interspersed base pairs. These conclusions are inconsistent with all other experimental observations concerning electron transfer in DNA.^[14–21] Furthermore, X-ray structural data^[22] indicate that repair photoenzymes operate over short distances and there is no obvious biological function that requires DNA to be an effective conduit for long-range electron tunnelling.

The number of experiments that address electron transfer in DNA remains limited, however, while theoretical evaluations^[23–25] appear contradictory. Unlike proteins,^[23] little is known about the ability of the DNA duplex to support electron transfer and, in particular, the size of the reorganization energy (λ) that accompanies electron transfer is unknown. This latter parameter, being crucial for an improved theoretical understanding of long-range electron tunneling, is a key feature of nonadiabatic electron-transfer reactions^[26] and ranges from about 0 eV in a frozen glass to almost 2 eV in polar solvents.^[27] It becomes of special significance when the thermodynamic driving force for electron transfer (ΔG^0) is small, since it is the interplay between λ and ΔG^0 that determines the activation energy for the electron-transfer event.^[26] Herein we measure λ values for both interfacial and through-strand electron transfer and show that the derived value is similar to that found for protein matrices.^[28]

Electron tunneling in DNA can be categorized according to the mutual positioning of the redox partners. The simplest case concerns interfacial electron transfer from an intercalated dye molecule to a positively-charged electron acceptor bound to the external phosphate chain. An appropriate redox pair, first studied by Fromherz and Rieger,^[29] comprises intercalated ethidium bromide (EB^+) as the donor and N,N' -dimethyl-4,4'-bipyridinium dichloride (MV^{2+}) as the surface-bound acceptor. Electron transfer from the singlet excited state of intercalated EB^+ to MV^{2+} ($\Delta G^0 \approx -0.08$ eV) can be monitored by steady-state and time-resolved fluorescence spectroscopy.^[15] The quenching of EB^+ fluorescence increases progressively with increasing concentration of MV^{2+} until the phosphate chain is saturated with bound MV^{2+} . Beyond this point, further increases in MV^{2+} concentration have no effect (Figure 1 A). At saturation, the position of MV^{2+} is frozen on the time scale of electron transfer, with the shortest edge-to-edge separation distance ($R \approx 4$ Å) being set by the occupancy number of each reagent. Under these conditions the rate constant for interfacial electron transfer between nearest neighbors at 20 °C ($k_{\text{ET}} = 6.3 \times 10^8 \text{ s}^{-1}$) can be determined according to Equation (1) from fluorescence lifetimes measured in the presence ($\tau_{\text{F}} = 1.5$ ns) and absence ($\tau_{\text{F}}^0 = 25.5$ ns) of MV^{2+} (Figure 1 B). The derived rate constant was found to be

$$k_{\text{ET}} = \frac{1}{\tau_{\text{F}}} - \frac{1}{\tau_{\text{F}}^0} \quad (1)$$

independent of ionic strength, type of background electrolyte, and concentration of MV^{2+} provided saturation prevailed, but was markedly dependent on temperature over the range $8 < T < 50$ °C. In fact, k_{ET} increases smoothly with increasing

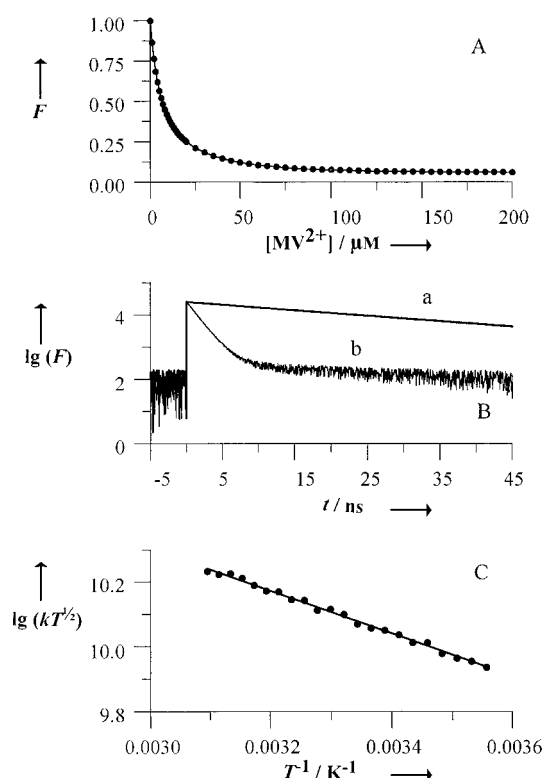


Figure 1. A) Effect of added MV^{2+} on the relative fluorescence yield F for EB^+ ($4 \mu\text{M}$) intercalated into calf-thymus DNA ($100 \mu\text{M}$). The solid line drawn through the data points corresponds to a binding constant of $1 \times 10^5 \text{ M}^{-1}$ for association of MV^{2+} to the exterior of the duplex; the ratio of initial and saturation F values is 16. B) Fluorescence decay profiles for EB^+ ($4 \mu\text{M}$) intercalated into DNA ($100 \mu\text{M}$) a) in the absence of MV^{2+} and b) at saturation. C) Effect of temperature on the rate constant for electron transfer from intercalated EB^+ to surface-bound MV^{2+} expressed in the form of Equation (2).

temperature (Figure 1 C) as expected for activated nonadiabatic electron transfer.^[26] This allows calculation of the free energy change of activation ($\Delta G^\ddagger$) from Equation (2) as being 0.129 ± 0.005 eV and giving a pre-exponential factor A of $(1.84 \pm 0.05) \times 10^{12} \text{ s}^{-1} \text{ K}^{1/2}$.

$$k_{\text{ET}} \sqrt{T} = A \exp\left(-\frac{\Delta G^\ddagger}{k_{\text{B}} T}\right) \quad (2)$$

Marcus theory^[26] expresses $\Delta G^\ddagger$ in terms of the thermodynamic driving force (ΔG^0) and the total reorganization energy (λ) that accompanies interfacial electron transfer [Eq. (3)]. Thus, the reorganization energy is approximately

$$\Delta G^\ddagger = \frac{(\lambda + \Delta G^0)^2}{4\lambda} \quad (3)$$

0.66 eV, which is much closer to that of a protein ($\lambda \approx 0.4$ eV^[28]) than a polar solvent ($\lambda \approx 1.5$ eV).^[27] This result suggests that the duplex both screens the acceptor from solvent effects and minimizes structural distortion during electron transfer. Replacing EB^+ with acridine orange ($\lambda = 0.70$ eV), palladium tetrakis(N -methyl-4-pyridinium)porphyrin ($\lambda = 0.67$ eV), or 9-methylacridinium cations ($\lambda = 0.68$ eV) results in an average λ value of 0.68 ± 0.04 eV. Furthermore,

evaluation of the pre-exponential factor according to Equation (4)^[26] shows that the electronic coupling matrix element

$$A = \frac{2\pi V^2}{\hbar \sqrt{4\pi\lambda k_B}} \quad (4)$$

V for the $\text{EB}^+/\text{MV}^{2+}$ system has a value of approximately 18 cm^{-1} . This latter value indicates weak coupling occurs between the closely spaced reactants, which gives rise to an activationless rate constant of about $1 \times 10^{11} \text{ s}^{-1}$ at 20°C , and confirms the validity of Marcus theory to this system.

A second case concerns electron transfer between intercalated reagents separated by several base pairs.^[14] To ensure consistency of the interspersed medium DNA was replaced with the synthetic polynucleotides poly[dGdC]·poly[dGdC] (poly[dGdC]) and poly[dAdT]·poly[dAdT] (poly[dAdT]) while MV^{2+} was replaced by N,N' -dimethyl-2,9-diazapyrenium dichloride (DAP^{2+}). This latter acceptor intercalates avidly but randomly into the polynucleotide^[30] relative to an incumbent EB^+ molecule. The closest approach between these reactants, which is set by the mutual exclusion principle, corresponds to three interspersed base pairs such that, assuming a regular duplex, the minimum edge-to-edge separation will be $10.2 \text{ \AA}$. Larger separations abound but these will be quantized since there must be a whole number of interspersed base pairs. This latter realization is the key element in our experimental attempt to measure the electronic conductivity of DNA by high-resolution, time-resolved fluorescence spectroscopy. Thus, if the duplex provides an extremely effective pathway for electron transfer between intercalated reagents the fluorescence lifetime of EB^+ will show only a shallow dependence on the separation distance and the decay profile will appear as a distribution of similar decay rates. Detailed analysis of such profiles can be made only if the shape of the distribution function is known. However, in the event that the duplex is a poor electronic conductor the fluorescence lifetime of intercalated EB^+ will depend strongly on the separation distance and, since only certain separations are possible, the decay profile will appear as a series of exponential functions. In this case individual lifetimes extracted from computer fitting of the decay data can be attributed to pairs of reactants separated by discrete distances, each being a multiple of $3.4 \text{ \AA}$, so that the difference between lifetimes provides a direct measure of the damping effect of the duplex.

In all our experiments the fluorescence decay profiles recorded in the presence of intercalated DAP^{2+} correspond to the sum of three exponential components, compared to the single-exponential decay observed in the absence of DAP^{2+} (Figure 2A). The derived lifetimes remain independent of the amount of DAP^{2+} loaded onto the strand, but the contribution of the shortest lifetime increases as more DAP^{2+} is bound. Laser flash photolysis studies have confirmed that fluorescence quenching is a consequence of electron transfer to the intercalated acceptor. Equation (1) then allows three k_{ET} values to be derived for each concentration of DAP^{2+} . Each rate constant can be assigned to electron transfer across a given number of interspersed base pairs, with the rate decreasing as the reactants become more remote. These

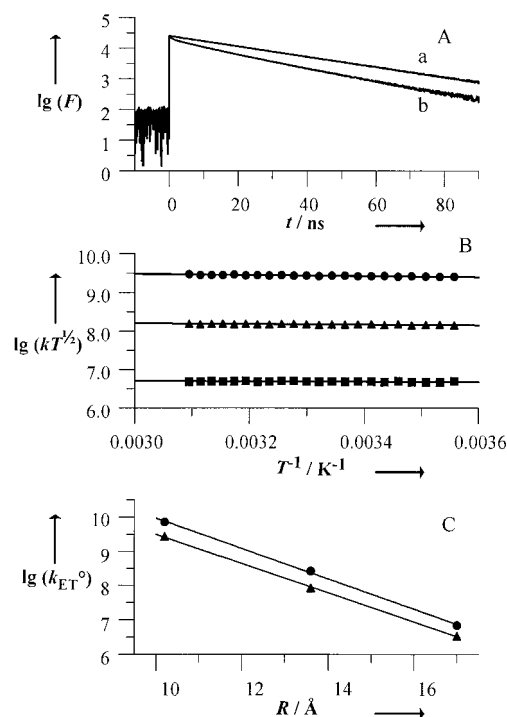


Figure 2. A) Fluorescence decay profiles for EB^+ ($4 \mu\text{M}$) intercalated into poly[dAdT] ($100 \mu\text{M}$) a) in the absence of an electron acceptor and b) in the presence of intercalated DAP^{2+} ($10 \mu\text{M}$). B) Effect of temperature on the rate constant for electron transfer from EB^+ ($4 \mu\text{M}$) to DAP^{2+} ($15 \mu\text{M}$) when both are intercalated into poly[dAdT] as measured over separation distances of 10.2 ($\bullet$), 13.6 ($\blacktriangle$), and $17.0 \text{ \AA}$ ($\blacksquare$). C) Relationship between the activationless rate constant for electron transfer from intercalated EB^+ to intercalated DAP^{2+} and the separation distance (R). Data refer to poly[dAdT] ($\bullet$) and poly[dGdC] ($\blacktriangle$) at 20°C . The solid lines correspond to fits to Equation (5) with $\beta = 1.0 \text{ \AA}^{-1}$.

derived k_{ET} values, therefore, must be associated with reactant pairs separated by three, four, or five base pairs.^[14] At larger separations the fluorescence lifetime remains unquenched because light-induced electron transfer cannot compete with the inherent deactivation processes when the reactants are separated by more than six base pairs.

This strategy allows rates of through-strand electron tunneling to be derived for the three shortest separation distances R of 10.2 , 13.6 , and $17.0 \text{ \AA}$. The rates found at 20°C decrease markedly with increasing separation over the limited range available, and it is immediately made clear that DNA is a relatively poor conduit for electrons. More surprisingly is the observation that the rate is quite sensitive to the composition of the duplex (Table 1). The rate of electron tunneling is faster for poly[dAdT] than for poly[dGdC] at each separation distance. These rates are weakly dependent on temperature (Figure 2B), such that Equations (1)–(4) allow calculation of λ and V values for the two polynucleotides (Table 1).

It is notable that $\Delta G^\ddagger$ decreases with increasing separation and, assuming the constancy of ΔG^0 ($= -0.26 \text{ eV}$), this must be a consequence of changes in the λ values. This latter effect is most likely caused by slight distortion of the double helix at short separations^[31] and, as the duplex becomes more regular, λ approaches a value of approximately 0.4 eV . Thus, the

Table 1. Parameters derived for through-strand electron tunneling involving intercalated reagents at 20 °C. Experimental conditions are as described in the legend to Figure 2.

R [Å] ^[a]	k_{ET} [s ⁻¹] ^[b]	$\Delta G^\ddagger$ [eV] ^[c]	λ [eV] ^[d]	V [cm ⁻¹] ^[e]	k_{ET}^0 [s ⁻¹] ^[f]
poly[dAdT]					
10.2	2.7×10^9	0.025	0.48	4.4	7.2×10^9
13.6	1.5×10^8	0.015	0.42	0.82	2.7×10^8
17.0	4.8×10^6	0.0095	0.38	0.13	7.0×10^6
poly[dGdC]					
10.2	1.1×10^9	0.023	0.47	2.7	2.7×10^9
13.6	4.3×10^7	0.018	0.44	0.47	8.6×10^7
17.0	2.0×10^6	0.014	0.41	0.09	3.4×10^6

[a] Edge-to-edge distance separating the intercalated reagents calculated from the binding isotherms and assuming the validity of the mutual exclusion principle. [b] Rate constant for electron transfer at 20 °C measured by time-resolved fluorescence spectroscopy, $\pm 10\%$. [c] Activation free-energy change for through-strand electron tunneling derived from Equation (2), $\pm 10\%$. [d] Total reorganization energy accompanying electron transfer as calculated from Equation (3), $\pm 10\%$. [e] Electronic coupling matrix element as calculated from Equation (4), $\pm 15\%$. [f] Activationless rate constant for electron transfer, $\pm 15\%$.

reorganization energy is remarkably similar to that found for proteins and is much smaller than normally observed for electron transfer in polar solvents. A small λ value, a natural consequence of a rigid matrix that is not readily polarized, favors rapid electron tunneling at modest thermodynamic driving forces, since such reactions will take place close to the apex of a Marcus rate versus energy-gap profile, and lowers the activation energy. In this respect, we might anticipate fast rates of both through-strand and interfacial electron transfer for DNA-bound reagents.

The derived activationless rate constants k_{ET}^0 , these being corrected for variations in both λ and $\Delta G^\ddagger$, can be used to refine earlier estimates of how the duplex attenuates electron tunneling.^[14, 32] Thus, the distance dependence for the rate of electron transfer can be expressed in terms of Equation (5)^[26] where β is an attenuation coefficient that quantifies the

$$k_{\text{ET}}^0 = k^0 \exp^{-\beta R} \quad (5)$$

damping effect of the interspersed medium. From the k_{ET}^0 values derived for each polynucleotide at discrete separation distances (Table 1), we obtain $\beta = 1.00 \pm 0.06 \text{ Å}^{-1}$, regardless of the composition of the strand. Again, this β value lies close to that reported for protein matrices^[23] and is inconsistent with fast long-range electron transfer. That the rate shows an exponential dependence on separation distance (Figure 2C) in both cases is indicative of poor connectivity between stacked base pairs and not because of restricted coupling between intercalated reagent and adjacent nucleotide.

Our analysis shows that poly[dAdT] provides better electronic coupling than does poly[dGdC]. This can be seen by comparing the rates at any fixed distance (Table 1) and is also apparent from the derived rate constants for electron tunneling at orbital contact (k^0). The inherent rate for poly[dAdT] is some fourfold faster than that for poly[dGdC] ($k^0 = 2.5 \times 10^{14} \text{ s}^{-1}$ and $k^0 = 5.8 \times 10^{13} \text{ s}^{-1}$, respectively). Given that β is identical for both polynucleotides, the enhanced rate must arise from an improved blending of LUMOs localized on the reactants and adjacent nucleotides, either with respect to

relative energy levels or coupling coefficients, if electron tunneling occurs by way of superexchange interactions.^[33] The derived k^0 values are very high, but are offset by the protein-like β value, which causes V to decrease rapidly with increasing separation.

The examination of the ability of polynucleotides to conduct electrons over quite short ($< 20 \text{ Å}$) distances in this study has revealed two antagonistic aspects of the duplex. The inherent rigidity and restricted solvation combine to lower the total reorganization energy that accompanies electron transfer and this facilitates fast electron tunneling, even at low temperature and with minimal thermodynamic driving force. This is restricted to short-range interactions, however, by weak electronic coupling between reactants, regardless of whether electron transfer is through-strand or interfacial. In the former case the problem arises from poor connectivity between stacked base pairs and is not associated with the choice of reactants. Restricted orbital overlap is responsible for the limited electronic communication when the reactants are separated by an interface and, in this case, the non-intercalated reagent must migrate along the phosphate backbone until it reaches the optimum site for electron transfer. The net conclusion is that DNA is a highly appropriate medium for fast, but close-range electron tunneling and this provides the rationale for the manner in which repair photoenzymes operate.^[22, 34] The poor connectivity between stacked base pairs can be considered as a protective measure to inhibit long-range electron tunneling and to restrict all such reactions to short-range events.

Experimental Section

Materials were obtained from Sigma Chemicals or were available from earlier investigations. Solutions of the polynucleotides were prepared in freshly-distilled water containing 5 mM phosphate buffer (pH 7) and 5 mM sodium sulfate and concentrations, expressed on the basis of nucleotide phosphate, were determined by absorption spectroscopy.^[14, 15] An aliquot of the intercalator, prepared in the same medium, was injected into the polynucleotide solution and equilibrated for 1 h. The final concentration was measured by absorption spectroscopy. A titration was made by adding aliquots of standard solutions of MV²⁺ or DAP²⁺ and the course of the reaction was followed by steady-state fluorescence spectroscopy. The temperature was controlled to within 0.1 °C by a circulating pump. Fluorescence lifetimes were measured by time-correlated, single-photon counting methodology using an Antares 76S Nd-YAG laser to pump a Rhodamine 6G or Styryl-9 dye laser that allowed selective excitation of intercalated dye. The temporal resolution of this instrument, after deconvolution of the laser pulse, was approximately 20 ps. See references [14, 15] for more details.

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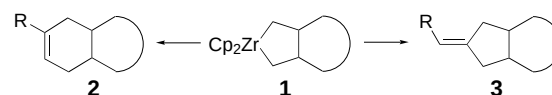
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 [31] The increased λ value found at shorter separations suggests that the reactants are more exposed to solvent molecules and reside in a less rigid environment.
 [32] The literature includes several reports of β values (given in units of $\text{\AA}^{-1}$) for electron tunneling in DNA or short synthetic duplexes: $\beta = 0.88^{[14]}$, $0.73^{[17]}$, $0.64^{[19]}$, $1.42^{[20]}$, $1.00^{[21]}$, $< 0.2^{[9]}$, $< 0.2^{[10]}$. A value of $\beta = 1.9 \text{ \AA}^{-1}$ has been given for light-induced electron exchange (that is, simultaneous two-site electron and hole transfer).^[15]
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Inter- or Intramolecular Carbometalation of Nonactivated Alkynes by Zirconacyclopentanes in the Presence of Copper Chloride**

Yuanhong Liu, Baojian Shen, Martin Kotora, and Tamotsu Takahashi*

Addition of metal–carbon bonds to alkynes is an attractive methodology for the construction of complex molecules.^[1, 2] This approach has been successfully applied to the formation of stereo-defined alkene derivatives. Alkylzirconocene derivatives have been useful in organic synthesis because they are easily prepared by hydrozirconation of alkenes,^[3] by alkylation of $[\text{Cp}_2\text{ZrCl}_2]$ ($\text{Cp} = \text{C}_5\text{H}_5$), or by olefin coupling on zirconocenes.^[4] Carbometalation of nonactivated alkynes with alkylzirconocenes would be a useful synthetic tool. However, to the best of our knowledge, no such reactions have been reported. Here we would like to fill this void and present a novel procedure for the direct conversion of zirconacyclopentanes into six- and five-membered ring carbocycles by means of inter- and intramolecular carbometalation of nonactivated alkynes (Scheme 1).



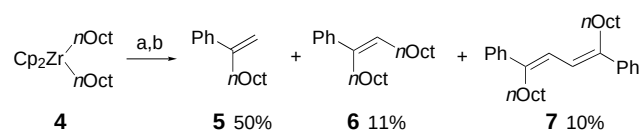
Scheme 1. Conversion of zirconacycles **1** into five- and six-membered ring carbocycles.

Initially, we investigated the carbometalation^[5] of nonactivated terminal alkynes with alkyl- and dialkylzirconocenes in the presence of CuCl because this type of reaction had not been reported previously. The reaction of alkylzirconocenes such as $[\text{Cp}_2\text{Zr}(n\text{Bu})\text{Cl}]$ and $[\text{Cp}_2\text{Zr}(n\text{Oct})\text{Cl}]$, prepared by hydrozirconation of alkenes, with alkynes gave the carbometalated products in low yields (<25%). Further investigation, however, revealed interesting reactivity patterns of dialkylzirconocenes. Thus the reaction of dialkylzirconocenes such as $[\text{Cp}_2\text{Zr}(n\text{Bu})_2]$ and $[\text{Cp}_2\text{Zr}(n\text{Oct})_2]$ (**4**) with phenylacetylene in the presence of CuCl afforded, after hydrolysis, a mixture of three products. As shown in Scheme 2, **5–7** are obtained when **4** was used as the substrate. Despite the fact that a mixture of products was formed, this result clearly indicated that the carbometalation of nonactivated alkynes by dialkylzirconocene had taken place.

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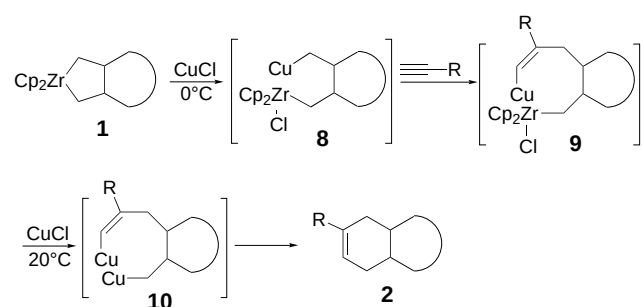
Supplementary information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 2. Reaction of **4** with phenylacetylene. a) $\text{PhC}\equiv\text{CH}$ (2 equiv), CuCl (2 equiv), $0^\circ\text{C}\rightarrow\text{room temperature}$, 3 h; b) H^+ .

Especially attractive seemed to be the formation of compound **6** which showed the feasibility of carbometalation of an alkyne followed by coupling of alkyl and alkenyl metals.^[6, 7]

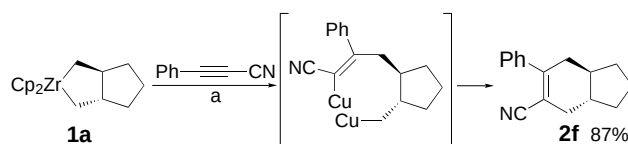
This result encouraged us to check the reaction of zirconacyclopentanes with nonactivated terminal alkynes in the presence of CuCl , because the second carbon–carbon bond formation after transmetalation is expected to form cyclic products as shown in Scheme 3.^[6, 7] In the first step,



Scheme 3. Formation of six-membered ring compounds by the reaction of zirconacycle **1** with alkynes in the presence of CuCl .

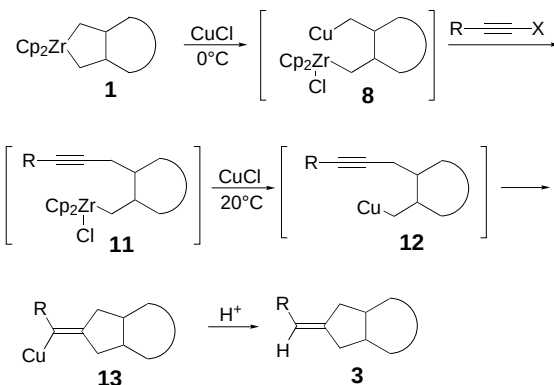
zirconacyclopentane **1** is transmetalated with CuCl to give the monocopper compound **8** (selective transmetalation of only one $\text{Zr}-\text{C}$ bond occurs at 0°C)^[8, 9] followed by the *syn* addition^[1] of the $\text{Cu}-\text{C}$ bond to alkyne to yield the intermediate **9**. In **9**, the second $\text{Zr}-\text{C}$ bond is transmetalated at 20°C to give the intermediate **10**. This species contains alkenylcopper and alkylcopper moieties and reductively couples to form the six-membered ring compound **2** with concomitant precipitation of copper powder from the reaction mixture.

Indeed, the reaction of zirconacyclopentanes of type **1** with alkynes to give compounds of type **2** proceeded as expected, and results are summarized in Table 1. Thus, the reaction of **1a** with phenylacetylene afforded **2a**. The reaction of **1b** with phenylacetylene afforded **2b** as a single product in 70% yield, and **1b** reacted with ethynylcyclohexene to give **2d** in 85% yield. Zirconacyclopentane **1c** reacted with phenylacetylene to afford **2e** in 64% yield. The formation of six-membered ring compounds was also achieved with activated alkynes (Scheme 4). In this case, the first reaction step is Michael addition followed by reductive coupling.



Scheme 4. Reaction of **1a** with 3-phenylpropynitrile: a) CuCl (2 equiv), $0^\circ\text{C}\rightarrow\text{room temperature}$, 6 h.

On the basis of these results, we envisioned that the formation of five-membered ring compounds could be achieved by intramolecular carbometalation of alkylzirconocene to a nonactivated alkynyl moiety in the presence of CuCl . However, in this case, we had first to develop a method for the preparation of alkylzirconocenes which contain an alkynyl moiety. This step was easily solved by the selective coupling of one $\text{Zr}-\text{C}$ bond of zirconacyclopentane with an alkynyl bromide.^[10] The overall reaction mechanism was assumed to be as described in the Scheme 5, although further



Scheme 5. Formation of five-membered ring compounds by the reaction of zirconacycle **1** with alkynyl bromides in the presence of CuCl .

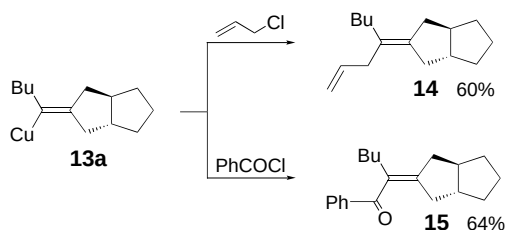
Table 1. Results of the reaction of zirconacyclopentanes with nonactivated alkynes in the presence of CuCl .^[a]

Zirconacycle	Alkyne	<i>t</i> [h]	Product	Yield [%] ^[b]
1a	$\text{Ph}-\text{C}\equiv\text{C}-\text{H}$	3	2a	56 ^[c] (50)
1b	$\text{Ph}-\text{C}\equiv\text{C}-\text{H}$	6	2b	70 ^[d] (61)
1b	$\text{Bu}-\text{C}\equiv\text{C}-\text{H}$	12	2c	– (52)
1b	C_6H_9	1	2d	85 ^[d] (78)
1c	$\text{Ph}-\text{C}\equiv\text{C}-\text{H}$	12	2e	64 ^[c] (56)

[a] Conditions: 2 equiv of CuCl ; solvent THF. [b] Yield of isolated products in parentheses. [c] Yield determined by gas chromatography (GC). [d] Yield determined by ^1H NMR spectroscopy.

investigation is necessary to elucidate the mechanism. In the first step, zirconacyclopentane **1** is transmetalated with CuCl to monocopper compound **8** followed by cross-coupling with alkynyl bromide to give an intermediate **11**, of which the second Zr–C bond undergoes transmetalation at 0 °C to give **12**. Then, the intermediate **12** undergoes intramolecular carbometalation to give **13**, which after hydrolysis affords the five-membered ring compound **3**.

The vinylcopper intermediate **13a** reacted with electrophiles such as allyl chloride and benzoyl chloride at room temperature to give the corresponding products **14** and **15** in 60 and 64% yields (Scheme 6). The reaction proceeded as



Scheme 6. Reaction of **13a** with electrophiles such as allyl chloride and benzoyl chloride at 20 °C for 2 h.

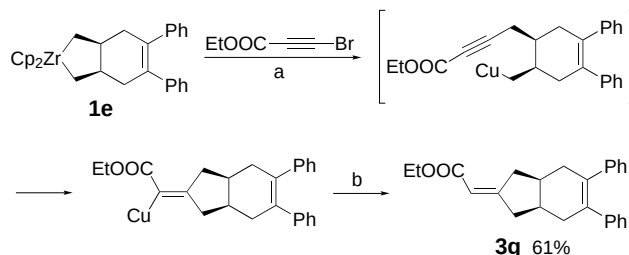
expected, and results are summarized in Table 2. Thus, the reaction of **1a–e** with alkynyl bromides gave the corresponding five-membered ring compounds **3a–e** in good yield by means of intramolecular carbometalation of the nonactivated alkyne moiety. When 1-bromo-8-phenyl-1,7-octadiyne was used as a substrate, reaction with **1a** resulted in the formation of **3f** by tandem double intramolecular carbometalation. As in the previous case, this intramolecular cyclization also

Table 2. Results of the reaction of zirconacyclopentanes with alkynyl bromides in the presence of CuCl.^[a]

Zirconacycle	Alkyne	<i>t</i> [h]	Product	Yield [%] ^[b]
	Bu–C≡C–Br	3		75 (58)
	Ph–C≡C–Br	1		71 (56)
	Bu–C≡C–Br	1		72 (55)
	TMS–C≡C–Br	1		72 (63)
	Bu–C≡C–Br	3		84 (78)
	Br–C≡C–(CH2)6–C≡C–Ph	9 ^[c]		– (55)

[a] Conditions: 2 equiv of CuCl; solvent THF. [b] Yield determined by GC. Yield of isolated product in parentheses. [c] Reaction carried out at 50 °C.

proceeds with activated alkynes. Thus, the reaction of **1e** with ethyl-3-bromopropynoate resulted in the formation of **3g** in 61% yield (Scheme 7). However, in this case the reaction with the triple bond is a Michael reaction.



Scheme 7. Reaction of **1e** with 3-bromopropynoate: a) CuCl (2 equiv), 0 °C → room temperature, 3 h. b) H⁺.

Experimental Section

2d: Ethynylcyclohexene (106 mg, 1.0 mmol) and CuCl (198 mg, 2 mmol) were added at 0 °C to a solution of **1b**, prepared from [Cp₂ZrBu₂] (1.0 mmol) and diallyldiphenylsilane (1.0 mmol). The reaction mixture was warmed up to 20 °C, stirred for 1 h, quenched with 3 N HCl, and extracted with hexane. The combined organic extracts were washed with aqueous NaHCO₃ and water, then dried (MgSO₄) and concentrated in vacuum. Column chromatography on silica gel (hexane) afforded 289 mg (78%) of **2d** as a colorless oil: ¹H NMR (CDCl₃, Me₄Si): δ = 0.72–0.84 (m, 2H), 1.46–1.70 (m, 8H), 1.96–2.30 (m, 6H), 2.52–2.60 (m, 1H), 2.69 (dd, *J* = 14.8, 3.8 Hz, 1H), 5.74 (d, *J* = 5.1 Hz, 1H), 5.81 (brs, 1H), 7.31–7.55 (m, 10H); ¹³C NMR (CDCl₃, Me₄Si): δ = 20.47, 20.93, 22.46, 23.13, 25.76, 25.85, 36.92 (2C), 41.29, 42.03, 121.05, 121.69, 127.85 (4C), 129.21 (2C), 134.71 (4C), 136.43, 136.88, 136.90, 137.01; HR-MS calcd for C₂₆H₃₀Si: 370.2115, found: 370.2130.

3e: Alkynyl bromide (161 mg, 1.0 mmol) and CuCl (198 mg, 2 mmol) were added at 0 °C to a solution of **1d**, prepared from [Cp₂ZrBu₂] (1.0 mmol) and 4,5,6,7-tetraethyl-1,4,6,9-decatetraene (1.0 mmol). The reaction mixture was warmed up to 20 °C, stirred for 3 h, quenched with 3 N HCl, and extracted with hexane. The combined organic extracts were washed with aqueous NaHCO₃ and water, then dried (MgSO₄) and concentrated in vacuum. Column chromatography on silica gel (hexane) afforded 256 mg (78%) of **3e** as a colorless liquid: ¹H NMR (CDCl₃, Me₄Si): δ = 0.81–1.40 (m, 21H), 1.66–2.12 (m, 12H), 2.12–2.32 (m, 6H), 5.18 (t, *J* = 1.8 Hz, 1H); ¹³C NMR (CDCl₃, Me₄Si): δ = 12.55 (2C), 12.79 (2C), 14.04, 22.49, 22.61 (2C), 25.65 (2C), 28.95, 31.96, 35.60, 35.85, 36.78, 41.02, 45.39, 45.45, 121.16, 133.86, 133.91, 135.98, 135.99, 140.96; HR-MS calcd for C₂₄H₄₀: 328.3128, found: 328.3131.

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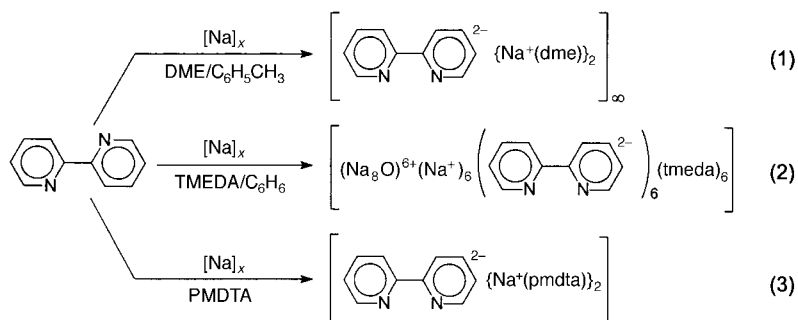
Keywords: alkynes • copper • cyclizations • metalations • zirconium

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structures of alkali metal salts with bipyridine ligands. The preparation and crystallization of contact-ion pairs containing the most advantageous Na^+ ion^[3] can be accomplished by the reduction in aprotic solution at a sodium metal mirror; a method employed on numerous occasions for organic compounds.^[3,4] In the reactions (1)–(3) contact-ion multiples are formed whose structures are decisively dependent on the cation solvation by the solvent.^[5] From dimethoxyethane (DME) and toluene a polymer crystallizes [reaction (1)], from *N,N,N',N'*-tetramethylethylenediamine (TMEDA) and benzene a novel $\text{Na}_4^+\text{O}^{2-}$ cluster [reaction (2)], and from pentamethyldiethylenetriamine (PMDTA) a monomeric sodium salt of the bipyridine dianion [reaction (3)].



The polymeric contact-ion triple crystallizes as deep red platelets, which according to their structure determination^[6] contain two additional toluene molecules in the unit cell. The sodium cations in the chains stretching in y direction (Figure 1A and B) are alternately bridged by a bipyridine dianion and two dme ligands; the $\text{Na}^+ \cdots \text{Na}^+ \cdots \text{Na}^+$ angles are 165° .

The structure determination confirms that the bipyridine dianions are planar (torsion angle $\omega(\text{NC}-\text{CN}) = 0^\circ$) and that their nitrogen centers adopt a cisoid arrangement. The sodium metal reduction of bipyridine^[7,8] shortens the C–C connection between the pyridine rings by 11 pm and stretches the C–N bonds to the bridge carbon centers by 9 pm and the C–C ring bonds by 5 pm. The internal angles of the ring at the carbon bridge centers are compressed by 6° . The sodium cations coordinate to both nitrogen centers and to the bridge carbon centers of the bipyridine dianion with $\text{Na}^+ \cdots \text{N}$ distances of 237 and an 240 pm and a $\text{Na}^+ \cdots \text{C}$ distance of 287 pm. Additional $\text{Na}^+ \cdots \text{O}$ contacts exist to both oxygen centers of one dme ligand and two further ones to an inversion symmetric second solvent ligand (Figure 1C). The mirror-symmetric sodium cations $\text{Na}^+ \cdots \text{Na}^+$ coordinated to the same bipyridine dianion are 332 pm apart, and the $\text{Na}^+ \cdots \text{O}$ contact distances to the second ligand are 374 pm.

As a continuation of our studies into ion aggregation,^[8] from a TMEDA/benzene solution under optimized conditions a novel sodium cluster $[(\text{Na}_8^+\text{O}^{2-})(\text{Na}^+)_6]^{12+}$ has been crystallized as deep red blocks^[9] (Figure 2). The cluster is lipophilically wrapped by six bipyridine dianions and six tmeda ligands. The inversion-symmetric Na_{14} cluster, wrapped by six bipyridine dianions and six tmeda ligands, contains a hexagonal bipyramid of Na^+ ions with a disordered O^{2-} dianion in its center (Figure 2B), which balances the charge of 14 sodium cations and only six bipyridine dianions.

Sodium Salts of the Bipyridine Dianion: Polymer $[(\text{bpy})^{2-}\{\text{Na}^+(\text{dme})\}_2]_\infty$, Cluster $[(\text{Na}_8\text{O})^{6+}\text{Na}_6^+(\text{bpy})_6^{2-}(\text{tmeda})_6]$, and Monomer $[(\text{bpy})^{2-}\{\text{Na}^+(\text{pmdta})\}_2]^*$

Hans Bock,* Jean-Marie Lehn,* Jochen Pauls, Sven Holl, and Volker Krenzel

Dedicated to Professor Gottfried Huttner

Polypyridines $\text{H}_4\text{NC}_5-(\text{C}_5\text{NH}_3)_n-\text{C}_5\text{NH}_4$ are excellent chelate ligands for metal cations, and the resulting complexes are suitable model compounds for studying supramolecular self-recognition and ion-pair aggregation to chains, ladders, or networks.^[1,2] Despite the numerous known metal cation bipyridyl complexes, a search in the Cambridge Structural Database (version 5.10) produced no hit with regard to

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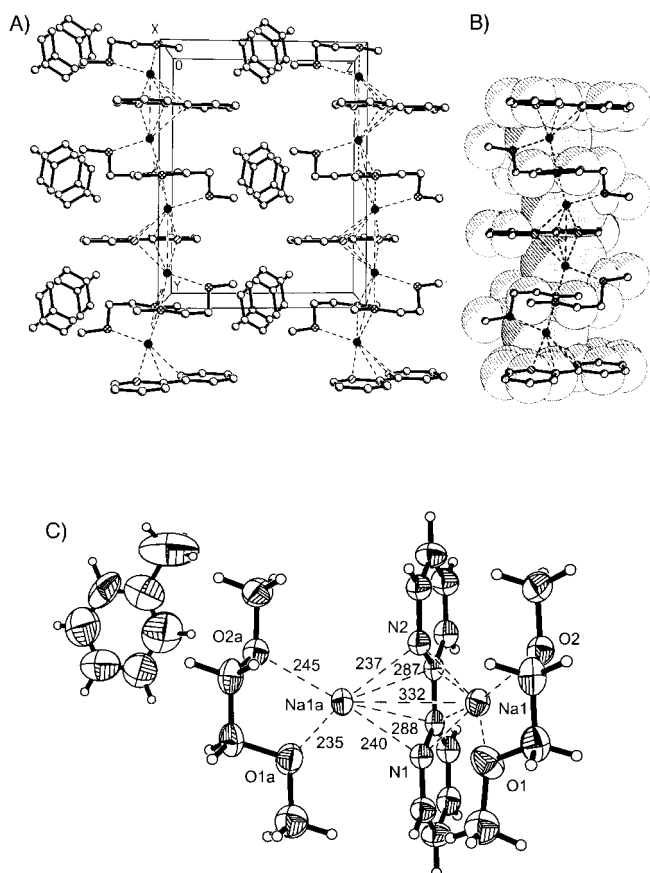


Figure 1. Crystal structure of $[(bpy)^{2-}][Na^+(dme)]_2 \cdot C_6H_5CH_3$ at 200 K: A) Unit cell (monoclinic $P2_1/m$, $Z=2$) with packing in x direction, B) space-filling representation of the polymer strand, and C) one chain link (50% thermal ellipsoids) with numbering of the N, O, and Na centers as well as with Na^+ contact distances.

According to the triclinic lattice symmetry of the crystal, the clusters are stacked in all three directions of the unit cell, and eight clusters form a parallelepiped with four benzene molecules enclosed in a diamond-shaped fashion.

In the cluster, the six equatorial cations (Figure 2B, Na1–Na3) surround the disordered oxygen dianion in a slightly pronounced chair conformation with torsion angles of only about 8° . The $Na^+ \cdots O^{2-}$ distances to the three different equatorial cations are between 257 and 277 pm, and the ones to the axial cations of 290 and 296 pm are, despite the reduced precision of the measurements due to the disorder of the O^{2-} anion, significantly longer. The contact distances $Na^+ \cdots Na^+$ vary over a wide range: they are between 296 and 306 pm within the equatorial ring and between 392 and 422 pm to the axial sodium cations, which are shifted by 13 pm out of the central position above the ring. Another sodium cation is coordinated to each of the equatorial Na^+ centers, the positions of which alternate above and below the idealized plane of the Na_6^+ six-membered ring. The angles between this plane and the outwardly oriented cation contacts are between 118° and 120° . Each two centers $Na^+ \cdots Na^+$ are bridged by a bipyridine dianion; the contact distances are: $Na^+ \cdots Na^+$ 312–316 pm, $Na^+ \cdots N$ 236–254, and $Na^+ \cdots C$ 276–290 and 313 pm. In the bipyridine dianion, the approximately planar pyridine rings are twisted by between 5 and 8° around the

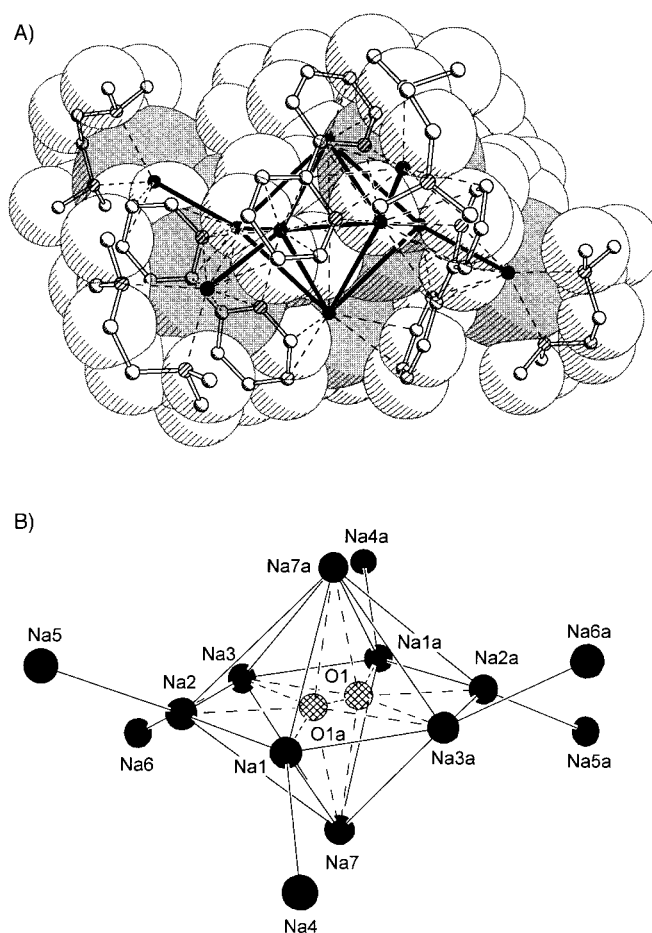


Figure 2. Crystal structure of the lipophilically wrapped sodium cluster $[(Na_8O)^{6+}Na_2^+(bpy)_6^{2-}(tmeda)_6] \cdot 4C_6H_6$ at 150 K: A) A side of view of a space-filling representation with partly sketched complex skeleton and B) arrangement of the Na^+ centers as well as of the disordered dianion O^{2-} in the cluster nucleus.

central C–C axis. The outer Na^+ ions are each additionally solvated by a tmeda ligand with contact distances $Na^+ \cdots N$ of between 247 and 269 pm.

The monomeric contact-ion triple $[(bpy)^{2-}[Na^+(pmdta)]_2]$ was crystallized from a solution in the more strongly Na^+ -solvating PMDTA.^[10] The structure determination of the deep red blocks^[10] confirms a monoclinic unit cell containing four complexes, which are separated by their solvent shells and packed in layers within the crystal (Figure 3A).

The two sodium ions coordinate as in the polymer (see Figure 1C) mirror-symmetrically to the two nitrogen and two bridge carbon centers of the bipyridine dianion (Figure 3C). The $Na^+ \cdots N$ distances are 237 and 244 pm as well as 239 and 243 pm on both sides, the $Na^+ \cdots C$ distances are 278 and 279 pm as well as 274 and 278 pm, and the $Na^+ \cdots Na^+$ distance is 366 pm. The bipyridine dianion exhibits slightly distorted pyridyl rings that are twisted relative to each other around the C–C axis by a torsion angle $\omega(NC-CN)$ of 3° , and the ring centers C2 and C5 are shifted out of the idealized double ring plane by 2 and 6° . The remaining bond lengths and angles correspond to those in the polymer (Figure 1), the C–C bond between the two pyridyl rings is similarly shortened by 11 pm and the C–N bond lengths extended by 9 pm. The sodium

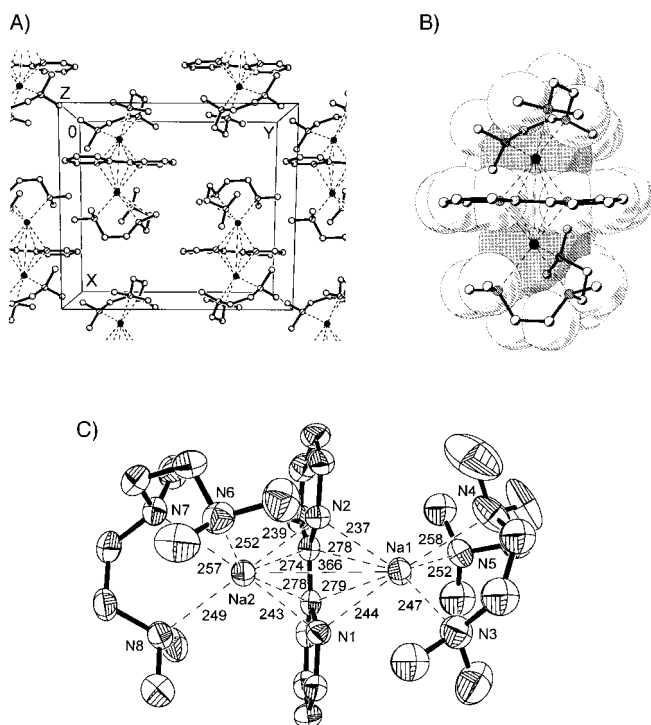
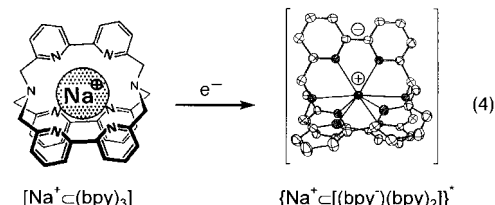


Figure 3. Crystal structure of $[(bpy)^{2-}[Na^+(pmdta)]_2]$ at 200 K: A) Unit cell (monoclinic $P2_1/c$, $Z=2$) packed in y direction, B) space-filling representation of the complex, and C) side view (50% thermal ellipsoids) with numbering of the Na and N centers as well as with Na^+ contact distances.

cation coordination spheres are completed by three $Na^+ \cdots N$ contacts to the solvating pmtda ligand; the $Na^+ \cdots N$ distances are between 247 and 258 pm (Figure 3C).

The charge distribution in the complex $[(bpy)^{2-}[Na^+(pmdta)]_2]$ has been approximated by a density functional theory (DFT) calculation (B3LYP with basis set 6-31G*)^[11] including a "natural bond orbital" analysis (Scheme 1). The negative charges are predicted to be delocalized over the bipyridine dianion, and the positive ones

to be localized at the Na^+ ions.^[12] In contrast to complexes of π -hydrocarbon anions,^[3,4] the cations coordinate here almost exclusively to the nitrogen centers of the dianion and the pmtda ligands. A similar arrangement has been reported previously for the "sodium cryptatium",^[13] the bipyridine radical anion salt that crystallizes during the electrochemical reduction of sodium tris(bipyridyl)cryptate at a platinum cathode [reaction (4)]. The contact distances $Na^+ \cdots N$ to the



different bipyridine ligand units are only 259 pm for the radical anion bridge, whereas the connections to the neutral bipyridine links are stretched by almost 20 pm to 278 pm.^[13]

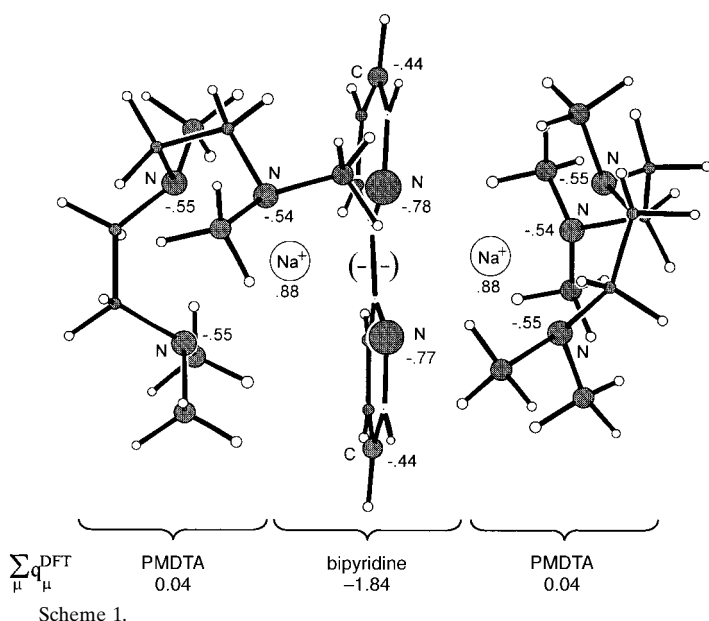
The different sodium salts of the bipyridine dianion, which crystallize depending on the sodium solvation from polyethers or polyamines as chain polymer (Figure 1), as Na^+_{14} cluster (Figure 2), or as monomeric contact-ion triple (Figure 3), together with the tris(bipyridine)cryptate radical anion confirm that bipyridine is well-suited to serve as a model ligand in solvent-dependent self-aggregation studies. Of special advantage in crystal growth experiments is the observation that twofold reduction of bipyridine at an alkali metal mirror in usual solvents is accompanied by a brownish violet color. Further investigation will be predominantly directed to polypyridines of greater length, for which these bipyridine experiments served as a preliminary study.

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- [6] Preparation and single crystal growth of $[(C_{10}H_8N_2^+)]_2[Na^+(dme)]_2 \cdot C_7H_8$: Freshly cut sodium (200 mg, 8.7 mmol) was weighed into a

Schlenk trap, dried by heating at 10^{-2} mbar, and evaporated to a metal mirror at 10^{-6} mbar. Under argon pure 2,2'-bipyridine (78 mg, 0.5 mmol) was added and this was covered with toluene (10 mL) and dimethoxyethane (5 mL). The trap was fixed in a diagonal position such that the bipyridyl solution was always in contact with the metal mirror. Deep red air-sensitive platelets grew from the violet-brown solution within 3 d. Crystal structure determination: Deep red platelets, $C_{10}H_8N_2Na_2 \cdot 2C_4H_{10}O_2 \cdot C_7H_8$ ($M_r = 474.54$), $a = 865.0(1)$, $b = 1386.2(1)$, $c = 1077.2(2)$ pm, $\beta = 95.22(3)^\circ$, $V = 1286.3(3) \times 10^6$ pm³ ($T = 200$ K), $\rho_{\text{calcd}} = 1.225$ g cm⁻³, monoclinic, $P2_1/m$ (No. 11), $Z = 2$, Siemens P4 four-circle diffractometer, MoK_{α} radiation, $\mu = 0.11$ mm⁻¹, 3090 measured reflections within $3^\circ \leq 2\theta \leq 50^\circ$, of which 2355 are independent ($R_{\text{int}} = 0.0237$). Structure solution by direct methods and difference Fourier technique (SHELXS-86), structure refinement against F^2 (SHELXL-93), 198 parameters, $w = 1/[\sigma^2(F_o^2) + (0.0690P)^2 + 0.4713P]$, R for 1647 $F_o > 4\sigma(F_o) = 0.0492$, wR_2 for all 2355 data = 0.1383, GOOF = 1.034, residual electron density 0.39/−0.22 e Å⁻³. Centers C, N, and O were refined by using anisotropic displacement parameters, disordered C center with isotropic displacement parameters, and hydrogen centers (in geometrically ideal positions) with fixed isotropic displacement parameters [$U_{\text{iso(H)}} = 1.2 \times U_{\text{eq(C)}}$ (CH/CH₂)]; [$U_{\text{iso(H)}} = 1.5 \times U_{\text{eq(C)}}$ (CH₃)] using the riding model. The free refinement of the carbon centers in the toluene molecule, disordered around a twofold axis did not provide a chemically reasonable arrangement of the centers and, therefore, as starting point a planar structure was chosen with equal C–C distances in the phenyl ring and a methyl bond length of 149 pm. One methyl group of the dme ligand is disordered in two positions, which are occupied by 71 % and 29 %.^[10b]

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- [9] Preparation and single crystal growth of [(Na₈O)⁶⁺Na₄⁺(bpy)₆⁻-(tmeda)₆]⁺·4C₆H₆: Freshly cut sodium (224 mg, 9.74 mmol) was weighed into a Schlenk trap, dried by heating at 10^{-3} mbar, and evaporated to a metal mirror at 10^{-6} mbar. Under argon pure 2,2'-bipyridine (78 mg, 0.5 mmol) was added and this was covered with TMEDA (3 mL) and benzene (10 mL). The trap was fixed in a diagonal position such that the bipyridyl solution was always in contact with the metal mirror. Black air-sensitive blocks grew from the violet-brown solution within 3 d. Crystal structure determination: Black blocks [(C₁₀H₈N₂Na₂·C₆H₁₆N₂)₆·O·2Na]·4C₆H₆ ($M_r = 2284.64$), $a = 1427.8(2)$, $b = 1442.3(3)$, $c = 1667.1(2)$ pm, $\alpha = 90.74(2)$, $\beta = 111.97(1)$, $\gamma = 92.03(2)^\circ$, $V = 3180.5(9) \times 10^6$ pm³ ($T = 150$ K), $\rho_{\text{calcd}} = 1.193$ g cm⁻³, triclinic, $P\bar{1}$ (No. 2), $Z = 1$, Siemens P4 four-circle diffractometer, MoK_{α} radiation, $\mu = 0.114$ mm⁻¹, 12552 measured reflections within $3^\circ \leq 2\theta \leq 50^\circ$, of which 11096 are independent ($R_{\text{int}} = 0.0274$). Structure solution by direct methods and difference Fourier technique (SHELXS-86); atomic form factors for O²⁻: P. Azavant, A. Lichanot, *Acta Crystallogr. A*, **1993**, *49*, 91, basic set B; all others from *International Tables*, **1992**, Vol. C, Table 6.1.1.4. Structure refinement against F^2 (SHELXL-93), 721 parameters, $w = 1/[\sigma^2(F_o^2) + (0.0525P)^2 + 2.8532P]$, R for 6930 $F_o > 4\sigma(F_o) = 0.0568$, wR_2 for all 11096 data = 0.1509, GOOF = 1.014, residual electron density 0.42/−0.28 e Å⁻³. N and Na centers, as well as non-disordered C centers refined by using anisotropic displacement parameters, disordered C and O centers with isotropic displacement parameters, and hydrogen centers (in geometrically ideal positions) with fixed isotropic displacement parameters [$U_{\text{iso(H)}} = 1.2 \times U_{\text{eq(C)}}$ (CH/CH₂)]; [$U_{\text{iso(H)}} = 1.5 \times U_{\text{eq(C)}}$ (CH₃)] using the riding model. The anisotropic displacement parameters of the benzene molecules indicate a rota-

tional disordering in the bonding plane, which however could not be refined. The carbon centers C60 and C61 of one dimethylamino group are disordered at two positions with occupancies of 45 % and 55 %, and the central oxygen center at two positions with occupancies of 28 % and 71 %. The oxygen center was refined as a dianion and to achieve charge neutrality Na⁺ as a cation. The significantly different isotropic displacement parameters of the O split positions together with the residual electron densities within the cluster point to a diffuse disordering, which cannot be dealt with satisfactorily using a simple split model.^[10b]

- [10] a) Preparation and single crystal growth of [(C₁₀H₈N₂⁺)-[Na⁺(pmdta)]₂]: Freshly cut sodium (189 mg, 8.2 mmol) was weighed into a Schlenk trap, dried by heating at 10^{-3} mbar, and evaporated to a metal mirror at 10^{-6} mbar. Under argon pure 2,2'-bipyridine (52 mg, 0.33 mmol) was added and this was covered with PMDTA (5 mL). The trap was fixed in a diagonal position such that the bipyridyl solution was always in contact with the metal mirror. Deep red air-sensitive blocks grew from the violet-brown solution within 3 d. Crystal structure determination: Deep red blocks C₁₀H₈N₂Na₂·2C₉H₂₃N₃ ($M_r = 548.77$), $a = 1322.7(2)$, $b = 1427.6(2)$, $c = 1803.2(2)$ pm, $\beta = 105.380(8)^\circ$, $V = 3283.0(8) \times 10^6$ pm³ ($T = 200$ K), $\rho_{\text{calcd}} = 1.110$ g cm⁻³, monoclinic, $P2_1/c$ (No. 14), $Z = 4$, Siemens P4 four-circle diffractometer, MoK_{α} radiation, $\mu = 0.091$ mm⁻¹, 6376 measured reflections within $3^\circ \leq 2\theta \leq 48^\circ$, of which 5103 are independent ($R_{\text{int}} = 0.0289$). Structure solution by direct methods and difference Fourier technique (SHELXS-86). Structure refinement against F^2 (SHELXL-93), 342 parameters, $w = 1/[\sigma^2(F_o^2) + (0.0634P)^2 + 2.8032P]$, R for 3120 $F_o > 4\sigma(F_o) = 0.0592$, wR_2 for all 5103 data = 0.1625, GOOF = 1.008, residual electron density 0.33/−0.25 e Å⁻³. N and Na centers, as well as non-disordered C centers with isotropic displacement parameters, hydrogen centers (in geometrically ideal positions) refined with fixed isotropic displacement parameters [$U_{\text{iso(H)}} = 1.2 \times U_{\text{eq(C)}}$ (CH/CH₂)]; [$U_{\text{iso(H)}} = 1.5 \times U_{\text{eq(C)}}$ (CH₃)] using the riding model. In one of the two PMDTA molecules several carbon centers are displaced over two positions exhibiting occupancies of at least 30 % and 70 %.
- b) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication nos. CCDC-101832 [(C₁₀H₈N₂⁺)[Na⁺(dme)]₂·C₇H₈), CCDC-101842 [(C₁₀H₈N₂Na₂·C₆H₁₆N₂)₆·O·2Na]·4C₆H₆), and CCDC-101833 [(C₁₀H₈N₂⁺)[Na⁺(pmdta)]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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The Proton Cryptate of Hexaethylenetetramine**

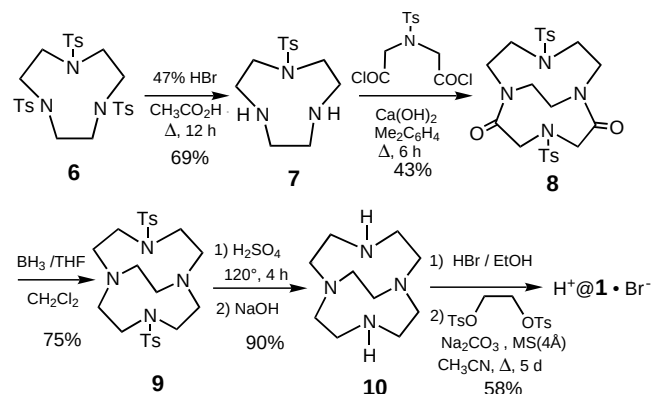
Yuji Miyahara,* Yurika Tanaka, Kiichi Amimoto, Takamitsu Akazawa, Tsutomu Sakuragi, Haruhito Kobayashi, Kazuomi Kubota, Masahiko Suenaga, Hiroyuki Koyama, and Takahiko Inazu*

Highly symmetrical molecules have always been fascinating targets for synthetic chemists.^[1] Hexaethylenetetramine (**1**) has been our goal ever since 1,4,7-triazacyclononane (**2**) was first synthesized in our laboratory,^[2] not just because of its beauty as a *T*-symmetric molecule, but because the electron density of its molecular cavity was expected to be exceptionally high as a result of accumulation of the four nitrogen lone pairs at the center of the small cage. The latter is in sharp contrast to the case for the well-known hexamethylenetetramine (**3**), where the lone pairs are directed outward in the *T_d*-symmetric adamantane structure.

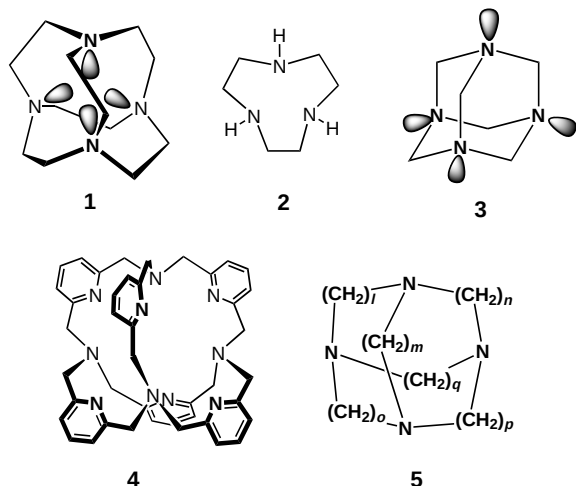
While **3** forms easily upon simple mixing of ammonia and formalin, our synthetic approaches to **1** ended in failure for many years due to its significant strain^[3] and the weakness of

carbon atoms.^[6] Here we report that the synthesis of **1** has been achieved, though as the proton cryptate $H^+@1 \cdot Br^-$, by utilization of the method of Springborg et al. in the final cyclization.

The synthetic route to the penultimate tetramine **10** is rather conventional (Scheme 1), but required special caution in each step. Two of the tosyl groups in **6** were removed by



Scheme 1. Synthesis of $H^+@1 \cdot Br^-$. Ts = tosyl = toluenesulfonyl.



C–N bonds. Although a highly electron rich cavity could be realized with **4**,^[4] which is also *T*-symmetric, we have been all the more fascinated by the smaller and simpler **1**. Springborg et al. have reported the synthesis of **5** ($l = 3, m = n = o = p = 2, q = 3$),^[5] which is larger than our target molecule by only two

heating under reflux in 47 % HBr/ CH_3CO_2H (2/1) for 5 h; the bis(hydrogen bromide) salt of **7** crystallized out of the solution.^[7] Treatment of the salt with solid KOH in refluxing benzene with azeotropic removal of water gave **7** as a pale yellow solid in 69 % yield. The most critical step of the synthesis was the coupling of **7** with *N*-(*p*-tosyl)iminodiacetyl chloride to give the bicyclic diamide **8**. Presumably to overcome the build-up of strain in going from **7** to **8**, use of high-boiling xylene as the solvent for the reaction under high dilution conditions was essential to obtain **8** in acceptable yields of 30–43 %.^[8] The side reactions of the base-sensitive acid chloride, particularly at the high reaction temperature, could be minimized by use of $Ca(OH)_2$ as a mild and nonvolatile base together with 4-Å molecular sieves as a scavenger of the water formed.

Reduction of the diamide **8** proceeded smoothly in 75 % yield by means of $BH_3 \cdot THF$ in dichloromethane. Detosylation of **9** in H_2SO_4 at 120 °C, followed by treatment with KOH, provided **10** as colorless crystals (m.p. 97–99 °C after sublimation at 50 °C/20 Torr) in 90 % yield.

The final ring closure was carried out under the conditions of Springborg et al., except that ethylene glycol di-*p*-tosylate was used for the ethylene bridging instead of trimethylene glycol di-*p*-tosylate. Thus, a mixture of **5**·3HBr, the di-*p*-tosylate, Na_2CO_3 , and powdered 4-Å molecular sieves in acetonitrile was heated under reflux with stirring for five days. Filtration of the reaction mixture, evaporation of acetonitrile, and crystallization of the residue from chloroform gave colorless rectangular plates which readily lost the solvent of crystallization and deliquesced in the air. On the basis of the following analytical and spectroscopic data, the product proved to be the proton cryptate of the target compound **1** with, in spite of the use of the di-*p*-tosylate in the cyclization, Br^- as the counterion and may be represented as $H^+@1 \cdot Br^-$. A yield as high as 58 % could be obtained if due precautions

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were taken for the considerable sensitivity of $\text{H}^+\text{@1}\cdot\text{Br}^-$ toward acid, air, light, and heat, particularly in solution.

The FAB mass spectrum of the product showed the parent ion peak at m/z 225.3, which is assignable to $\text{H}^+\text{@1}$. The elemental analysis found for a vacuum-dried sample (C 46.16, H 7.94, N 17.37) is satisfactory if residual chloroform is taken into account (calcd for $\text{C}_{12}\text{H}_{24}\text{N}_4\cdot\text{HBr}\cdot 0.09\text{CHCl}_3$: C 45.95, H 8.00, N 17.73).

The most definitive proof for the structure of the cryptate $\text{H}^+\text{@1}\cdot\text{Br}^-$ came from X-ray crystallographic analysis. A crystal grown in chloroform was sealed in a capillary and held at -120°C for collection of reflection data by means of an area detector to minimize radiation damage.^[9] As shown in Figure 1, the cage is slightly deformed from the expected T

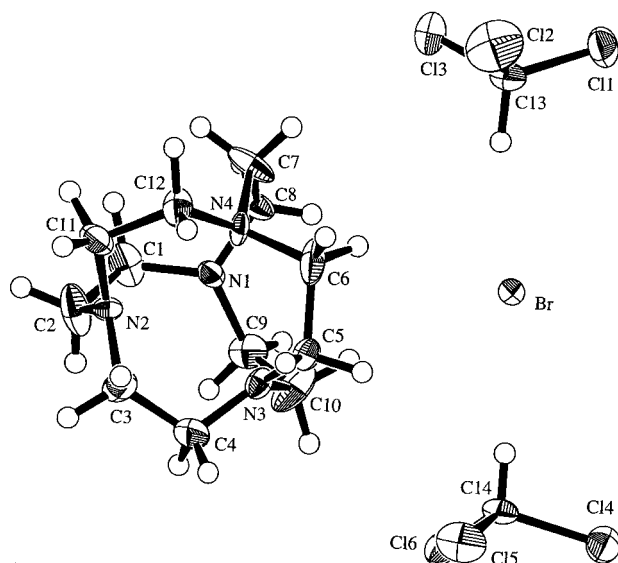


Figure 1. ORTEP representation of $\text{H}^+\text{@1}\cdot\text{Br}^- \cdot 2\text{CHCl}_3$ (thermal ellipsoids are drawn at the 50% probability level).

symmetry. Unfortunately, the encrypted proton could not be located on the difference Fourier map. However, one of the four nitrogen atoms, N4, is different from the others in several respects: 1) The $\text{N}\cdots\text{N}$ distances to the other nitrogen atoms are almost equal ($\text{N4}\cdots\text{N1}$ 2.650, $\text{N4}\cdots\text{N2}$ 2.598, $\text{N4}\cdots\text{N3}$ 2.603 Å) and shorter than those between the other nitrogen atoms ($\text{N1}\cdots\text{N2}$ 2.857, $\text{N1}\cdots\text{N3}$ 2.860, $\text{N2}\cdots\text{N3}$ 2.827 Å), 2) the $\text{N}-\text{C}$ bonds at N4 are all relatively long ($\text{N4}-\text{C7}$ 1.491, $\text{N4}-\text{C6}$ 1.455, $\text{N4}-\text{C12}$ 1.484 Å), and 3) the $\text{N}-\text{C}$ bonds at the "ends" of the chains radiating from N4 are all unusually short ($\text{C8}-\text{N1}$ 1.372, $\text{C5}-\text{N3}$ 1.376, $\text{C11}-\text{N2}$ 1.393 Å). Therefore, the encrypted proton should be present in the neighborhood of N4 in the solid state due to electrostatic interactions with the counterion.

It is interesting that the crystal contains two molecules of chloroform which are hydrogen-bonded to the bromide ion ($\text{C13}\cdots\text{Br}$ 3.553, $\text{C14}\cdots\text{Br}$ 3.627 Å), and the atoms C13, Br, and C14 are aligned almost in a straight line (175.7°). In agreement with the X-ray results, a chloroform-containing sample of $\text{H}^+\text{@1}\cdot\text{Br}^-$ in Nujol mull showed an absorption at 2450 cm^{-1} in the IR spectrum (Figure 2C). This absorption

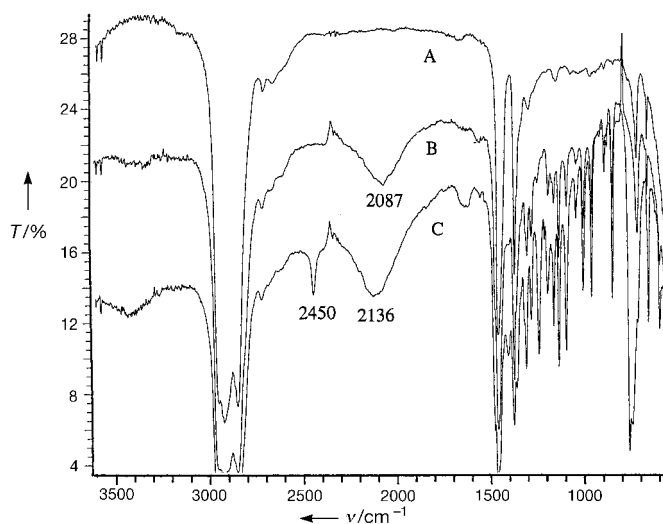


Figure 2. IR spectra of Nujol (A), $\text{H}^+\text{@1}\cdot\text{Br}^-$ in Nujol (B), and $\text{H}^+\text{@1}\cdot\text{Br}^- \cdot 2\text{CHCl}_3$ in Nujol (C). T = transmittance.

was absent when the same sample was measured in CH_2Cl_2 or when the included CHCl_3 was removed (Figure 2B). The pronounced shift of the $\text{C}-\text{H}$ stretching band to lower frequency compared to that for free CHCl_3 (3040 cm^{-1}) should be due to the presence of hydrogen bonding.

The most noticeable feature of the IR spectra, however, is the occurrence of a broad but distinctive band at about 2100 cm^{-1} . The shape and position of this band were almost unaffected when the measurements were carried out in CH_2Cl_2 (2132 cm^{-1}), suggesting that it is isolated from external effects. Extensive studies by Alder et al. on intra-bridgehead hydrogen-bonded ions in bicyclic diamines have shown that the position of the stretching band for hydrogen-bonded $\text{N}-\text{H}$ groups ranges from 2450 cm^{-1} for systems with a $\text{N}-\text{H}\cdots\text{N}$ angle of 130° down to $1400-1900\text{ cm}^{-1}$ for a highly hydrogen bonded linear $\text{N}-\text{H}\cdots\text{N}$ system.^[10] Thus, the present system shows favorable hydrogen-bonding interactions even with the less favorable tetrahedral bond angles of 109° , under the assumption that the encapsulated proton is at the center of the cage.

As expected, NMR spectroscopy provided more information on the structure of $\text{H}^+\text{@1}\cdot\text{Br}^-$. In contrast to the complex multiplets observed for the CH_2 protons of **10** and its salts, the ^1H NMR spectrum (400 MHz) of $\text{H}^+\text{@1}\cdot\text{Br}^-$ in CDCl_3 is very simple. It consists of a singlet at $\delta = 15.00$ for the included proton and a sharp signal at $\delta = 3.04$ for the CH_2 protons in a ratio of 1:24, and it is free from characteristic *p*-tosyl peaks. Most significantly, the CH_2 proton signal is not a singlet, but a narrow doublet separated by 1.0 Hz. That this splitting is caused by coupling with the included proton was confirmed by a decoupling experiment with irradiation at $\delta = 15.00$. The appearance of only one peak at $\delta = 48.05$ in the ^{13}C NMR spectrum of $\text{H}^+\text{@1}\cdot\text{Br}^-$ also indicates a symmetrical structure.

The apparent T_d symmetry of $\text{H}^+\text{@1}\cdot\text{Br}^-$ is evidently derived from averaging of signals on the NMR time scale. ^1H NMR studies (400 MHz) at various temperatures revealed that, as the temperature was lowered, the closely spaced doublet for the CH_2 protons collapsed to a broad singlet and,

below the coalescence temperature of -65°C , split to an AA'BB' system (Figure 3) in a similar fashion as the signals for axial and equatorial protons in cyclohexane.

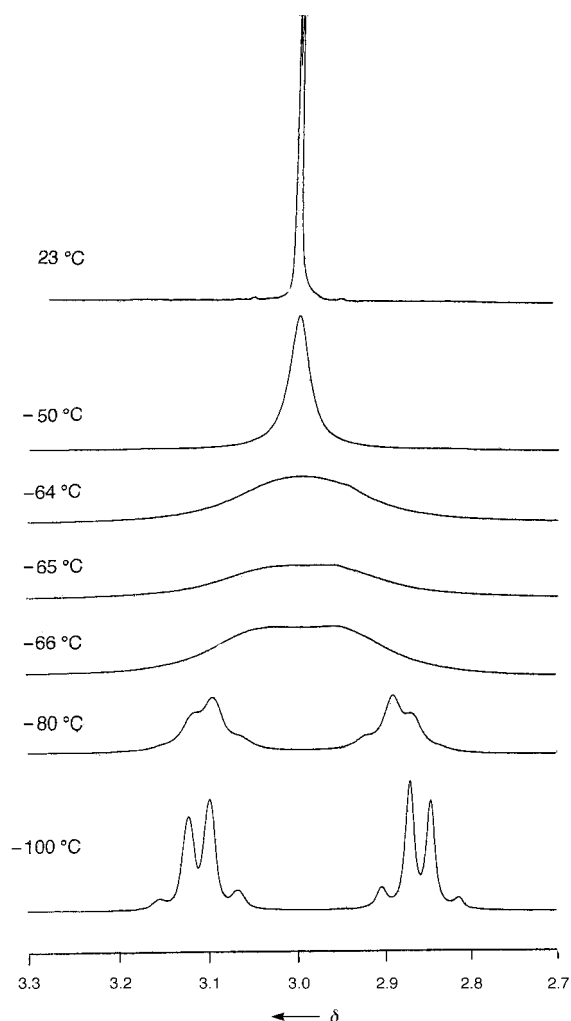


Figure 3. ^1H NMR spectra (400 MHz) of $\text{H}^+\text{@}1 \cdot \text{Br}^-$ in $\text{CD}_2\text{Cl}_2/\text{CS}_2$ (3/2).

As shown in Figure 4, the most interesting features of $\text{H}^+\text{@}1$ as a T -symmetric species are its chirality and the interconversion of its enantiomers. When the chiral shift agent (*R*)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol was added to a solution of $\text{H}^+\text{@}1 \cdot \text{Br}^-$ in CD_2Cl_2 at room temperature and the ^1H NMR spectrum was measured immediately at -90°C , the peaks for

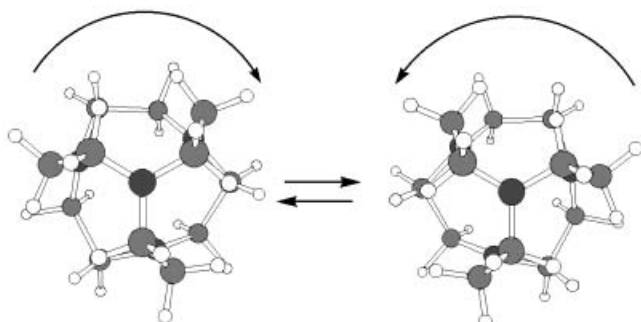


Figure 4. Schematic representation of the interconversion of the enantiomers of T -symmetrical $\text{H}^+\text{@}1$.

the encapsulated proton and one of the CH_2 protons split into two peaks of roughly equal intensity (Figure 5). The activation energy ΔG_{208} for the process was estimated to be about $9.8 \text{ kcal mol}^{-1}$ from the difference in chemical shift at -100°C and a line-shape analysis based on the AA'BB' spin system.

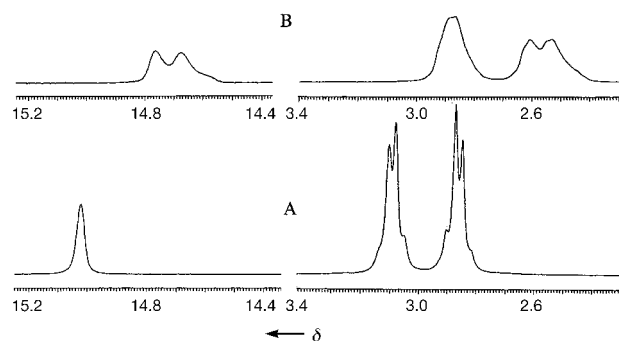


Figure 5. ^1H NMR spectra of $\text{H}^+\text{@}1 \cdot \text{Br}^-$ in CD_2Cl_2 at -90°C before (A) and after addition of the chiral shift reagent (*R*)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (B).

The proton signal appearing at such a low field as $\delta = 15.00$ in the NMR spectrum is typical of a hydrogen-bonded proton.^[10] Even in the larger **5** ($l=3$, $m=n=o=p=2$, $q=3$; $\delta = 15.2$ in D_2O) the encrypted proton is very strongly held in the cavity by the hydrogen bonds, and could not be removed by treatment with aqueous base.^[5] Likewise, the encrypted proton of $\text{H}^+\text{@}1 \cdot \text{Br}^-$ ($\delta = 15.17$ in D_2O) did not show any sign of being removed to form the vacant **1** or of exchanging with an external deuteron in a strongly alkaline solution (3M NaOD), even after standing at room temperature for 14 days followed by heating at 95°C for 3 days.

In addition to continued efforts at making proton-free **1**, we are trying to include an alkaline or a transition metal ion in **1**, with encapsulation of a metal atom as our ultimate goal.

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Keywords: cage compounds • cryptates • nitrogen heterocycles

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- [8] The yield of **8** increased as the boiling temperature of the solvent was raised on going from benzene to toluene to xylene. In addition, when one of the ethylene bridges in **7** was replaced by a less sterically demanding *o*-phenylene bridge, the cyclization occurred readily at room temperature in 53% yield: Y. Miyahara, K. Handou, T. Inazu, unpublished results.
- [9] X-ray structure analysis of $H^+@1 \cdot Br^-$: Measurements were carried out on a colorless prism ($0.3 \times 0.3 \times 0.2 \text{ mm}^3$) in a sealed capillary at -120°C ; Rigaku RAXIS II instrument with an imaging plate area detector, graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.7107 \text{ \AA}$) up to $2\theta = 54.9^\circ$. Of 5363 total reflections collected, 3733 were observed [$I > 3.0\sigma(I)$]; Lorentz polarization corrections were made. Crystal data: $C_{12}H_{24}N_4 \cdot Br \cdot 2CHCl_3$, $M_r = 543.01$, orthorhombic, space group *Pbca* (no. 61), $a = 21.078(3)$, $b = 17.378(3)$, $c = 12.560(3) \text{ \AA}$, $V = 4600(2) \text{ \AA}^3$, $Z = 8$, $\rho_{\text{calcd}} = 1.57 \text{ g cm}^{-3}$, $\mu(Mo_{K\alpha}) = 24.95 \text{ cm}^{-1}$. The structure was solved by direct methods (SHELXS86). All the hydrogen atoms (except the encrypted proton) were found, but refined as fixed at idealized positions. The final residuals $R = 0.051$ and $R_w = 0.025$ were obtained after refinement against $|F|$; min./max. residual electron density $-1.25/1.08 \text{ e \AA}^{-3}$. 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-103234. 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 Potassium Complex of a Fluorine-Containing Macrocyclic Cage Compound: Interactions between Fluorine Atoms and Metal Ions**

Hiroyuki Takemura,* Noriyoshi Kon, Mikio Yasutake, Hiroyuki Kariyazono, Teruo Shinmyozu, and Takahiko Inazu

Interactions between covalently bonded fluorine atoms (C–F) and metal cations have been observed in the solid state and discussed based on crystallographic data.^[1] Theoretical studies also predict such interactions in solution.^[2] On the

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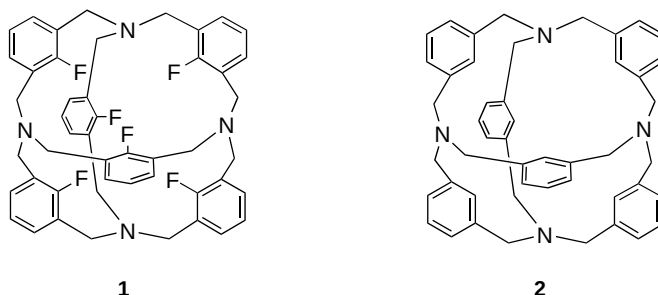


Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

other hand, few reports are available of experimental elucidation of these interactions.^[3] The first attempt to obtain experimental evidence was made in 1984 by Cram and co-workers.^[4] However, the fluorinated spherands did not show any complexation phenomena toward alkali metal cations.

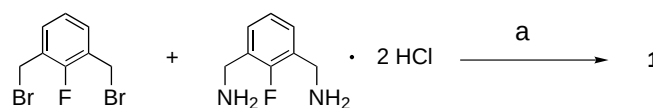
Most recently, Plenio et al. synthesized several types of macrocyclic compounds which contain oxygen and fluorine as the donor atoms.^[5] Based on ^{13}C and ^{19}F NMR spectral data, X-ray crystallographic analyses, and determinations of stability constants, they concluded that the fluorine atom plays an important role in the complexation of alkali and alkaline earth metal cations. These results are probably the first experimental proof of $\text{C-F} \cdots \text{M}^+$ interactions. However, the molecules contain the ethyleneoxa moiety, and there is no doubt that the oxygen atoms significantly contribute to the trapping of metal cations. Therefore, it is a drawback that the effect of oxygen has to be subtracted from the total cation affinities, and careful handling of each datum is required to discuss the $\text{C-F} \cdots \text{M}^+$ interactions.

Is it possible to observe the interactions with the fluorine atom as the sole donor? To give an unequivocal answer to this question, we began to design a host molecule in which only the fluorine atom acts as a donor. Previously, we reported the simple synthesis of the cage compound **2** and its analogues.^[6] The pyridine analogue of **2** is an ideal host molecule for spherical cations because it has a preorganized structure and an “enforced” cavity. Actually, the pyridine analogue showed



strong binding phenomena toward alkali metal cations, ammonium ions, and protons. We applied these structures to capture metal cations by fluorine; replacing pyridine or benzene units by fluorobenzene promises a preorganized cavity in which six C–F donor units are pointing toward the cavity (**1**). We now report the synthesis of the host molecule **1** and its ability to complex cations as well as the structure elucidation of the potassium complex of **1**.

Compound **1** was synthesized in a manner similar to that for **2**.^[6f] The reaction of 2-fluoro-1,3-bis(bromomethyl)benzene and 2-fluoro-1,3-bis(aminomethyl)benzene dihydrochloride under phase-transfer conditions ($\text{CH}_2\text{Cl}_2/\text{Bu}_4\text{NBr}/\text{aq. KOH}$) afforded **1** in 2.3% yield (Scheme 1). To estimate the complexation ability of **1**, extraction experiments with metal



Scheme 1. Synthesis of cage compound **1**. a) $\text{CH}_2\text{Cl}_2/\text{aq. KOH}$, Bu_4NBr .

picrates (metal = Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺, Ag⁺, NH₄⁺; picrate = Pic⁻ = 2,4,6-(NO₂)₃C₆H₂O⁻) were carried out. Figure 1 shows the distribution ratios of the picrates between the aqueous phase and the CHCl₃ phase. Compound **1** shows K⁺ selectivity among alkali metal ions, and the affinities for Ag⁺ and NH₄⁺ are strong. The cation affinity of **1** is size-sensitive,

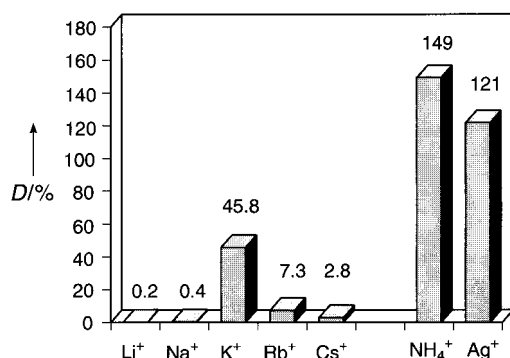


Figure 1. Distribution ratios $D[\%]$ of metal picrates. $D[\%] = [\text{Pic}^-]_{\text{org}} / ([\text{Pic}^-]_{\text{aq}} - [\text{Pic}^-]_{\text{org}}) \times 100$; $[\text{Pic}^-]_{\text{aq}}$ is the initial concentration of picrate in the aqueous phase, and $[\text{Pic}^-]_{\text{org}}$ is the observed concentration of picrate in the organic phase. Ligand **1**: 1.0×10^{-3} M in chloroform; the metal picrates are formed from 0.10 M metal chloride or nitrate (Ag⁺) and 1.01×10^{-3} M lithium picrate.

and this property implies the preorganized cavity of **1**. Analysis of the extraction data showed formation of a 1:1 complex for K⁺, Ag⁺, and NH₄⁺. Stability constants $\lg K_s$ were estimated to be 5.58, 4.57, and 4.37 for K⁺, Ag⁺, and NH₄⁺, respectively. As previously reported, **2**, which is free of fluorine atoms, does not show cation affinity.^[6] Compound **2** was used as a reference compound in these experiments, and it was confirmed that **2** did not extract metal picrates at all.

A solution of K⁺ **1** was prepared by addition of potassium picrate to **1** in CHCl₃/CH₃CN (1/1). Elemental analysis of crystals obtained from the solution gave a satisfactory result and showed the composition to be K⁺ **1** · C₆H₂N₃O₇ · 2H₂O. The K⁺ complex formation was also confirmed by the ¹H, ¹³C, and ¹⁹F NMR spectra. The ¹H NMR spectra of the complex in CDCl₃ showed a significant low-field shift ($\delta = 0.16$) of the methylene proton signals compared to those for metal-free **1**. The ¹⁹F NMR spectra displayed a characteristic high-field shift of the ¹⁹F signal (free ligand: $\delta = -110.50$, K⁺ **1**: $\delta = -126.26$). The features of the ¹³C NMR spectra are the signal of the C–F carbon atom, which was slightly shifted high field ($\delta = 1.1$), and the ¹J_{C–F} coupling, which is remarkably reduced upon complexation (free ligand: $J = 260.5$, K⁺ **1**: $J = 247.8$ Hz). These typical phenomena coincided with those reported by Plenio et al.^[5]

To clarify the coordination structure of K⁺ **1**, we carried out an X-ray crystallographic analysis (Figure 2). The single crystal suitable for the analysis was obtained by slow evaporation of the solution of the complex in CHCl₃. The six fluorine atoms are coordinated to K⁺ in a distorted octahedral fashion. The four equatorial fluorine atoms F2, F4, F5, and F6 as well as K⁺ are almost coplanar, and are capped by the two axial fluorine atoms F1 and F3. The distances between K⁺ and the fluorine atoms are in the range of 2.56–2.92 Å. To the best of our knowledge, the value of 2.56 Å is

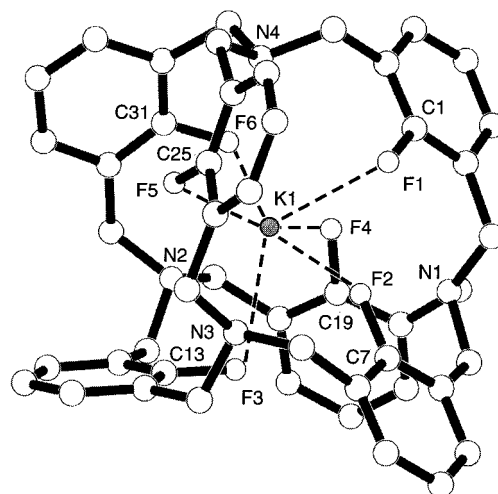


Figure 2. Crystal structure of K⁺ **1** (H atoms are omitted for clarity). Selected bond lengths [Å] and angles [°]: K1–F1 2.920(3), K1–F2 2.563(3), K1–F3 2.786(3), K1–F4 2.704(3), K1–F5 2.727(3), K1–F6 2.722(3), K1–N1 3.739(4), K1–N2 3.276(4), K1–N3 3.629(4), K1–N4 3.295(4); C1–F1–K1 95.1(2), C7–F2–K1 148.2(3), C13–F3–K1 105.4(2), C19–F4–K1 116.2(2), C25–F5–K1 109.9(2), C31–F6–K1 98.4(2).

among the shortest reported.^[3] It is noteworthy that a shorter K⁺...F distance tends to make the bond angle C–F...K closer to linear. The N...K⁺ distances (3.28–3.74 Å) are much longer than the F...K⁺ distances, and formation of the chelate –N...K⁺...F– is not observed. Bridgehead nitrogen atoms do not participate in the K⁺ coordination, and this feature is supported by the fact that the fluorine-free compound **2** does not show any ability to complex cations.

In conclusion, the C–F...M⁺ interaction was proven very simply with K⁺ **1**, and it was elucidated that the fluorine atom undoubtedly acts as a donor atom toward cations. A new donor (C–F) can now be added to the field of host–guest chemistry, and it can be widely used as a receptor unit for the design of host molecules.

Experimental Section

1: A mixture of 2-fluoro-1,3-bis(aminomethyl)benzene hydrochloride (4.70 g, 20.7 mmol), CH₂Cl₂ (350 mL), Bu₄NBr (0.37 g, 1.15 mmol), and 4 N aq. KOH (30 mL) was heated under reflux with vigorous stirring. A solution of 2-fluoro-1,3-bis(bromomethyl)benzene (6.78 g, 24.0 mmol) in CH₂Cl₂ (150 mL) was added to the mixture over a period of 3.5 h, and additional heating was continued overnight. The organic layer was separated and dried over MgSO₄. After evaporation of the solvent, the resultant material was purified by column chromatography on alumina with benzene as an eluent. The white powder obtained was recrystallized from CH₂Cl₂/benzene to afford colorless prisms (111 mg, 2.3%). M.p. 337.1–337.5 °C (decomp. in a sealed, Ar-filled tube); ¹H NMR (400.1 MHz, CDCl₃, TMS): $\delta = 7.14$ (brs, 12H, ArH), 6.95, 6.93, 6.90 (t, ³J(H,H) = 7 Hz, 6H, ArH), 3.49 (s, 24H, CH₂); ¹³C NMR (100.6 MHz, CDCl₃, TMS): $\delta = 162.30$, 159.71 (d, ¹J(C,F) = 260.5 Hz), 130.75 (s), 126.09, 125.94 (d, ²J(C,F) = 14.5 Hz), 121.38 (s), 52.34 (s); ¹⁹F NMR (564.7 MHz, CDCl₃, CFCl₃): $\delta = -110.50$; MS (FAB): m/z (%): 789 (16) [$M^+ + 1$]; elemental analysis of a sample recrystallized from CH₂Cl₂/hexane: calcd for C₄₈H₄₂N₄F₆ · CH₂Cl₂: C 67.35, H 5.08, N 6.41; found: C 67.60, H 5.18, N 6.41; the quantity of the solvent included in the analytical sample was confirmed by means of the ¹H NMR spectrum.

K⁺ **1** · Pic⁻: M.p. > 271.2 °C (decomp. in a sealed, N₂-filled tube); ¹H NMR (400.1 MHz, CDCl₃, TMS): $\delta = 7.24$, 7.22 (d, ³J(H,H) = 8 Hz, 12H, ArH), 7.09, 7.07, 7.05 (t, ³J(H,H) = 8 Hz, 6H, ArH), 3.65 (s, 24H, CH₂); ¹³C NMR

(100.6 MHz, CDCl₃, TMS): δ = 161.16, 158.70 (d, $^1J(\text{C},\text{F})$ = 247.8 Hz), 131.89 (s), 126.56 (s), 124.31 (s), 55.89 (s); ^{19}F NMR (564.7 MHz, CDCl₃, CFCl₃): δ = -126.26; MS (FAB): m/z (%): 827 (100) [$M+K^+$]; elemental analysis of a sample recrystallized from CHCl₃: calcd for C₄₈H₄₈N₄F₆·C₆H₂N₃O₇K·2H₂O: C 59.39, H 4.43, N 8.98; found: C 59.47, H 4.30, N 8.93.

Crystallographic data for K⁺·1·Pic⁻·2H₂O (C₅₄H₄₈N₇O₉F₆K): M_r = 1092.10, T = 23 °C, Mo $\text{K}\alpha$ (Rigaku RAXIS-IV imaging plate area detector, λ = 0.71070 Å), crystal dimensions 0.40 × 0.30 × 0.50 mm³ (yellow prism); triclinic, space group P $\bar{1}$ (no. 2), a = 13.980(4), b = 18.336(8), c = 11.198(5) Å, α = 98.38(1), β = 110.54(3), γ = 102.04(2)°, Z = 2, μ_{Mo} = 1.87 cm⁻¹, V = 2552.6(8) Å³, ρ_{calcd} = 1.391 g cm⁻³, $2\theta_{\text{max}}$ = 55°, $F(000)$ = 1106. Indexing was performed from three oscillations which were exposed for four minutes. A total of 31 oscillation images (5.00°) were collected, each being exposed for ten minutes. Of the 8043 reflections collected, 6178 were unique and 8000 were independent with $I > 3\sigma(I)$. The structure was solved by direct methods and refined on SAPI 91. Data were corrected for Lorentz and polarization effects. The data/parameter ratio was 6.86; R = 0.071, R_w = 0.065, GOF = 1.55, max./min. residual density +0.95/−0.58 e Å⁻³. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-103215. 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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Novel Aluminum-Containing Ring Systems: An Octanuclear Structural Analogue of a Calix[4]pyrrole**

Vernon C. Gibson,* Carl Redshaw, Andrew J. P. White, and David J. Williams

The study of the coordination chemistry of Group 13 alkyl derivatives to give complexes of differing nuclearities is being actively pursued, prompted by the relevance of such species to alkylaluminoxanes, (RAlO)_n,^[1] and as single-source precursors to semiconducting and electronic materials, for example GaN.^[2] In recent studies, a number of unusual aluminum-containing ring and cage structures have been described. For example, difunctional molecules such as 1,2-diaminobenzene (1,2-(NH₂)₂C₆H₄), salicylic acid (1-(CO₂H)-2-(OH)C₆H₄), and 1,2-diphosphanylbenzenes (1,2-(PR₂)₂C₆H₄; R = H, Me) have been shown to react with Me₃M (M = Al, Ga) to give ring and cage structures for both aluminum and gallium;^[3] other ring and cage structures have been accessed from reactions involving the use of primary or secondary amines^[4] and phenylhydrazine.^[5]

In separate studies, gallium-mediated coupling reactions involving acetonitrile have recently been described. For example, the trimethylgallium-induced trimerization of acetonitrile, in the presence of a catalytic amount of caesium salt, was supported by the isolation and crystal structure determination of the heterocycle [Me₂Ga{HNC(Me)}₂C(CN)];^[6] further reaction with Me₃Ga/MeCN gave [(Me₂Ga)₄(Me-Ga)₂{HNC(Me)C(CN)CH(NCMe)₂}]₂. Here we describe two remarkable reactions of Me₃Al with 1,1-disubstituted hydrazines to give novel macrocyclic ring structures arising by reaction of Me₃Al with the hydrazine followed by insertion of acetonitrile into the so-generated Al–N bonds.

Addition of two molar equivalents of Me₃Al to 1,1-dimethylhydrazine (Me₂NNH₂) in toluene results in rapid evolution of methane and formation of a colorless solution. After removal of the solvent, the residue was dissolved in acetonitrile, warmed at 80 °C for 10 min, and then cooled to −20 °C to give large colorless prisms of complex **1**. The crystal structure^[7] of **1** (Figure 1 a) shows the molecule to be made up of four five-membered AlN₃C rings linked by Me₂Al bridges, and it may be viewed as a structural analogue of the calix[4]pyrroles.^[8] The molecule has non-crystallographic S₄ symmetry, adopting a 1,3-*alternate* conformation (Figure 1 b) very similar to the aforementioned calix[4]pyrroles. The four bridging aluminum atoms of the 16-membered ring are coplanar to within 0.19 Å, with the four near-planar AlN₃C rings inclined by 59–67° to this plane. The geometries at the bridging aluminum centers are only slightly

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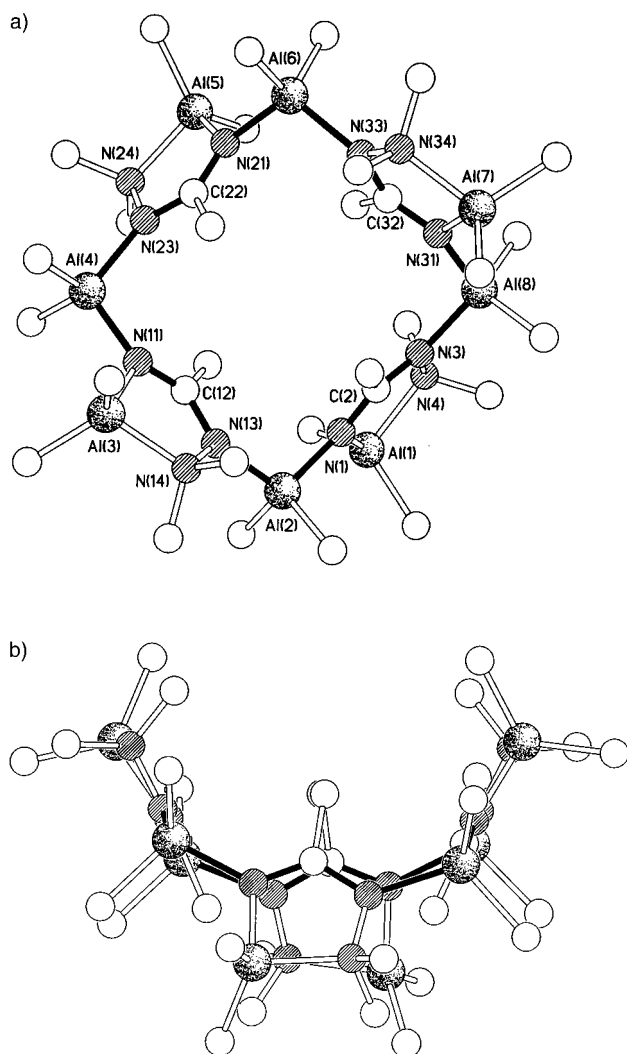
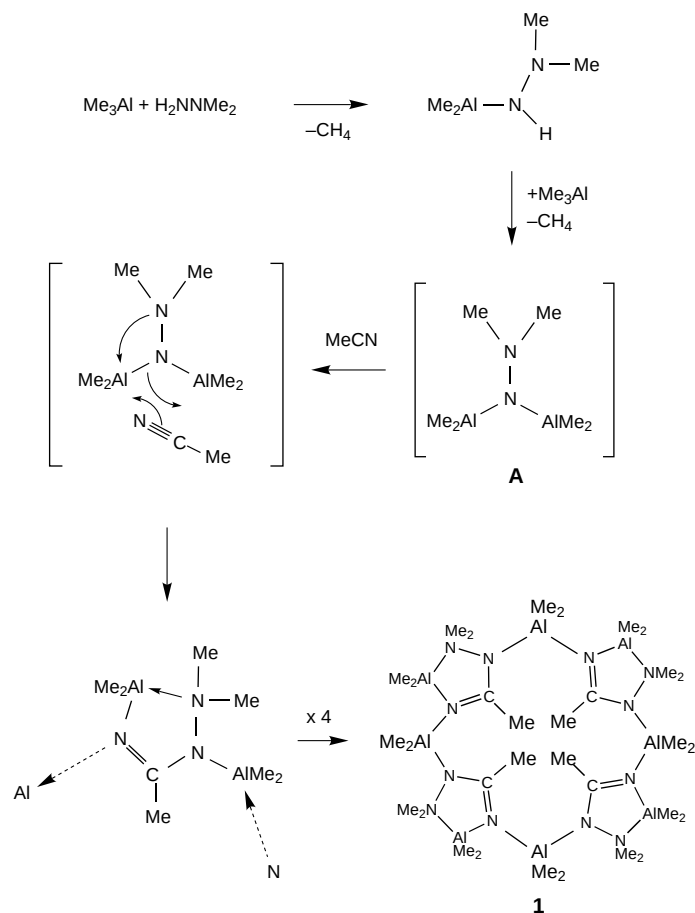


Figure 1. a) The molecular structure of **1** (top view). Selected bond lengths [Å]: Al(1)–N(1) 1.877(3), Al(1)–N(4) 2.042(3), Al(2)–N(1) 1.899(3), Al(2)–N(13) 1.972(3), N(1)–C(2) 1.313(4), C(2)–N(3) 1.356(4); b) Side view of **1**.

distorted from tetrahedral, with angles ranging between 107 and 115°. By contrast, the aluminum atoms within the five-membered rings have pseudo-trigonal-pyramidal geometries with the Me₂N moiety occupying the apical position and with the aluminum atoms lying between 0.37 and 0.39 Å out of the basal plane.^[9] This geometry, coupled with the noticeably long Al–N(apical) linkages (2.020(3)–2.042(3) Å), is comparable to that observed in related Al–NR₃ systems^[10] and reflects the formal dative character of these bonds; the other Al–N distance within the five-membered rings is about 0.14 Å shorter (av 1.886 Å). The bridging Al–N distances alternate in length, with those to N(1), N(11), N(21), and N(31) (av 1.893 Å) being significantly shorter than those to N(3), N(13), N(23), and N(33) (av 1.975 Å); in each case the shorter distance is to the nitrogen atom involved in a double bond to the ring carbon atom (av 1.308 Å). The Al–Me distances are unexceptional (1.952(4)–1.981(4) Å). The presence of the bulky methyl substituents results in an essential self-filling of the potential macrocyclic cavity, there being no significant free pathway through the ring center.

Any mechanism for the synthesis of **1** has to account for (after the loss of methane) the presence of two extra carbon atoms and a nitrogen atom in each of the five-membered rings. A plausible sequence is shown in Scheme 1, where the first two



Scheme 1. Proposed mechanism for the formation of **1**.

steps involve loss of methane and formation of a bridged hydrazido(2–) species **A**. This intermediate is then postulated to react by insertion of acetonitrile into an Al–N bond to afford the five-membered ring structural motif found in **1**.

The macrocycle **1** displays broadened signals in the ¹H NMR spectrum ([D₈]toluene, 500 MHz) for the Me₂N (δ = 2.31; ω_{1/2} = 80 Hz) and Me₂Al groups (δ = –0.43, –0.58; ω_{1/2} = 60 Hz) at ambient temperature. At the low temperature limit (265 K) each of the broadened resonances is resolved into two signals of approximately equal intensity. The ΔG[‡] value for this fluxional process, which we attribute to being a flipping of the five-membered rings in the 1,3-alternate conformation, is calculated to be 64 kJ mol^{–1} (T_c = 295 K, rate constant *k* = 27 s^{–1}). On warming to 325 K all signals are sharp. In the ambient-temperature ¹³C NMR spectrum, the Me₂N group appears as two broadened resonances (δ = 47.5, 49.0), while the Me₂Al units give rise to two sets of broadened signals (δ = –9.7, –8.4 and –4.9, –2.3). All three sets coalesce to singlet resonances on warming (T_c = 350 K).

Encouraged by the successful synthesis of the novel macrocyclic ring system **1**, we then investigated the reaction of Me₃Al with methylphenylhydrazine. Slow addition of a solution of Me₃Al (2 equiv) in toluene to MePhNNH₂,

followed by heating under reflux for 12 h in acetonitrile, afforded a pale brown solution from which colorless plates of **2** were isolated in about 55 % yield after recrystallization from hot acetonitrile. The X-ray analysis^[7] reveals that the product is the C_i -symmetric macrocyclic complex illustrated in Figure 2. The 12-membered macro-ring is self-filling and comprises four aluminum atoms (two bridging and two chelating), six nitrogen atoms, and two carbon atoms. The two

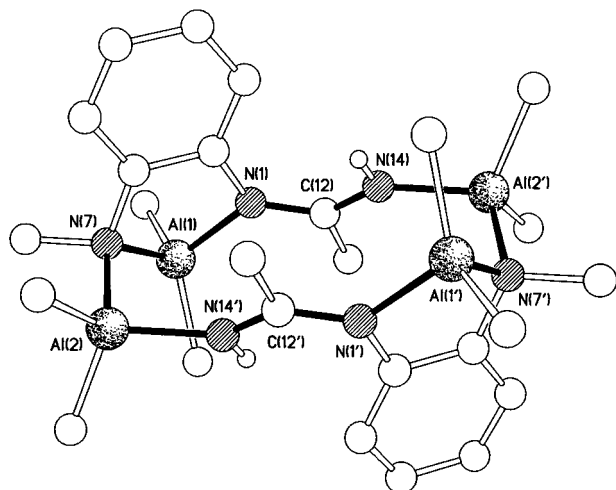


Figure 2. The molecular structure of **2**. Selected bond lengths [Å]: Al(1)–N(1) 1.930(2), Al(1)–N(7) 1.961(3), N(7)–Al(2) 2.000(3), Al(2)–N(14') 1.909(2), N(14)–C(12) 1.313(4), C(12)–N(1) 1.342(4).

disubstituted chelating phenylene rings are oriented approximately axially with respect to the macro-ring plane. The geometries at Al(1) and Al(2) are distorted tetrahedral, with angles of 86.4(1)–116.4(1)° and 99.4(1)–115.4(1)°, respectively. There is also a marked variation in the Al–N bond lengths within the ring; the Al(2)–N(7) linkage (2.000(3) Å) is significantly longer than the others (1.909(2)–1.961(3) Å). However, these values lie within the broad range of Al–N distances reported in the literature. The five-membered chelate rings are folded so as to produce an axial/equatorial geometry for the two methyl substituents, the aluminum atom lying 0.67 Å out of the plane of the other four ring atoms.

The ¹H NMR spectrum (300 MHz) of **2** at ambient temperature shows sharp signals and consists of four singlets of equal intensity in the Al–methyl region ($\delta = -0.2$ to -0.7) together with two further singlets (again of similar intensity) at $\delta = 1.38$ and 2.57. On warming, two of the Al–methyl resonances (at ca. $\delta = -0.5$) coalesce ($T_c = 368$ K), which we attribute to wagging of the bridging Me₂Al groups (Al(2) and Al(2')) in Figure 2); $\Delta G^\ddagger = 79$ kJ mol⁻¹, $k = 52$ s⁻¹. The mechanism of formation of **2** again clearly involves incorporation of acetonitrile, but in this case the process is accompanied by N–N hydrazine bond scission along with cleavage of one of the *ortho*-phenyl C–H bonds. The detailed pathway remains to be established.

In conclusion, these results exploiting the ready insertion of acetonitrile into aluminum–nitrogen bonds highlight a potentially powerful synthetic approach to macrocyclic aluminum-containing ring systems. Further work is in progress to explore and extend the synthetic scope of this reaction.^[11]

Experimental Section

Complexes **1** and **2** were prepared by heating Me₃Al (50 mL, 2.0 M, 0.1 mol) and the appropriate hydrazine (0.05 mol) in toluene (30 mL) under reflux for 12 h. Volatile components were then removed in vacuo, and the products obtained by treatment with excess hot acetonitrile (50–60 mL). Large colorless prisms of **1** and **2** are obtained on prolonged cooling at -20°C .

1: Yield: 3.2 g; further crops can be obtained from the mother liquor (overall yield 40%). Elemental analysis calcd for C₃₂H₈₄N₁₂Al₆: C 45.1, H 9.9, N 19.7; found: C 45.1, H 9.5, N 19.9; ¹H NMR (500 MHz, [D₈]toluene, 298 K): $\delta = -0.58$ (brs, 24H, Me–Al), -0.43 (brs, 24H, Me–Al), 0.63 (s, 0.5 MeCN), 2.16 (s, 12H, C(Me)), 2.31 (brs, 24H, Me₂N); ¹³C NMR (100.6 MHz, C₆D₆, 298 K): $\delta = -9.66$, -8.42 , -4.90 , -2.25 (4 × brs, Me–Al), 23.41 (s, C(Me)), 47.48, 48.96 (2 × brs, Me₂N), 172.00 (s, C(Me)).

2: Yield: 55 %. Elemental analysis calcd for C₂₆H₄₆N₆Al₄: C 56.7, H 8.4, N 15.7; found: C 56.4, H 8.4, N 15.2; ¹H NMR (300 MHz, C₆D₆, 298 K): $\delta = -0.66$, -0.41 , -0.27 , -0.20 (4 × s, 4 × 3H, Me–Al), 1.38 (2 × s, 2 × 3H, MeC), 2.57 (2 × s, 2 × 3H, MeN), 6.48–7.27 (3 × m, 8H, aryl H); the signal for NH₂ was not observed; ¹³C NMR (100.6 MHz, C₆D₆, 298 K): $\delta = -12.24$, -12.81 (br, Me–Al), -8.91 (br, Me–Al), -5.99 (br, Me–Al); IR: $\tilde{\nu}(\text{N–H}) = 3345\text{cm}^{-1}$; MS (EI): m/z : 550 [M^+].

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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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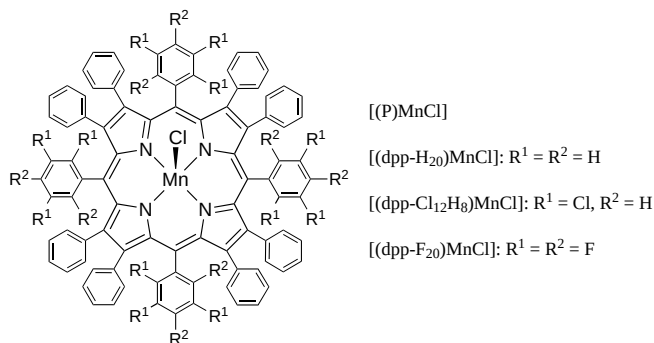
Decreased Electron Transfer Rates of Manganese Porphyrins with Conformational Distortion of the Macrocycle**

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Jean-Michel Barbe, Roger Guilard,*
Eric Van Caemelbecke, Ning Guo, and
Karl M. Kadish*

Nonplanar conformations of porphyrins have been suggested as being related to their functions in biological systems.^[1–4] For example, a deformation of the porphyrin skeleton in heme and photosynthetic proteins is believed to arise from environmental effects.^[3, 5, 6] Synthetic dodecaphenylporphyrins which contain a phenyl ring at each β -pyrrole and *meso* position of the porphyrin macrocycle are known to adopt a nonplanar conformation.^[3, 7–9] Nonplanarity of the porphyrin ring due to steric repulsion between ring substituents in dodecasubstituted porphyrins affects their optical properties.^[7–11] Manganese is an essential metal in several biological systems that are involved in electron transfer reactions,^[12] and extensive efforts have therefore been devoted

to elucidating electron transfer processes in synthetic manganese porphyrins.^[13–16] However, there have been so far no reports on how conformational distortions of the porphyrin ring affect the rates of homogeneous electron transfer reactions in manganese or other transition metal porphyrins.

Here we report the homogeneous electron transfer kinetics for reduction of [(P)MnCl], where P represents the dianion of tetraphenylporphyrin (tpp) or of the dodecaphenylporphyrins (dpp-X, X = H₂₀, Cl₁₂H₈, F₂₀). The relationship between the



logarithms of the rate constants of electron transfer and the free energy change of electron transfer gives an initial evaluation of reorganization energies λ for reduction of manganese porphyrins with a planar macrocycle, as in [(tpp)MnCl], and a nonplanar macrocycle, as in [(dpp-X)MnCl].

Previous studies of the electrochemistry of [(dpp-X)MnCl] in benzonitrile show that the reduction of Mn^{III} to Mn^{II} is quasireversible^[16] and that the potential separation between the cathodic and anodic peak potentials, $\Delta E_p = |E_{pc} - E_{pa}|$, increases in the order [(tpp)MnCl] < [(dpp-H₂₀)MnCl] < [(dpp-Cl₁₂H₈)MnCl] < [(dpp-F₂₀)MnCl] (Table 1).^[17] The large ΔE_p value (0.52 V) for the reduction of [(dpp-F₂₀)MnCl] may be related to the dissociation of Cl⁻ after reduction of Mn^{III} to Mn^{II}. However, the fact that the magnitude of ΔE_p remains unchanged after addition of an excess of Cl⁻ (0.1 M) suggests that the large ΔE_p value is due to slow electron transfer kinetics, as reported for other [(P)MnCl] complexes^[15, 18] whose kinetics have been determined by electrochemical methods.^[15, 18, 19]

The slow electron transfer in the reduction of [(dpp-X)MnCl] and [(tpp)MnCl] was confirmed in the present study by measuring the kinetics of the homogeneous electron transfer reduction with semiquinone radical anions as chemical reductants whose oxidation potentials^[20] are low enough to reduce each Mn^{III} porphyrin to its Mn^{II} form. These reducing agents were prepared by comproportionation of the *p*-benzoquinone derivatives with the corresponding hydroquinone dianions, which were generated by reactions of hydroquinones with tetramethylammonium hydroxide.^[21] The kinetics of electron transfer from the semiquinone radical anion to [(tpp)MnCl] or [(dpp-X)MnCl] were then followed by monitoring the increase in absorbance of the Mn^{II} Soret band (e.g., at 488 nm for [(dpp-H₂₀)MnCl]).

The electron transfer rate obeys a second-order rate law when the initial concentrations of [(dpp-X)MnCl] and the semiquinone radical anion are equal. The observed second-

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Table 1. Rate constants k_{et} and free energy changes ΔG_{et}^0 for electron transfer reduction of [(P)MnCl] with semiquinone radical anions $\text{XQ}^{\cdot-}$ in deaerated MeCN at 298 K, their one-electron redox potentials E_{red}^0 and E_{ox}^0 versus SCE, and reorganization energies λ_{22} for the self-exchange reaction of [(P)MnCl]/[(P)MnCl] $^-$.

[(P)MnCl]	E_{red}^0 (ΔE_p) ^[c] [V]	$\text{XQ}^{\cdot-}$ ^[d]	E_{ox}^0 [V]	k_{et} ^[e] [M $^{-1}$ s $^{-1}$]	ΔG_{et}^0 [eV]	λ_{22} ^[f] [kcal mol $^{-1}$]
[(dpp-H ₂₀)MnCl] (1)	−0.36 (0.13)	2,6-Me ₂ Q $^{\cdot-}$	−0.67	4.7×10^5	−0.31	67
		MeQ $^{\cdot-}$	−0.58	4.1×10^4	−0.22	72
		Q $^{\cdot-}$	−0.50	1.3×10^4	−0.14	71
[(dpp-Cl ₁₂ H ₈)MnCl] (2)	−0.15 (0.25)	2,6-Me ₂ Q $^{\cdot-}$	−0.67	2.8×10^5	−0.52	86
		MeQ $^{\cdot-}$	−0.58	1.2×10^5	−0.43	84
		Q $^{\cdot-}$	−0.50	8.6×10^4	−0.35	79
[(dpp-F ₂₀)MnCl] (3)	0.04 (0.52)	2,6-Me ₂ Q $^{\cdot-}$	−0.67	2.3×10^6	−0.71	89
		MeQ $^{\cdot-}$	−0.58	1.3×10^6	−0.62	86
		Q $^{\cdot-}$	−0.50	2.0×10^5	−0.54	89
		ClQ $^{\cdot-}$	−0.34	1.2×10^5	−0.38	79
[(tpp)MnCl] (4)	−0.22 (0.07) ^[g]	Q $^{\cdot-}$	−0.50	5.4×10^7	−0.28	41
		ClQ $^{\cdot-}$	−0.34	3.4×10^7	−0.12	31
		2,5-Cl ₂ Q $^{\cdot-}$	−0.18	6.1×10^5	0.04	38

[a] Determined in PhCN unless otherwise noted; 0.1M TBAP, scan rate 0.1 V s $^{-1}$,^[14] the experimental error is within ± 0.005 V. [b] Taken from ref. [18]; the experimental error is within ± 0.005 V. [c] The values of peak potential separation expressed in volts are given in parentheses. [d] 2,6-Me₂Q = 2,6-dimethyl-*p*-benzoquinone, MeQ = methyl-*p*-benzoquinone, Q = *p*-benzoquinone, ClQ = chloro-*p*-benzoquinone, 2,5-Cl₂Q = 2,5-dichloro-*p*-benzoquinone. [e] The experimental error is within $\pm 5\%$. [f] Obtained from Equation (1). [g] Determined in MeCN, 0.1M TBAP, scan rate 0.1 V s $^{-1}$.^[17]

order rate constants k_{et} of the electron transfer reactions are summarized in Table 1, which also lists redox potentials of the porphyrin and the reducing agent as well as the free energy change of electron transfer from the semiquinone radical anion to [(P)MnCl] (ΔG_{et}^0). It was confirmed that the k_{et} value for electron transfer from semiquinone radical anion (Q $^{\cdot-}$) to [(dpp-F₂₀)MnCl] in the presence of 0.1M tetrabutylammonium chloride (1.8×10^5 M $^{-1}$ s $^{-1}$) is essentially the same as the value in the absence of excess Cl $^-$ (2.0×10^5 M $^{-1}$ s $^{-1}$). The ΔG_{et}^0 values were calculated from the one-electron reduction potentials of [(P)MnCl] (E_{red}^0) and the one-electron oxidation potentials of the semiquinone radical anions (E_{ox}^0), which are equivalent to the one-electron reduction potentials of the corresponding quinones.^[20]

Reorganization energies λ_{22} for the self-exchange reaction of [(P)MnCl]/[(P)MnCl] $^-$ are listed in Table 1 and were determined by using Equation (1), which is readily derived from the Marcus equation;^[22] $\Delta G^\ddagger$ is the free energy of

$$\lambda_{22} = 2(2\Delta G^\ddagger - \Delta G_{\text{et}}^0 + 2[\Delta G^\ddagger(\Delta G^\ddagger - \Delta G_{\text{et}}^0)]^{1/2}) - \lambda_{11} \quad (1)$$

$$\Delta G^\ddagger = (2.3 RT/F) \lg[Z(k_{\text{et}}^{-1} - k_{\text{diff}}^{-1})] \quad (2)$$

activation, and λ_{11} is the reorganization energy for the self-exchange reaction of XQ/XQ $^{\cdot-}$. A λ_{11} value of 16.9 kcal mol $^{-1}$ for Q/Q $^{\cdot-}$ was determined from the linewidth variation of the EPR spectrum of semiquinone radical anion (Q $^{\cdot-}$) in the presence of various concentrations of *p*-benzoquinone (Q) in PhCN at 298 K.^[23] The λ_{11} values of other semiquinone radical anion derivatives were assumed to be the same as that of Q/Q $^{\cdot-}$. The $\Delta G^\ddagger$ values were obtained from the observed rate constant of electron transfer k_{et} and the diffusion rate constant k_{diff} by Equation (2); Z is the collision frequency (taken as 1×10^{11} M $^{-1}$ s $^{-1}$), F is the Faraday constant, and the k_{diff} value in MeCN is 2.0×10^{10} M $^{-1}$ s $^{-1}$.

The reorganization energies depend significantly on the type of porphyrin macrocycle, and the λ_{22} values (mean values, in kcal mol $^{-1}$) increase in the order [(tpp)MnCl] (37 ± 4) <

[(dpp-H₂₀)MnCl] (70 ± 2) < [(dpp-Cl₁₂H₈)MnCl] (83 ± 3) $\approx$ [(dpp-F₂₀)MnCl] (86 ± 4). Hence, the electron transfer rate at a given free energy change of electron transfer should decrease in this order. This is clearly shown in Figure 1, in

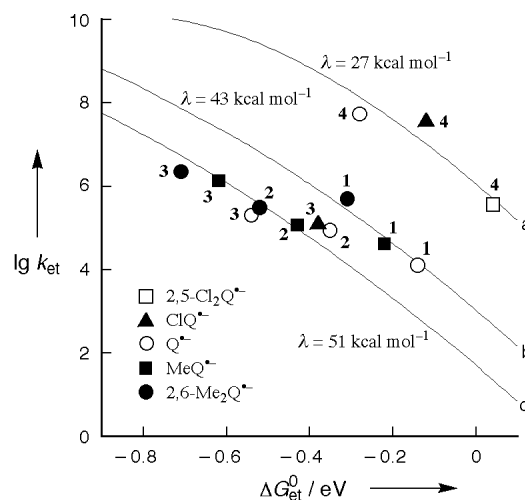


Figure 1. Dependence of $\lg k_{\text{et}}$ on ΔG_{et}^0 for the electron transfer reductions of the [(P)MnCl] derivatives **1–4** by semiquinone radical anions in deaerated MeCN at 298 K. Curve a: P = tpp (**4**); curve b: P = dpp-H₂₀ (**1**); curve c: P = dpp-Cl₁₂H₈ (**2**), P = dpp-F₂₀ (**3**). For explanation of abbreviations, see Table 1. The λ values are calculated by using the Marcus theory of adiabatic outer-sphere electron transfer.

which the $\lg k_{\text{et}}$ values are plotted against ΔG_{et}^0 for electron transfer from XQ $^{\cdot-}$ to [(P)MnCl] in MeCN at 298 K. The fit of the curves to the Marcus theory of adiabatic outer-sphere electron transfer^[22] for the reorganization energies of the cross-reactions between [(P)MnCl] and XQ $^{\cdot-}$ ($\lambda = (\lambda_{11} + \lambda_{22})/2$) indicates that the rate variations at a given ΔG_{et}^0 value arise from the difference in the λ value and not from the non-adiabaticity.

The crystal structure of [(dpp-F₂₀)MnCl] reveals a non-planar conformation of the macrocycle because of peripheral

congestion of the porphyrin.^[16] The Mn^{III} center is located inside the curved surface of the nonplanar porphyrin macrocycle. Such a distorted conformation results in appreciably shorter Mn^{III}–N distances (1.99(1) Å)^[16] than in planar [(tpp)MnCl] (2.009 Å).^[24] Such strong binding of Mn^{III} to the nonplanar porphyrin results in major bond reorganization on reduction of Mn^{III} to Mn^{II}, which is accompanied by out-of-plane displacement since an electron is added to the d_{x²–y²} orbital, which directly interacts with the orbitals of the pyrrole nitrogen atoms.^[24] Hence, the greater the nonplanarity of the porphyrin ligand, the stronger the binding of Mn^{III} to the ligand and the larger the reorganization energy of the electron transfer reduction, as was experimentally observed in the present study. The fact that the E_{red}^0 value of [(dpp-H₂₀)MnCl] (–0.36 V) is more negative than that of [(tpp)MnCl] (–0.22 V), in spite of the electron-withdrawing effect of the additional phenyl groups in dpp-H₂₀ (Table 1), is also ascribed to the stronger Mn^{III}–N binding of the nonplanar conformation of the dpp-H₂₀ ligand, as compared with the planar tpp ligand.

In conclusion, the rate of electron transfer reduction of a Mn^{III} to a Mn^{II} porphyrin is significantly slowed by conformational distortions of the porphyrin ring.

Experimental Section

Syntheses and characterization of the [(P)MnCl] complexes have been previously reported.^[16] Redox potentials of [(P)MnCl] in PhCN or MeCN containing 0.1 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte were determined at room temperature by cyclic voltammetry under deaerated conditions with a three-electrode system and a BAS 100B electrochemical analyzer. Kinetic measurements for electron transfer from XQ^{•–} to [(P)MnCl] were carried out with a Union RA-103 stopped-flow spectrophotometer under deaerated conditions. The rates of electron transfer were followed by monitoring the increase in spectral absorbance of the reduced porphyrin under second-order conditions with equal initial concentrations of [(P)MnCl] and the XQ^{•–}. Second-order rate constants were determined by least-squares curve fitting on a Macintosh microcomputer. The second-order plots of $(A_{\infty} - A)^{-1}$ versus time (A_{∞} and A are the final absorbance and the absorbance at the reaction time, respectively) were linear for three or more half-lives (correlation coefficient $\rho > 0.999$).

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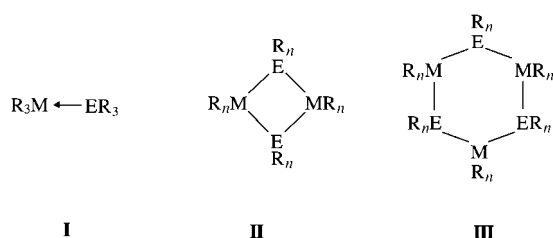
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[Me₂AlBi(SiMe₃)₂]₃—The First Structurally Characterized Organometallic Compound Containing a Bond between an Element of Group 13 and Bi**

Stephan Schulz* and Martin Nieger

*Dedicated to Professor Edgar Niecke
on the occasion of his 60th birthday*

Over the past two decades compounds containing elements of Groups 13 and 15 (III–V compounds) have attracted much attention due to their potential applications as single-source precursors for the deposition of thin films of the corresponding semiconducting material by metal organic chemical vapor deposition (MOCVD). Numerous amides, phosphides, and arsenides of Al, Ga, and In, usually in the form of simple 1:1 Lewis acid–base adducts (type I) or ring compounds (type II and III), have been synthesized.^[1]

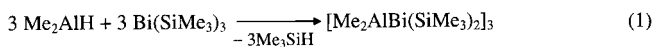


M = Al, Ga, In; E = N, P, As, Sb; R = Alkyl

Fundamental investigations concerning the synthesis and structural characterization of Ga and In antimonides were performed by Wells et al.^[2] and by other groups.^[3] Bisorganogallium and -indium chlorides reacted with Sb(SiMe₃)₃ under elimination of Me₃SiCl (dehalosilylation) to form four-membered ring systems (type II). Recently, we described the synthesis and structural characterization of the first organometallic Al–Sb compounds.^[4] Sb(SiMe₃)₃ reacted with bisorganoaluminum hydrides R₂AlH under elimination of Me₃SiH (dehydrosilylation) to give the ring systems [R₂AlSb(SiMe₃)₂]_x, while trialkylaluminum compounds form simple adducts R₃Al ← Sb(SiMe₃)₃.

Organometallic Bi heterocycles [R_xBiMR_x]_x containing Bi and the main group element M in 1:1 stoichiometry were synthesized and structurally characterized in form of four-membered ring systems (type II; M = main group element, E = Bi). In these compounds the Bi atoms are linked by an electronegative element such as oxygen^[5] or nitrogen.^[6] [Cp₂^{*}SmBi]₂, the only structurally characterized ring compound showing Bi atoms linked by an electropositive element, also contains a four-membered Sm₂Bi₂ core.^[7]

We report herein on the synthesis of the first III–V compound with the higher homologue Bi. Equimolar amounts of Me₂AlH and Bi(SiMe₃)₃ react quantitatively under dehydrosilylation to give [Me₂AlBi(SiMe₃)₂]₃, a six-membered ring system of type III containing Al–Bi bonds [Eq. (1)]. [Me₂AlBi(SiMe₃)₂]₃, which is very sensitive towards air and moisture,



was characterized by elemental analysis, as well as ¹H NMR, ¹³C NMR, and IR spectroscopy. In addition, the solid-state structure was determined by a single-crystal X-ray diffraction study.

The ¹H NMR spectrum shows resonances for the Me and SiMe₃ groups at δ = 0.32 and 0.76, respectively, the ¹³C NMR spectrum only shows the resonance for the SiMe₃ groups at δ = 7.02.^[8] In the mass spectra the peaks with the highest mass observed are at m/z 710 (Bi₂(SiMe₃)₄⁺) and m/z 428 (Bi(SiMe₃)₃⁺). A signal at m/z 57 corresponds to the AlMe₂⁺ fragment. The molecular ion peak [Me₂AlBi(SiMe₃)₂]₃⁺ could not be detected. Evidently, [Me₂AlBi(SiMe₃)₂]₃ is thermally unstable and decomposes under the measurement conditions to the observed compounds. The ¹H NMR spectrum of [Me₂AlBi(SiMe₃)₂]₃ showed no contamination with Bi(SiMe₃)₃ or Bi₂(SiMe₃)₄.^[9]

Single crystals suitable for an X-ray structure analysis were obtained from pentane at 0 °C.^[10] [Me₂AlBi(SiMe₃)₂]₃ crystallizes in the monoclinic space group P2(1)/n (Figure 1). Within

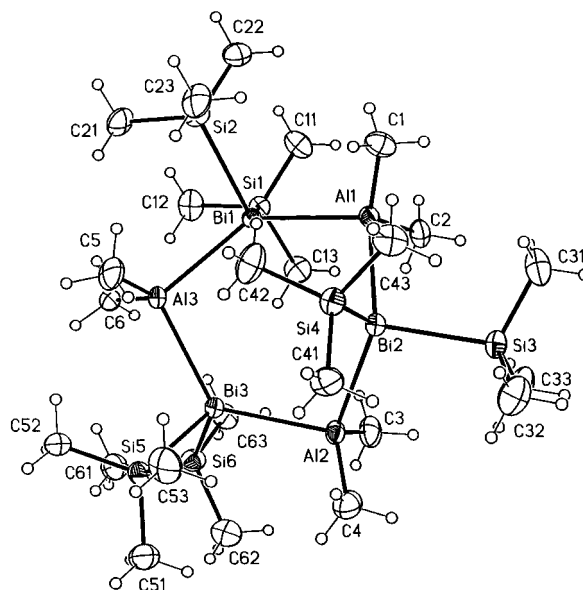


Figure 1. Solid-state structure of [Me₂AlBi(SiMe₃)₂]₃. Selected bond lengths [Å] and angles [°]: Bi1–Al1 2.793(3), Bi1–Al3 2.790(3), Bi2–Al1 2.755(3), Bi2–Al2 2.766(3), Bi3–Al2 2.766(3), Bi3–Al3 2.773(3), Bi1–Si1 2.646(3), Bi1–Si2 2.640(3), Al1–C1 1.967(9), Al1–C2 1.960(9); Al1–Bi1–Al3 130.48(9), Al1–Bi2–Al2 121.67(9), Al2–Bi3–Al3 128.40(8), Bi1–Al1–Bi2 101.00(9), Bi2–Al2–Bi3 101.78(9), Bi1–Al3–Bi3 104.13(8), C1–Al1–C2 122.4(4), Si1–Bi1–Si2 100.75(8).

the six-membered Al₃Bi₃ ring, the Al and Bi atoms adopt a distorted tetrahedral environment. The Al–Bi bond lengths range from 2.755(3) to 2.793(3) Å, which is slightly longer

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than the sum of the covalent radii of these atoms (2.72 Å).^[11] The Al–Bi distances observed at Bi2 and Bi3 are about 3 pm shorter than those found at Bi1. Analogous structural findings were observed in the isostructural compounds [Me₂Ga–Sb(SiMe₃)₂]₃^[12] and [Me₂AlSb(SiMe₃)₂]₃.^[4b] Due to the lack of organometallic Al–Bi compounds, only a comparison with the Zintl phase anion [Al₂Bi₆]^{10–} in Ca₅Al₂Bi₆, showing comparable bond lengths of 2.800–2.869 Å, can be made.^[13] The endocyclic Al–Bi–Al bond angles span a wide range of 121.7(1) to 130.5(1)°, while the Bi–Al–Bi bond angles only vary between 101.0(1) and 104.1(1)°. The greater angle at Al and the smaller angle at Bi are in agreement with the VSEPR model. Comparable values were found in six-membered rings with an Al₃E₃ core (E = element of Group 15), such as [Me₂AlNH₂]₃,^[14] [(Me₃Si)₂AlP(H)(cyclo-C₆H₁₁)]₃,^[15] [H₂Al–P(SiMe₃)₂]₃,^[16] [Me₂AlAsPh₂]₃·2 C₇H₈,^[17] and [Me₂AlSb–(SiMe₃)₂]₃.^[4b] The average Al–C (1.958(9) Å) and Bi–Si (2.635(3) Å) bond lengths are within the expected range.

The dehydrosilylation pathway allows a ready access to Al compounds with the higher homologues of Group 15, Sb and Bi, which could not be synthesized by established methods like salt and alkane elimination or dehalosilylation. Since the corresponding P and As compounds can also be synthesized by dehydrosilylation, this pathway seems generally applicable for the synthesis of Al–pnictogen compounds. Its potential with respect to the synthesis of the corresponding Ga and In compounds is currently under investigation.

Experimental Section

Neat Me₂AlH^[18] (0.12 g, 2 mmol) and Bi(SiMe₃)₃^[19] (0.86 g, 2 mmol) were combined at room temperature. Gas evolution followed immediately, and after 30 s, a light brown solid was formed, which was heated to 50 °C (10 min) and then dissolved in hot pentane. Colorless crystals (0.78 g, 0.63 mmol, 94 %) were formed at 0 °C. M.p. 132 °C (decomp). Elemental analysis for C₂₄H₇₂Al₃Bi₃Si₆ (%): calcd: C 23.30, H 5.87; found: C 23.09, H 5.78; ¹H NMR (300 MHz, C₆D₆, 25 °C): δ = 0.32 (s, 3 H, Me), 0.76 (s, 9 H, SiMe₃); ¹³C NMR (75 MHz, C₆D₆, 25 °C): δ = 7.02 (SiMe₃); MS (EI, 20 eV): *m/z* (%): 710 (5) [Bi₂(SiMe₃)₄]⁺, 428 (60) [Bi(SiMe₃)₃]⁺, 73 (100) [SiMe₃]⁺, 57 (40) [Me₂Al]⁺; IR (Nujol): $\tilde{\nu}$ = 1259, 1245, 1172, 1100, 842, 822, 670, 622 cm^{–1}.

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- [10] [Me₂AlBi(SiMe₃)₂]₃ (C₂₄H₇₂Al₃Bi₃Si₆), colorless crystals, crystal size 0.18 × 0.12 × 0.05 mm, *M_r* = 1237.2, monoclinic, space group *P2(1)/n* (no. 14), *a* = 9.5523(3), *b* = 20.9233(8), *c* = 24.4374(6) Å, β = 96.257(2)°, *V* = 4855.1(3) Å³, *Z* = 4, λ (MoKα) = 0.71073 Å, μ = 11.06 mm^{–1}, ρ_{calcd} = 1.69 g cm^{–3}, *T* = 123(2) K, *F*(000) = 2352. Using a Nonius Kappa CCD diffractometer, 34105 reflections (2θ_{max} = 50°) were collected (8158 unique, *R_{int}* = 0.0978). The structure was solved by direct methods (SHELXS-90)^[20] and refined by full-matrix least-squares on *F*² (SHELXL-97).^[21] All non-hydrogen atoms were refined anisotropically and hydrogen atoms by a riding model. *R*₁ = 0.043 (*I* > 2σ(*I*)), *wR*₂ = 0.093, 331 parameters, largest min./max. residual electron density in the final difference Fourier synthesis: –3.021 e Å^{–3}/2.308 e Å^{–3} (absorption effects). An empirical absorption correction was applied (min./max. transmission 0.2890/0.4466). 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-106174. 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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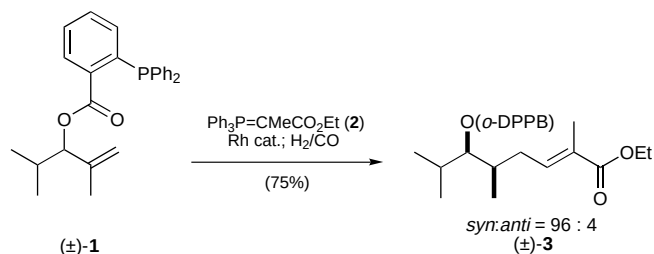
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Domino Hydroformylation-Wittig Reactions**

Bernhard Breit* and Stephan K. Zahn

Efficient organic synthetic transformations are those capable of combining any two given complex building blocks in a convergent synthesis. An important example is the Wittig olefination, a reliable operation for forming carbon–carbon double bonds.^[1] This reaction requires the generation of an aldehyde as a coupling partner, which is typically realized via a redox functional group interconversion or a deprotection. However, according to a definition by Hendrickson only those steps that elaborate the framework of the desired target are deemed synthetically efficient.^[2] Those framework constructing reactions that simultaneously provide a new stereogenic center in a selective manner are, according to Corey, even more efficient.^[3] Hence, a reaction in agreement with these efficiency criteria would be a merged stereoselective hydroformylation, Wittig reaction process. Herein we report the first domino hydroformylation–Wittig as well as the first domino hydroformylation–Wittig–hydrogenation process with both methallyl and homomethallyl alcohols as alkenic substrates.^[4]

When the methallyl-*o*-DPPB ester ($\pm$)-**1** (*o*-DPPB = *ortho*-diphenylphosphanylbenzene) was subjected to hydroformylation conditions in the presence of 1.1 equivalents of the stabilized disubstituted Wittig ylide **2** the 1,6-functionalized trisubstituted alkene ($\pm$)-**3** was isolated in good yield and diastereoselectivity (*syn:anti* = 96:4; Scheme 1).^[5–7] As we have shown previously, diastereoselectivity in the course of the hydroformylation reaction is controlled by the substrate-bound, catalyst-directing *o*-DPPB group.^[8,9] The intrinsic *trans* selectivity of the Wittig olefination when using stabilized



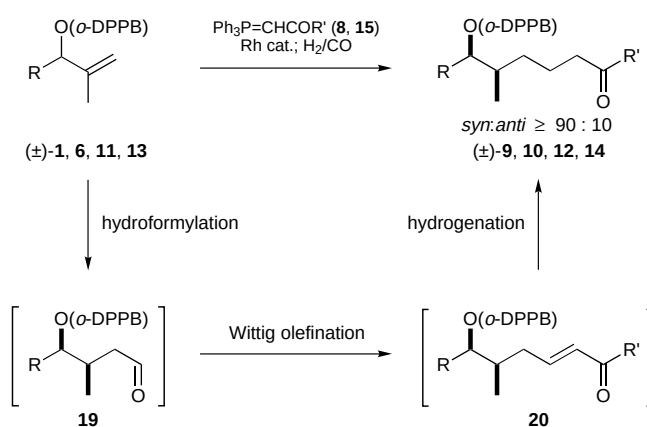
Scheme 1. Reaction conditions: 1.1 equiv $\text{Ph}_3\text{P}=\text{CMeCO}_2\text{Et}$ (**2**), 0.7 mol % $[\text{Rh}(\text{H})(\text{CO})(\text{PPh}_3)_3]$, toluene, 90 °C, 48 h, 20 bar H_2/CO (1/1).

ylides dictates the *E*-olefin geometry. Hence, this process allows the stereoselective formation of a trisubstituted double bond and the formation of a carbon–carbon single bond in a single operational step. In addition, a new stereogenic center

is formed in the acyclic system with high levels of stereoselectivity.

When the disubstituted acetyl Wittig ylide **4** was used, the trisubstituted α,β -unsaturated ketone ($\pm$)-**5** was obtained in good yield and diastereoselectivity (Table 1, entry 2). The presence of a second methyl group bearing a stereocenter within the starting *o*-DPPB ester substrate ($\pm$)-**6** (Piv = pivaloyl, $(\text{H}_3\text{C})_3\text{CCO}$) enabled the construction of a building block with three consecutive stereogenic centers (entry 3). However, a limitation of this domino reaction was set by the basicity of the Wittig ylide employed. Nonstabilized ylides lead to inactivation of the rhodium catalyst.

Interestingly, when the mono-substituted stabilized Wittig ylide **8** was used, the saturated derivatives ($\pm$)-**9**, ($\pm$)-**10**, ($\pm$)-**12**, and ($\pm$)-**14** were obtained as single reaction products in satisfactory to good yields (Scheme 2) and with diastereoselectivities ranging from 9:1 (entry 6) up to >98:2 (entry 7).



Scheme 2. Reaction conditions: 1.5 equiv $\text{Ph}_3\text{P}=\text{CHCOR}'$ (**8** or **15**), 0.7 mol % $[\text{Rh}(\text{H})(\text{CO})(\text{PPh}_3)_3]$, toluene, 90 °C, 48 h, 20 bar H_2/CO (1/1).

The apparent mechanism of this sequential transformation consists of three separate steps. First *o*-DPPB-directed stereoselective hydroformylation provides the aldehyde **19**,^[8] which should react immediately with the Wittig ylide present to form the corresponding α,β -unsaturated carbonyl derivative **20**. Rhodium-catalyzed hydrogenation of this alkene concludes the sequence of events, and affords the saturated ketones ($\pm$)-**9**, ($\pm$)-**10**, ($\pm$)-**12**, and ($\pm$)-**14**.^[7] The reaction could be used for the construction of the all-*syn*, the *anti*–*syn* and the all-*anti* stereotriad sequences that are known as central building blocks of polyketide natural products.^[10] The 1,2-asymmetric induction was controlled in all those reactions within the hydroformylation step by making use of either the substrate-bound, catalyst-directing *o*-DPPB group (entries 4–6),^[7] or by making use of substrate control through conformational constraints (entry 7).^[11] In addition to the acetyl-substituted Wittig ylide **8**, the ester substituted ylide **15** could be employed. However, the yield of the corresponding saturated ester product ($\pm$)-**18** was low, presumably as a result of oligomer formation (entry 8). The reaction could also be applied to a homomethallyl-*o*-DPPB ester ($\pm$)-**17**; Scheme 3). The substrate-bound, catalyst-directing *o*-DPPB group allowed the control of the diastereoselectivity by

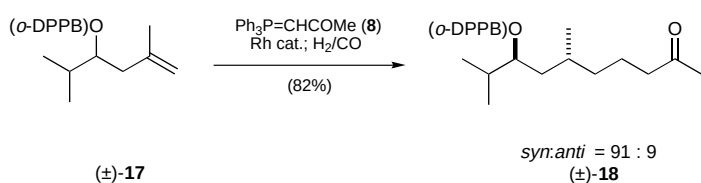
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Table 1. Diastereoselective domino hydroformylation-Wittig and domino hydroformylation-Wittig-hydrogenation reactions.

Entry ^[a]	Alkene	Ylide	Major diastereomer ^[7]	Yield ^[b] [%]	d.r. (<i>syn:anti</i>) ^[c]
1		Ph ₃ P=CMcCO ₂ Et 2		75	96:4
2		Ph ₃ P=CMcCOMe 4		78	92:8
3		2		60	92:8
4		Ph ₃ P=CHCOMe 8		70	92:8
5		8		68	92:8
6		8		60	90:10
7		8		78 ^[d]	> 98:2
8		Ph ₃ P=CHCO ₂ Et 15		36	92:8
9		8		82	91:9

[a] See ref. [5, 6] for a representative procedure and physical data for compounds (±)-**3** and (±)-**9**. [b] Yield of isolated product after column chromatography. [c] Determined by NMR spectroscopic analysis of the crude reaction mixture. [d] Based on 77% conversion (24h at 60°C).



Scheme 3. Reaction conditions: 1.5 equiv Ph₃P=CHCOMe (**8**) 0.7 mol % [Rh(H)(CO)(PPh₃)₃], toluene, 50°C, 4 d, 20 bar H₂/CO (1/1).

making efficient use of 1,3-asymmetric induction.^[9] Thus, the 1,7-functionalized *anti*-ketone (±)-**18** was obtained in good yield and diastereoselectivity (entry 9).

Hence, this sequential transformation allows in a single operational step the formation of two carbon–carbon single bonds, with concomitant generation of a new stereogenic center with high levels of acyclic stereocontrol. This reaction may potentially be used to couple any two given complex building blocks in the context of a convergent synthetic strategy.

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- [5] Representative procedure for the preparation of ester (±)-**3**: To a solution of [RhH(CO)(PPh₃)₃] (6.4 mg, 7 × 10^{−3} mmol, 0.7 mol %) in toluene (3 mL) at 20°C (exclusion of air and moisture), was added subsequently (±)-**1** (402 mg, 1.0 mmol) and ylide **2** (399 mg,

1.1 mmol), and the solution stirred for 5 min. The resulting solution was transferred by cannula with rinsing (toluene, 2 mL) into a carefully evacuated and argon filled stainless-steel autoclave. The autoclave was heated to 90 °C and subsequently pressurized with H₂/CO (1/1, 20 bar). After stirring the mixture for 48 h at this temperature the autoclave was cooled rapidly to 20 °C and depressurized. The reaction solution was filtered through a small pad of silica with *tert*-butyl methyl ether (50 mL). After evaporation of the solvent the crude product was analyzed by NMR spectroscopy to determine the diastereomer ratio (96:4). Subsequent column chromatography on silica (solvent: petrolether/*tert*-butyl methyl ether, 9/1) provided the unsaturated ester (±)-**3** (391 mg, 0.75 mmol) as a highly viscous oil.

- [6] All compounds were characterized by ¹H, ¹³C, ³¹P NMR spectroscopies and elemental analysis. Selected physical data for (±)-**3**: ¹H NMR (300.133 MHz, CDCl₃): δ = 0.66–0.75 (m, 9H, 3CH₃), 1.14 (t, *J* = 7.1 Hz, 3H, CH₃CH₂), 1.61 (s, 3H, CH₃), 1.73–2.01 (m, 4H), 4.03 (q, *J* = 7.1 Hz, 2H, CH₂), 4.72 (dd, *J* = 7.6 Hz, 3.7 Hz, 1H), 6.58 (m_c, 1H, CH-olefin), 6.79 (m_c, 1H, ArH), 7.11–7.16 (m, 11H, ArH), 7.23 (m_c, 1H, ArH), 7.99 (m_c, 1H, ArH); ¹³C NMR (75.469 MHz, CDCl₃): δ = 12.21, 13.34, 13.91, 17.93, 18.84, 29.38, 32.71, 34.28, 59.96, 81.87, 127.76, 128.01 (d, *J*_{C,P} = 7.1 Hz, 2C), 128.08 (d, *J*_{C,P} = 7.2 Hz, 2C), 128.25 (2C), 128.52, 130.10, 130.81 (2C), 133.53 (d, *J*_{C,P} = 20.8 Hz, 2C), 133.66 (d, *J*_{C,P} = 21.0 Hz, 2C), 133.93, 137.78 (d, *J*_{C,P} = 12.5 Hz), 137.81 (d, *J*_{C,P} = 11.9 Hz), 139.75, 140.88 (d, *J*_{C,P} = 28.2 Hz), 165.92, 167.60; ³¹P NMR (81.015 MHz, CDCl₃): δ = –2.9; elemental analysis calcd for C₃₂H₃₇O₄P (516.62): C 74.49, H 7.22; found: C 74.61, H 7.34. Selected physical data for (±)-**9**: ¹H NMR (300.133 MHz, CDCl₃): δ = 0.67–0.78 (m, 9H, 3CH₃), 0.97 (m, 1H, CH), 1.08 (m, 1H, CH), 1.43 (m, 2H, CH₂), 1.65 (m, 1H, CH), 1.80 (m, 1H, CH), 1.97 (s, 3H, CH₃), 2.10–2.20 (m, 2H, CH₂), 4.72 (dd, *J* = 7.8 Hz, 4.1 Hz, 1H), 6.82 (m_c, 1H, ArH), 7.10–7.20 (m, 11H, ArH), 7.26 (m_c, 1H, ArH), 8.01 (m_c, 1H, ArH); ¹³C NMR (75.469 MHz, CDCl₃): δ = 13.40, 18.08, 18.88, 21.04, 26.61, 29.20, 32.84, 34.01, 43.42, 81.55, 124.91, 127.76, 128.04 (d, *J*_{C,P} = 6.8 Hz, 2C), 128.12 (d, *J*_{C,P} = 5.7 Hz, 2C), 128.62, 130.08, 131.47 (2C), 133.52 (d, *J*_{C,P} = 20.8 Hz, 2C), 133.68 (d, *J*_{C,P} = 21.0 Hz, 2C), 133.88, 137.63 (d, *J*_{C,P} = 10.3 Hz), 137.96 (d, *J*_{C,P} = 11.8 Hz), 140.77 (d, *J*_{C,P} = 27.9 Hz), 165.97, 208.44; ³¹P NMR (81.015 MHz, CDCl₃): δ = –2.9; elemental analysis calcd for C₃₀H₃₅O₃P (474.58): C 75.93, H 7.43; found: C 75.77, H 7.57.

- [7] The relative configuration of the domino reaction products was determined at the aldehyde stage, since the stereochemistry-determining step is the hydroformylation.^[8, 9, 11] This was confirmed by stepwise transformation of the known aldehyde (±)-**19** derived from olefin (±)-**1**^[8] into both the domino hydroformylation-Wittig product (±)-**20** (Ph₃P=CHCOMe, CH₂Cl₂, RT, 2 d, 80 %) and the domino hydroformylation-wittig-hydrogenation product (±)-**9** (0.7 mol % [Rh(H)(CO)(PPh₃)₃], H₂ (20 bar), toluene, 60 °C, 24 h, 62 %).

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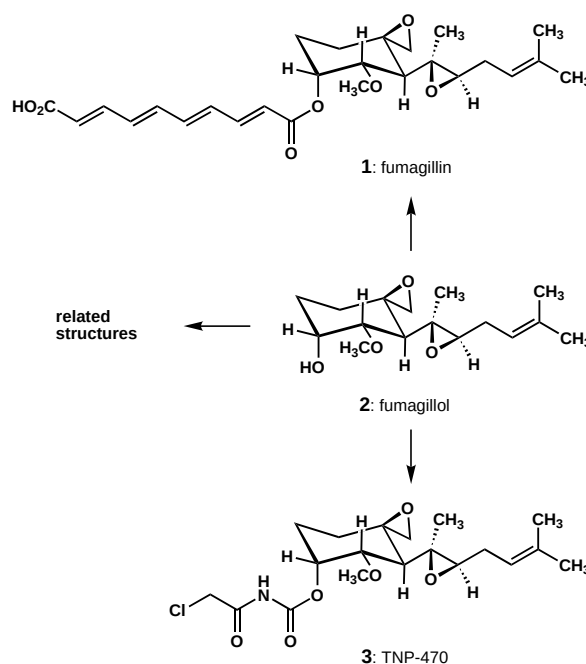
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A Concise Synthesis of Fumagillol**

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Fifty years ago Hanson and Eble reported that cultures of the fungus *Aspergillus fumigatus* inhibited *Staphylococcus aureus* 209 bacteriophage.^[1] The active constituent was named fumagillin, and was soon renowned for its potent anti-parasitic properties.^[2] The meritorious studies of Tarbell and co-workers yielded insight into the chemical behavior of fumagillin and a hypothesis about its constitution.^[3] An X-ray crystallographic analysis confirmed the outcome of the chemical degradation campaign and thus established the structures of fumagillin (**1**) and its saponification product fumagillol (**2**).^[4] Each substance is distinguished by two



epoxide functions, one of which is highly reactive, and six contiguous stereocenters. Fumagillin presented itself as an attractive objective for research in organic synthesis because of its novel structure and utility as an amebicide.^[5] In a landmark paper in 1972 Corey and Snider described their elegant studies that culminated in the first chemical synthesis of this natural product.^[6, 7]

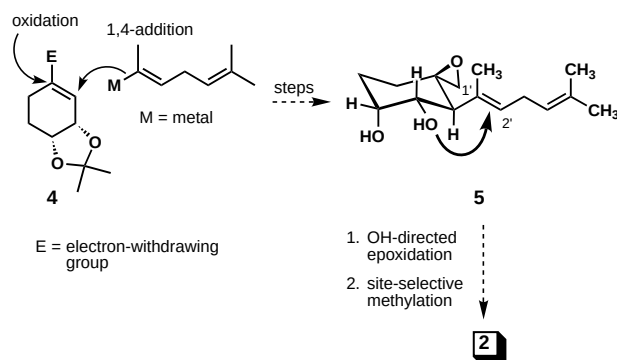
Interest in the biological properties of fumagillin experienced a renaissance when Judah Folkman and his group discovered that **1** and its semi-synthetic derivative TNP-470

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(3) have the remarkable capacity to inhibit the proliferation of endothelial cells in vitro and tumor-induced angiogenesis in vivo.^[8] Since unregulated angiogenesis can cause the growth and metastasis of malignant tumors and inflammatory diseases, inhibitors of this process are of considerable interest as potential chemotherapeutic agents.^[9] TNP-470 (3) is currently one of the most promising small molecule inhibitors of angiogenesis and is being evaluated in phase III clinical trials as a potential cancer drug. In recent work, fumagillin-derived affinity reagents allowed the identification of methionine aminopeptidase-2 (MetAP-2) as the molecular target of fumagillin's action.^[10–12] Fumagillin may prevent the myristoylation of a key endothelial cell regulatory protein^[9e, 13] by irreversibly inhibiting MetAP-2, a cobalt-containing metalloprotease that removes methionine residues from the N termini of proteins in a critical co-translational processing event.^[14] Research in our laboratory sought a concise solution to the chemical problem posed by these structures. Herein we describe a 13-step synthesis of (±)-fumagillol (2), the immediate precursor of fumagillin (1) and TNP-470 (3), from abundant and inexpensive starting materials.

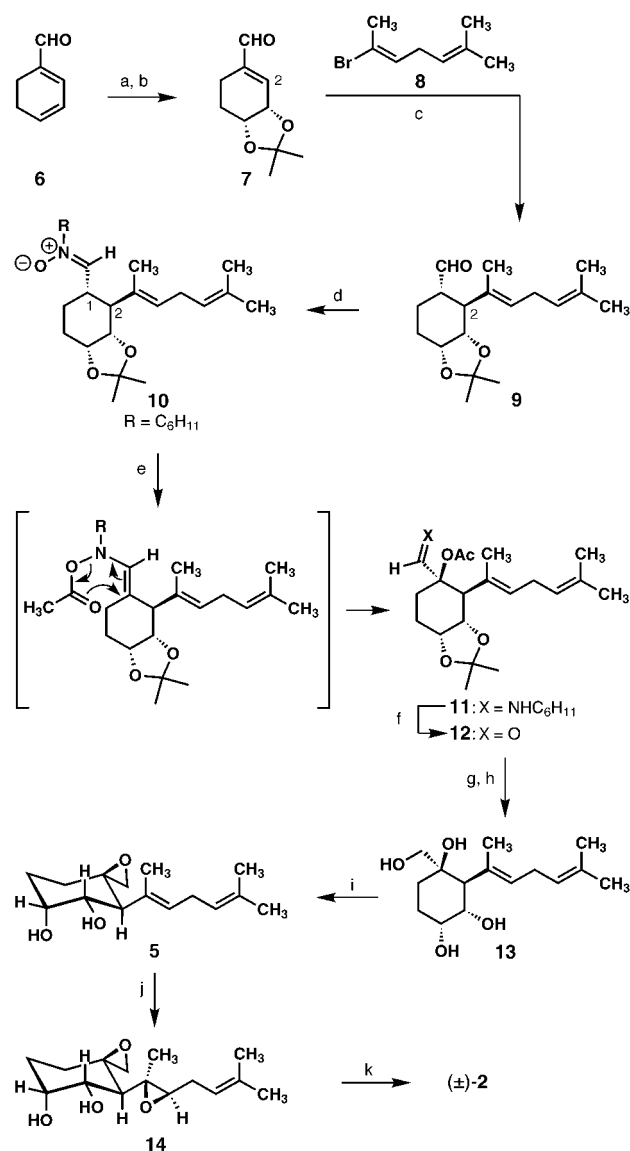
The essence of our strategy is a vicinal difunctionalization of the electron-deficient alkene of a compound of type 4 (E = electron-withdrawing group), after which a short reaction sequence could give rise to diol 5 (Scheme 1). Although 5



Scheme 1. An approach to the synthesis of 2, which features a vicinal difunctionalization of 4.

bears two trisubstituted alkenes, the C1'–C2' double bond is distinguished by its proximity to a free hydroxyl group. Our intention was to explore the feasibility of effecting a site- and diastereoselective hydroxyl-directed epoxidation of the C1'–C2' olefin.^[15] A selective methylation of the equatorial C-3 hydroxyl group, a reaction shown to be feasible by Corey and Snider,^[6] would then complete a synthesis of 2.

Our synthesis of (±)-2 was guided by this general analysis and achieved by the pathway presented in Scheme 2. Upjohn dihydroxylation^[16] of the more electron-rich γ,δ -double bond of 1,3-cyclohexadiene-1-carbaldehyde (6), a known substance^[17] that can be prepared in bulk from acrolein and 1-diethylamino-1,3-butadiene,^[18] afforded a diol (73% yield) that could be efficiently protected as isopropylidene ketal 7 (87% yield). By this sequence we could procure multi-gram quantities of enal 7.



Scheme 2. Synthesis of (±)-2: a) OsO₄ (0.5 mol %), NMO · H₂O (2.5 equiv), 2-propanol, RT, 1 h, 73 %; b) (CH₃)₂CO, *p*-TsOH (0.05 equiv), RT, 1 h, 87 %; c) 8, *t*BuLi (2.1 equiv), Et₂O, –78 °C, 1 h; then Li(2-thienyl)CuCN (1.2 equiv), 1 h; then 7, BF₃ · OEt₂ (1.0 equiv), Et₂O, 5 min, 46 %; d) C₆H₁₁NHOH (2.0 equiv), EtOH, NaHCO₃ (5.0 equiv), RT, 1 h; e) AcCl (1.0 equiv), Et₃N (1.0 equiv), Et₂O, 0 °C → RT, 0.5 h; f) AcOH, NaOAc, RT, 1 h, 51 % from 9; g) LiAlH₄ (6.0 equiv), Et₂O, 0 °C → RT, 1 h, 71 %; h) 2.0 N HCl/THF (4/1), RT, 1 h, 81 %; i) MsCl (2.0 equiv), 4-DMAP (0.2 equiv), Et₃N (5.0 equiv), CH₂Cl₂, –30 °C, 0.2 h; then 6.0 N NaOH (10.0 equiv), MeOH, RT, 0.5 h, 97 %; j) [VO(acac)₃] (0.2 equiv), *t*BuOOH (3.0 equiv), benzene, RT, 5 min, 75 % (4.3/1 ratio of epoxide diastereomers in favor of 14); k) *t*BuONa (1.4 equiv), MeI (3.0 equiv), THF, [15]crown-5, RT, 2 h, 40 % of (±)-2 plus 40 % recovered 14. NMO = 4-methylmorpholine *N*-oxide, *p*-TsOH = *p*-toluenesulfonic acid, MsCl = methanesulfonyl chloride, DMAP = 4-dimethylaminopyridine, [VO(acac)₃] = vanadyl acetylacetonate.

We hoped that the *cis*-ring fusion of bicycle 7 would permit a diastereocontrolled bond formation at C-2.^[19] Despite the presence of two electrophilic sites in enal 7, the organocuprate reagent derived from vinylbromide 8^[20–22] reacted with 7 in a highly regio- and diastereoselective fashion in the presence of BF₃ · OEt₂ and gave a 3/1 mixture of C-1 epimers in favor of aldehyde 9 (Table 1).^[23] While the minor epimer could be

Table 1. Selected physical properties of compounds **9**, **12**, and **2**.

9: TLC: R_f = 0.50 (silica gel, hexanes/EtOAc (5/1)); IR (film): $\tilde{\nu}$ = 1726 cm^{-1} (C=O); ^1H NMR (400 MHz, CDCl_3): δ = 9.43 (d, J = 3.5 Hz, 1H; CHO), 5.30–5.26 (m, 1H; H-2'), 5.07–5.03 (m, 1H; H-4'), 4.28–4.26 (m, 1H; H-4), 3.98 (dd, J = 4.8, 9.2 Hz, 1H; H-3), 2.73–2.70 (m, 2H; H-3'), 2.41 (dd, J = 9.3, 11.9 Hz, 1H; H-2), 2.27–2.19 (m, 2H), 1.77–1.71 (m, 3H), 1.68 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.55 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ = 203.2, 132.0, 128.8, 122.5, 108.4, 76.4, 72.8, 50.1, 49.8, 28.6, 28.5, 26.9, 26.2, 25.6, 25.5, 20.5, 17.7, 13.6; HR-MS (FAB): m/z calcd for $[M^+ + \text{Na}]$ 315.1936, found 315.1927.

12: TLC: R_f = 0.26 (silica gel, hexanes/EtOAc (5/1)); IR (film): $\tilde{\nu}$ = 1735 cm^{-1} (C=O); ^1H NMR (500 MHz, CDCl_3): δ = 9.33 (s, 1H; CHO), 5.31–5.28 (m, 1H; H-2'), 5.06–5.02 (m, 1H; H-4'), 4.39 (dd, J = 4.8, 9.9 Hz, 1H; H-3), 4.33–4.31 (m, 1H; H-4), 2.73–2.70 (m, 2H; H-3'), 2.29 (d, J = 9.9 Hz, 1H; H-2), 2.27–1.25 (m, 4H), 2.16 (s, 3H; C(O)CH₃), 1.77 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3): δ = 199.0, 170.0, 132.3, 128.7, 122.1, 108.5, 87.3, 74.8, 72.4, 53.4, 50.2, 28.5, 27.1, 26.3, 25.6, 23.2, 21.8, 20.9, 17.8, 15.4; HR-MS (FAB): m/z calcd for $[M^+ + \text{H}]$ 351.2171, found 351.2181.

($\pm$)-fumagillol (**2**): TLC: R_f = 0.41 (silica gel, CH_2Cl_2 /acetone (3/1)); IR (film): $\tilde{\nu}$ = 3430 cm^{-1} (OH); ^1H NMR (400 MHz, CDCl_3): δ = 5.21–5.17 (m, 1H; H-4'), 4.37–4.35 (m, 1H; H-4), 3.61 (dd, J = 3.0, 11.4 Hz, 1H; H-3), 3.48 (s, 3H; OCH₃), 2.92 (d, J = 4.0 Hz, 1H; exomethylene oxide H), 2.57 (t, J = 6.5 Hz, 1H; H-2'), 2.52 (d, J = 4.0 Hz, 1H; exomethylene oxide H), 2.39–2.33 (m, 2H; H-3'), 2.3–1.3 (m, 5H), 1.91 (d, J = 11.4 Hz, 1H; H-2), 1.73 (s, 3H), 1.64 (s, 3H), 1.20 (s, 3H; CH₃-1'); ^{13}C NMR (125 MHz, CDCl_3): δ = 134.9, 118.5, 80.9, 64.0, 61.2, 59.8, 58.5, 56.5, 50.7, 47.0, 28.5, 27.4, 26.5, 25.7, 18.0, 13.9; HR-MS (FAB): m/z calcd for $[M^+ + \text{Na}]$ 305.1729, found 305.1734.

converted into **9** on treatment with *t*BuOK in *t*BuOH, both stereoisomers could be converted into α -acetoxyaldehyde **12** (see below). Gratifyingly, the C-2 epimer of **9** was not observed. The cyclic protecting group of enal **7** thus dictated the stereochemical course of the crucial carbon–carbon bond formation.

Our efforts to introduce the needed C-1 oxygen atom via the enolate of aldehyde **9** encountered significant resistance. However, treatment of *N*-cyclohexylnitrone **10**, derived in one step from aldehyde **9**, with acetyl chloride and triethylamine yielded α -acetoxy-*N*-cyclohexylimine **11**, presumably via a [3,3] sigmatropic isomerization of a transitory *N*-vinyl-*O*-acetylhydroxylamine^[24] as shown in Scheme 2. While imine **11** could be isolated, it was convenient to convert crude **11** into aldehyde **12** by hydrolysis with mild aqueous acid. This under-utilized method, developed by Cummins and Coates^[24] for creating α -acetoxycarbonyl compounds, provided a simple solution to the nontrivial problem of introducing the requisite oxygen atom at C-1 in a diastereocontrolled fashion. We could create compound **12** in good yield and diastereoselectivity by this procedure (51 % over three steps; less than 5 % of the C-1 epimer was isolated). It is difficult to advance a compelling argument to explain the observed diastereoselectivity, but we note that the acetoxy group in **12** is situated on the convex face of the bicyclic framework.

The path to **2** from compound **12** was a straightforward one. After reduction of **12** with lithium aluminum hydride, acid-catalyzed hydrolysis of the isopropylidene ketal provided tetraol **13**. Although the primary alcohol moiety of **13** is flanked by a fully substituted carbon atom, it could be converted selectively into the corresponding methanesulfonate ester. Direct treatment of the reaction mixture with

methanolic sodium hydroxide solution then afforded spiroepoxide **5**. We hoped to exploit a documented side-chain conformational preference^[6] and the close spatial relationship between the free hydroxyl at C-3 and the interior side-chain double bond to achieve the introduction of the remaining epoxide function of fumagillol. When **5** was subjected to Sharpless's vanadium-based epoxidation protocol,^[25] **14** was isolated in 61 % yield (75 % yield of a 4.3/1 mixture of diastereomers in favor of **14**).^[26] Finally, exposure of **14** to *t*BuONa and iodomethane^[27] resulted in the formation of ($\pm$)-**2**, identical in all respects, except optical rotation, to a sample derived from natural fumagillin (**1**).

In summary, we have described a chemical synthesis of **2** by a pathway that is distinguished by its brevity and reliance on relatively simple starting materials and reagents. We are currently adapting our strategy to the production of fumagillol in enantiomerically pure form. In passing, we note that the hypothetical construct **4** wherein E = CO₂Me (Scheme 1) is available in optically active form^[28] and can be converted into enal **7** (Scheme 2) through a two-stage reduction/oxidation sequence. The convergent nature of the strategy described herein may facilitate the synthesis of manifold fumagillin-like small molecules that may show utility as inhibitors of angiogenesis.

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Dynamic Light Scattering Evidence for a Ligand-Induced Motion between the Two Domains of Glucoamylase G1 of *Aspergillus niger* with Heterobivalent Substrate Analogues**

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Glucoamylases (GAs) catalyze the hydrolytic release of β-D-glucose from the nonreducing ends of starch and related oligo- and polysaccharides. Most GAs possess a starch-binding domain (SBD) separated from the catalytic domain (CD) by a glycosylated peptide linker of variable length.^[1] Removal of the SBD reduces the activity of GA from *Aspergillus niger* on insoluble starch but not on soluble substrates.^[2] We have previously shown that 6^{II}-thiopanose and its higher oligomers bind essentially to the SBD and modulate GA activity on starch.^[3] This raised the possibility of an interaction between the CD and the SBD of GA, and these observations have suggested that a cooperativity of the two domains could be critical for optimal activity.^[4] The only low resolution structural information available so far on the entire GA was obtained by scanning tunneling microscopy,^[5] but the possible mobility of the two domains induced by substrate binding cannot be described by this technique. The three-dimensional structure of the CD of the GA from *Aspergillus awamori* X100 has been solved by X-ray crystallography,^[6] while that of the SBD of GA isolated from *Aspergillus niger* has been recently determined by NMR spectroscopy.^[7] Failure to crystallize the entire GA has repeatedly been observed and this is attributed to the inherent flexibility of the linker peptide that connects the two constitutive domains. Co-crystallization in the presence of a ligand targeted to both the CD and SBD may stabilize one conformer. Recently, closure of a flexible loop onto a substrate analogue has allowed the crystallization of a cellulase.^[8]

Herein we describe the design and synthesis of high affinity probes that bind the CD and SBD of GA at the same time, and hence get new insights into the structure/activity relationships of GA. The structural variations of GA between free and bound states were monitored by quasi-elastic light

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scattering, a technique that has recently been used with success for the determination of the hydrodynamic dimensions of a multidomain cellulase.^[9]

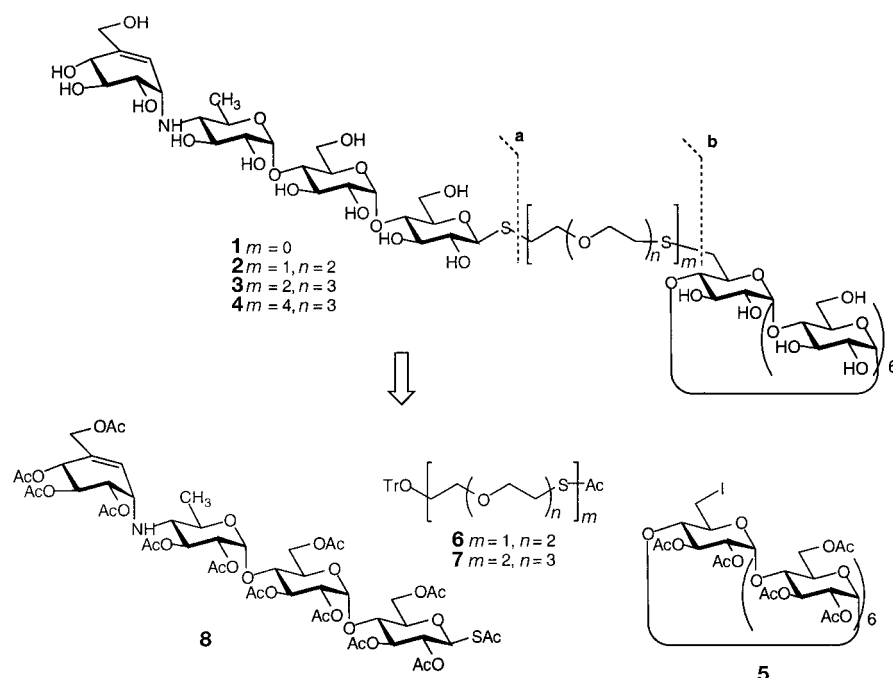
It is well known that acarbose is a potent inhibitor of GA and that two molecules of β -cyclodextrin may mimic amylose binding on two sites of the SBD but cannot fit into the pocket-shaped active site.^[10] These observations indicate that an amylose chain that interacts with the two constitutive domains of GA could be mimicked by an acarbose molecule tethered to a cyclodextrin molecule through flexible spacers of different lengths.

A few years ago we developed an efficient strategy for the synthesis of β -cyclodextrins that were substituted at the 6-position with a thioglucosyl moiety.^[11] The X-ray structure of a cyclodextrin glycosyltransferase complexed with one of these molecules was recently solved, and indicated that the presence of a thioglucosidic linkage did not hinder the binding to the protein.^[12] These results encouraged us to connect linkers to acarbose and β -cyclodextrin through sulfur linkages.

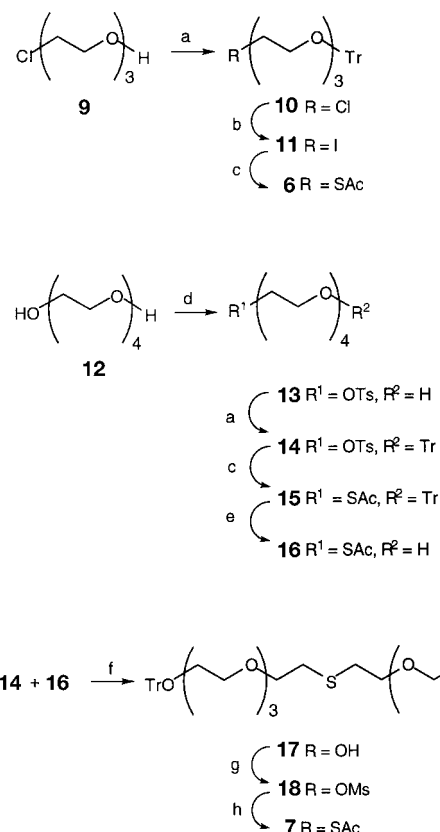
Scheme 1 presents the retro-synthetic analysis on which the synthesis was based. Disconnection at the indicated bonds led to the key building blocks **5**–**8** for the synthesis of **1**–**3**. The longest probe **4** was best prepared by elongation of the linker **7** already coupled to β -cyclodextrin. To allow coupling with 6-deoxy-6-iodo- β -cyclodextrin **5** the bifunctional spacers **6** and **7** had to carry a thiol function and a protected hydroxyl group that could be easily converted into a thiophilic group for the establishment of the thioglycosidic linkage with *S*-acetylacarbose (**8**).

Protection of the hydroxyl groups of commercially available monochlorotriethylene glycol **9** by tritylation (trityl = Tr = triphenylmethyl), followed by halogen exchange and nucleophilic displacement of iodine with potassium thioacetate, afforded the first spacer-arm **6** in 33% overall yield (Scheme 2). Tetraethylene glycol **12** was treated with tosyl chloride and the resulting monotosylate **13** was tritylated to give **14**. Substitution of the tosylate with thioacetate and cleavage of the trityl group gave **16**, which was coupled with **14** after *S*-deacetylation ($\rightarrow$ **17**). Mesylation (mesyl = Ms = methanesulfonyl) of **17** gave **18**, and substitution of the mesylate group with thioacetate gave the bifunctional spacer **7** in 22% overall yield (from **12**).

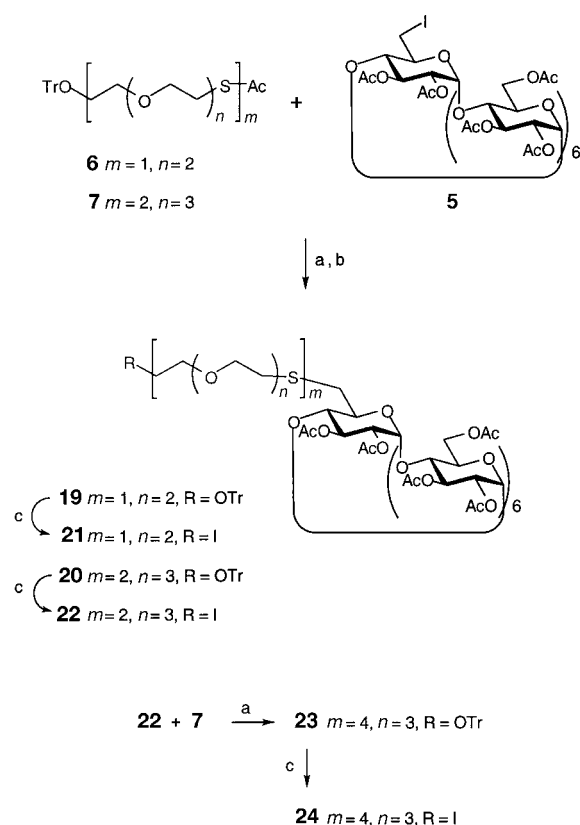
The *S*-deacetylation of compounds **6** or **7** gave the corresponding sodium thiolates, which were coupled, without characterization, with 6-deoxy-6-iodo-cyclodextrin **5** prepared as already described^[11] (Scheme 3). The branched cyclodextrins **19** and **20** were obtained in 76 and 64% yield, respectively. Detritylation, followed by mesylation and displacement of the mesyl group with iodine gave the



Scheme 1. Retrosynthetic pathway of the bifunctional ligands **1**–**4**.



Scheme 2. Preparation of the bifunctional spacers **6**–**7**. a) Ph_3CCl , CH_2Cl_2 , 4-DMAP, NEt_3 , RT, 20 h, **10**: 70%, **14**: 51%; b) NaI (1.1 equiv), DMF, 100 °C, 3 h, 75%; c) KSAc (6 equiv), DMF, 100 °C, 2 h, **6**: 63%, **15**: 87%; d) TsCl (1 equiv), pyridine, 0 °C $\rightarrow$ RT, 12 h, 52%; e) HBF_4 in H_2O , CH_3CN , RT, 1 h, 95%; f) **1**, MeONa (1.3 equiv), MeOH, 0 °C, 2 h, argon; 2. **14** (0.6 equiv), DMF, 45 °C, RT, argon, 73%; g) MeSO_3Cl , pyridine, 0 °C $\rightarrow$ RT, 84%; h) KSAc, DMF, RT, 2 h, argon, 87%. 4-DMAP = dimethylaminopyridine.

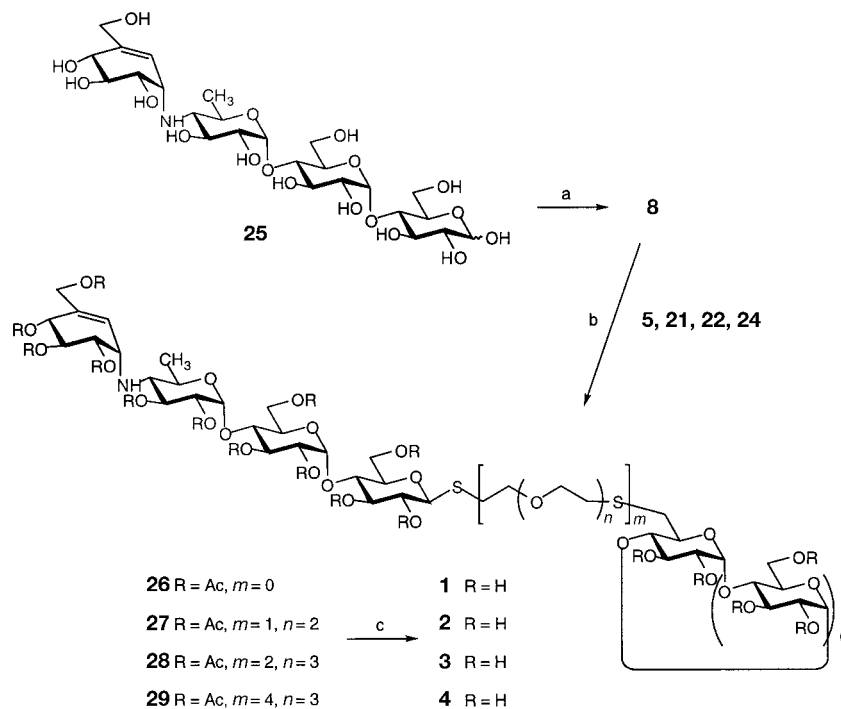


Scheme 3. Preparation of branched cyclodextrins **21**, **22**, and **24**. a) **6**, **7**, **22**, MeONa (10 equiv), 1 h, 0 °C, argon; b) 1. DMF, RT, 12 h, argon; 2. Ac₂O, pyridine, 4-DMAP, 60 °C, 12 h, **19**: 76 %, **20**: 64 %, **23**: 70 %; c) 1. HBF₄ in H₂O, CH₃CN, RT, 20 min; 2. MeSO₃Cl, pyridine, 0 °C → RT, 12 h; 3. NaI, DMF, 100 °C, 4 h, **21**: 51 %, **22**: 40 %, **24**: 43 %.

corresponding iodo compounds **21** and **22** in overall yields of 51 and 40 %, respectively. This stepwise protocol gave a higher yield than the direct OH → I substitution.^[13] Coupling of the iodo derivative **22** with the thiolate arising from S-deacetylation of **7**, as already described, gave compound **23** in 70 % yield. Transformation of **23** into **24** was obtained by sequential reactions as described for the synthesis of **21** and **22**. The longest synthon **24** was obtained in an overall yield of 43 % starting from **22**.

To synthesize donor **8**, acarbose (**25**) was first acetylated, then treated with HBr/AcOH,^[14] and the corresponding bromide was transformed into **8** in 63 % overall yield (Scheme 4).

Coupling of the acarbose derivative **8** with acceptors **5**, **21**, **22**, and **24** under the conditions of von Itzstein et al.^[15] led to the acetylated target molecules **26–29** in yields of 63, 88, 83, and 74 %, respectively. The final products **1–4** were obtained by O-deacetylation and were purified by reversed-phase chromatography on a C-18 column.



Scheme 4. Preparation of ligands **1–4**. a) 1. Ac₂O, pyridine, RT, 24 h; 2. CH₂Cl₂, HBr/AcOH, –10 °C → 0 °C, 1 h; 3. KSAc, DMF, RT, 18 h, 63 %; b) Et₃NH, DMF, RT, 4 h, **26**: 63 %, **27**: 88 %, **28**: 83 %, **29**: 74 %; c) MeONa, MeOH, RT, 1 h, then NH₄OH (1 M), RT, 12 h, **1**: 78 %, **2**: 74 %, **3**: 91 %, **4**: 74 %.

The thermodynamic parameters of ligand binding to GA, as well as to the CD and SBD of GA, have been studied previously by isothermal titration calorimetry.^[16] The ΔH^0 values of binding of the four heterobidentate ligands were, within experimental error, equal to the sum of the ΔH^0 values of the binding of free acarbose and β -cyclodextrin to the CD and SBD, respectively. These results showed that the catalytic and starch binding sites of GA are in close proximity in solution and this suggests considerable flexibility of the linker region.

Here the hydrodynamic dimensions of GA and the CD were determined by quasi elastic light scattering experiments. In accord with their three-dimensional structures the isolated SBD and CD were approximated as spheres, while the dumb-bell two-domain GA was approximated as an ellipsoid whose minor semi-axis corresponded to the hydrodynamic radius of the largest individual component, for example, the CD. The experiments were performed at different angles and several protein concentrations (0.3–1.1 mg mL^{–1}). The autocorrelation function was represented by a single exponential decay for all concentrations and scattering angles. A very small contribution from a slow process has been identified at high concentration, which was attributed to the presence of minor protein aggregates. Relaxation times were estimated from the correlation functions, and the variation of the frequencies versus the scattering angles allowed the determination of the translational diffusion coefficients $D_T(c)$ at different concentrations. The values of the diffusion coefficients at infinite dilution $D_T^0 = D_T(c)_{c \rightarrow 0}$, which represents the single particle property, were converted into their hydrodynamic radii (modified Stokes–Einstein relation). Neither the GA or CD systems showed any concentration dependence on the diffusion coefficient (Figure 1).

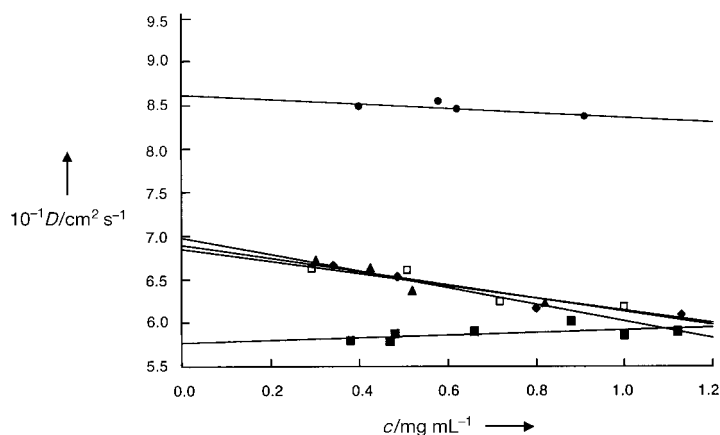


Figure 1. Variation of the translational diffusion coefficient $D_T(c)$ as a function of concentration c of GA (■), CD (●), GA + 1 (◆), GA + 2 (▲), and GA + 3 (□).

The extrapolated values at infinite dilution gave hydrodynamic sizes of 78.5 Å (major semi-axis) and 30 Å (semi-axis), for the GA and CD, respectively. These values are in good agreement with those obtained previously with other techniques (141 and 60–65 Å for the major and minor axis of GA, respectively).^[5, 6] The diffusion coefficients for **1**, **2**, and **3** in the presence of GA show a decrease as a function of concentration (Figure 1).

The three systems gave roughly the same concentration dependence within experimental errors. The associated diffusion coefficients decrease with the protein concentration and the extrapolated value at infinite dilution gave a hydrodynamic size of 62 Å (major semi-axis). However a value of 72.5 Å was found with the longer probe **4**. These values are intermediate between systems with a CD and GA only; and is in fact expected if the ligand somehow decreases the size of the GA system. The negative value of the slope of D_T versus c shows that there is a strong attraction between the particles and reflects the thermodynamics of the system; in general the value of the slope is pH dependent. It appears from these results that the presence of the bound bifunctional ligands stabilizes a more compact conformation of GA. If this motion of the two domains also occurs upon binding of the natural substrate it may achieve the processivity of this exo-enzyme: GA would bind to the polymer through its SBD and the motion of the CD would allow the cleavage of several glucose moieties at the nonreducing end of the same glucan chain before the release of the enzyme. This new concept is under investigation by using mutants of glucoamylase with linkers of various lengths.

Experimental Section

All compounds were homogeneous according to elemental analysis or HR-MS and ^1H and ^{13}C NMR spectra.

Dynamic light scattering experiments: Sample preparation, equipment, and data analysis are essentially the same as described in ref. [9], except that sodium acetate buffer (50 mM, pH 4.2) and a temperature of 27 °C were used. Equimolar ratios of ligands and enzyme were utilized.

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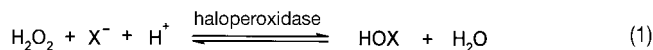
Keywords: carbohydrates • dynamic light scattering • enzyme inhibitors • protein structures • structure–activity relationships

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Metal-Free Haloperoxidases: Fact or Artifact?

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Haloperoxidases are enzymes that catalyze the oxidation of a halide ion (X^-) to the corresponding hypohalous acid [Eq. (1)]. The hypohalous acid produced can further react with different nucleophilic acceptors to form a diversity of halogenated compounds.^[1, 2] Different classes of haloperox-



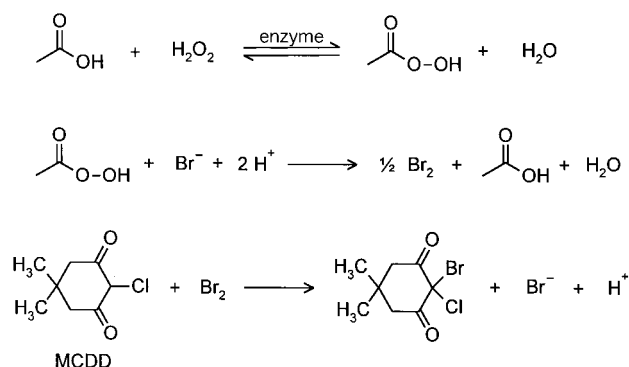
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idases have been identified. Two types of haloperoxidases carry a prosthetic group: a heme group in the case of the heme-containing haloperoxidases and a vanadate ion (VO_4^{3-}) in the case of the vanadium-containing haloperoxidases.^[1, 3]

Recently, a third class of haloperoxidases, the so-called metal-free haloperoxidases, was described by van Pée et al.^[4] The mode of action of these haloperoxidases was unknown although a proposal was made that involved the formation of an intermediate methionine sulfoxide in the active site.^[5] It was, therefore, very surprising that an X-ray structural analysis of a metal-free bromoperoxidase^[6] did reveal the presence of a catalytic triad (Ser-His-Asp) commonly found in lipases, esterases, and serine proteases.^[7] Combined with the fact that these enzymes exhibit activity only in acetate buffer, a mechanism was suggested by Berkessel, Krauss, van Pée, and co-workers^[8] in which the halogenation of monochlorodimedone (2-chloro-5,5-dimethyl-1,3-cyclohexadione, MCDD) in the presence of acetate, H_2O_2 , Br^- , and the enzyme is explained by the reaction sequence outlined in Scheme 1.



Scheme 1. The mechanism of MCDD halogenation when catalyzed by a "metal-free haloperoxidase". A low stationary-state concentration of peracetic acid is formed by perhydrolysis of acetic acid.^[8]

The enzyme-catalyzed step in the process is perhydrolysis (that is, lysis with hydrogen peroxide) of acetic acid to form peracetic acid. Unfortunately, the authors were not able to prove the presence of peracetic acid in their reactions because of a lack of sufficiently sensitive analytical tools. An indirect proof was, however, obtained when the metal-free haloperoxidase, which was also found to have esterase activity towards 4-nitrophenyl acetate, was found to degrade solutions of pre-formed peracetic acid. Four proteases as well as a choline esterase were also tested for haloperoxidase activity under the same experimental conditions by the authors, but only choline esterase gave a positive reaction. It was, therefore, concluded that the ability to catalyze perhydrolysis is not a general characteristic of enzymes with a catalytic mechanism based on a Ser-His-Asp catalytic triad, and that a novel enzymatic system had been introduced.

We have described previously a highly sensitive assay based on HPLC with electrochemical detection for the detection of peracids in complex mixtures.^[9] This system was, furthermore, used to monitor the ability of different hydrolases to catalyze both the degradation and formation of peracids.^[10] It could be concluded from this study that many different hydrolases with

a Ser-His-Asp catalytic triad are, indeed, able to catalyze perhydrolysis of relevant substrates. Thus, lipases were found to catalyze perhydrolysis of long-chain carboxylic acids while only esterases, including choline esterase, were able to catalyze perhydrolysis of acetic acid. These results somewhat contradict the proposal made by Berkessel et al. that the ability to catalyze perhydrolysis is an unusual activity. The aim of the present study was, therefore, to investigate if another class of enzymes, lipases, in general would be able to give a positive haloperoxidase reaction under the experimental conditions of Berkessel et al. and, if this was the case, to investigate if the expected peracid was formed during the course of the reaction.

As we have found previously (see above) that lipases are capable of catalyzing perhydrolysis of long-chain acyl substrates the experimental setup described by Berkessel et al. was adjusted by substituting the 1M acetate buffer used with a hexanoic acid buffer. This buffer had to be only 0.2M as a consequence of the more limited solubility of hexanoic acid. Three lipases derived from *Fusarium oxysporum*, *Humicola lanuginosa*, and *Candida antarctica* (B-component) were selected for the study. As indicated in Figure 1 both the

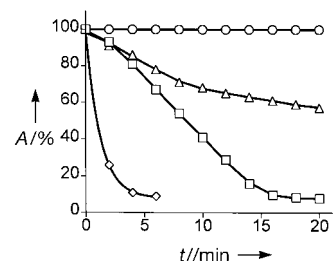


Figure 1. Profile of MCDD halogenation catalyzed by lipases. ○ control, ◇ 100 μM pre-formed peracid, □ *Fusarium* lipase, △ *Humicola* lipase.

Fusarium and the *Humicola* lipases gave a positive haloperoxidase reaction as measured by the bromination of MCDD. The *Fusarium* lipase appeared to be stable under the experimental conditions and capable of transforming all available MCDD. In contrast, the A_{290} curve for the *Humicola* lipase was only linear up to about seven minutes, while data obtained beyond this point suggests an inactivation of the enzyme. No conclusive data were, unfortunately, obtained with the *Candida* lipase as an impurity in the enzyme preparation gave rise to a strong UV absorption that interfered with the assay. All lipases were, however, able to produce perhexanoic acid under the experimental conditions used even though the concentrations, as expected, were very low (Table 1).

Table 1. Formation of peroxyhexanoic acid by using different lipase catalysts.

Lipase	$[\text{H}_2\text{O}_2]$ [mM]	[peroxyhexanoic acid] ^[a] [μM]
<i>Fusarium oxysporum</i>	250	372
	50	128
	7.2	17
<i>Humicola lanuginosa</i>	250	194
<i>Candida antarctica</i> B	250	147
(no enzyme)	250	0

[a] After 15 min.

As expected from the experiments in which the halogenation of MCDD was monitored, the *Fusarium* lipase was superior to the *Humicola* lipase with respect to peracid formation after 15 minutes. In the case of the *Fusarium* lipase, the effect of varying the concentration of hydrogen peroxide was investigated, and a clear dose–response effect was seen. The equilibrium concentration of only 17 μM measured under the conditions used for halogenation of MCDD should, however, be sufficient to account for the conversion of MCDD as this scavenger was present in a concentration of only 44 μM .

To further support the hypothesis that the peracid formed was, indeed, responsible for the observed halogenation of MCDD, pre-formed peracid was added in experiments performed without any enzyme. As indicated in Figure 1, addition of 100 μM pre-formed peracid did, as expected, result in a very rapid halogenation of MCDD. Finally, the lipase-based systems were compared to a vanadium-containing microbial haloperoxidase derived from *Curvularia verruculosa*.^[11] Addition of this enzyme in a concentration of 0.4 $\mu\text{g mL}^{-1}$ resulted in complete halogenation of MCDD in less than two minutes, both in the hexanoate buffer and in a phosphate buffer (results not shown). No peracid could, as expected, be detected in experiments performed in the hexanoate buffer. These experiments further highlight the difference between a true haloperoxidase and the apparent “metal-free haloperoxidase” activity exhibited by a hydrolase.

Our investigations show that lipases can also exhibit metal-free haloperoxidase activity in the presence of an acyl substrate, hydrogen peroxide, and halide ions. This apparent haloperoxidase activity is shown to result from the formation of a peracid, which can be detected in very low concentrations. We therefore propose that the postulated unique enzymatic activity of metal-free haloperoxidases previously described^[8] is, in fact, a common side-activity of hydrolases such as lipases and esterases.

Experimental Section

General: The lipases derived from *Fusarium oxysporum*, *Humicola lanuginosa*, and *Candida antarctica* were obtained from Novo Nordisk A/S (Denmark) and characterized as described previously.^[10] The haloperoxidase from *Curvularia verruculosa* was also obtained from Novo Nordisk A/S and prepared as described by Fuglsang et al.^[11] MCDD and potassium bromide were obtained from Sigma, while H_2O_2 and all other chemicals (all of analytical grade) were obtained from Merck. Reference samples of peroxyoctanoic acid and peroxyhexanoic acid were made according to literature procedures by H_2O_2 oxidation of the parent acid using acidic^[12] and lipase catalysis,^[13] respectively.

Assay for haloperoxidase activity: The experiments were performed according to the procedure described in ref. [8], but using a 0.2 M hexanoic acid buffer pH 5.5 instead of a 1 M acetate buffer. In short, a typical experiment was initiated by adding 100 LU mL^{-1} (LU = lipase unit; 1 LU is defined as the amount of lipase that liberates 1 μmol fatty acid from tributyrin under standard conditions. A detailed description of the assay is available from Novo Nordisk A/S as AF 95/5) lipase to a test solution containing MCDD (44 μM), H_2O_2 (7.2 mM), and KBr (82 mM) in the hexanoic acid buffer at 25 °C. The reaction was followed spectrophotometrically and halogenation of MCDD was evident from a decrease in absorbance at 290 nm.

HPLC assay to monitor the formation of peracid: The assay was performed as previously described^[9] using HPLC with electrochemical detection, a reversed phase column (LiChrosorb RP-18, Merck) with 20 mM phosphate

buffer pH 6/methanol (50/50) as the mobile phase. As the detector response of different peroxy-carboxylic acids has previously been found to be independent of the peracid chain-length^[9] the system was calibrated with peroxyoctanoic acid, which was available in highly pure crystalline form, while a reference sample of peroxyhexanoic acid was used to define the retention time. Experiments were performed as above, only varying the H_2O_2 concentration as indicated in Table 1 and omitting the addition of MCDD and KBr. As the very high concentration of salts gave some interference with the electrochemical detector, samples were extracted with hexane (2 mL sample extracted with 1 mL hexane) and 100 μL of the hexane phase was then diluted in 400 μL mobile phase prior to analysis. Formation of peroxyhexanoic acid was monitored after 0 and 15 min in all experiments.

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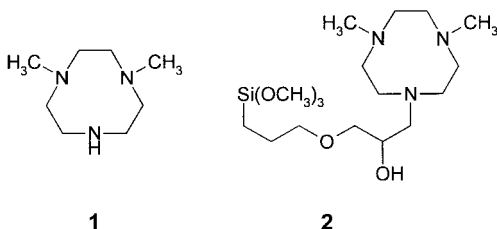
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Selective Alkene Oxidation with H₂O₂ and a Heterogenized Mn Catalyst: Epoxidation and a New Entry to Vicinal *cis*-Diols**

Dirk E. De Vos, Stefaan de Wildeman, Bert F. Sels, Piet J. Grobet and Pierre A. Jacobs*

Selective oxidation of olefins produces valuable intermediates including epoxides and *cis*-dihydroxylated products.^[1] Industrial epoxidations are frequently conducted with stoichiometric reagents (peracids, Payne's procedure).^[2] *cis*-Dihydroxylation is currently not practiced on a large scale because of the price and toxicity of Os catalysts.^[3] Aims in research on catalytic oxidation are: 1) using H₂O₂ instead of organic oxygen donors, 2) avoiding pollution with halides or toxic metals, for example by employing heterogenized reagents, and 3) generating stable products at, for example, nearly neutral pH to protect epoxides. In spite of impressive progress in this area, few systems, if any, meet all these requirements.^[4] Catalysis with complexes of manganese and cyclic triamines such as 1,4,7-trimethyl-1,4,7-triazacyclononane (tmtacn) recently became a true alternative to the known conversions mediated by homogeneous manganese complexes.^[5–8] For instance, the labile diepoxybutane is produced from H₂O₂ and butadiene monoxide in 80% yield with a Mn-tmtacn-oxalate catalyst (>90% selectivity, 530 Mn turnovers).^[7]

Here we describe the first satisfactory heterogenized equivalent of this new type of oxygen-transfer catalyst. A crucial element in the synthesis of the catalyst is the use of the asymmetrically substituted ligand 1,4-dimethyl-1,4,7-triazacyclononane (dmtacn, **1**). This molecule has not only the CH₃ substituents of the original tmtacn but also a secondary amino group for attaching a covalent tether. Even for α -olefins the



new heterogenized Mn catalyst displays high activity and epoxide selectivity (up to 90%, see Table 1). With disubstituted olefins the reaction yields (apart from the epoxide) a sizeable amount of the *cis*-diol (10–60 moles of *cis*-diol per mole of Mn). This is the first observation of a Mn-catalyzed *cis*-dihydroxylation.

Compound **1** was synthesized by selective detosylation of tritosylated 1,4,7-triazacyclononane (tacn), methylation of monotosylated tacn, and detosylation in H₂SO₄.^[9] The free amine base was obtained as a light yellow oil, which was pure based on its ¹H NMR spectrum. To compare the dimethylated dmtacn with trimethylated tmtacn, homogeneous catalysis experiments were performed with complexes formed in situ from MnSO₄·H₂O and dmtacn. The test reactions were the oxidation of styrene and 1-hexene in both acetone and acetonitrile (Table 1, entry 1). While the epoxidation of styrene with Mn-dmtacn in acetone was comparable to the reaction with the Mn-tmtacn catalyst,^[6a] homogeneous Mn-dmtacn in acetonitrile as such was practically inactive for the epoxidation of 1-hexene. In this case epoxidation was marginal in comparison to peroxide decomposition, which is reportedly a binuclear reaction.^[10]

Various approaches were followed to link dmtacn to a solid support: 1) reaction of dmtacn with commercial 3-chloropropylsilica and Et₃N ($\rightarrow$ Sil-p-dmtacn); 2) glycidylation of a mesoporous ordered silica (MCM-41)^[11] or an amorphous precipitated silica with (3-glycidyloxypropyl)trimethoxysilane, followed by addition of dmtacn ($\rightarrow$ MCM-41-gp-dmtacn, Sil-gp-dmtacn); and 3) a "reversed" synthesis, in which dmtacn is first treated with (3-glycidyloxypropyl)trimethoxysilane, and the resulting silylating agent **2** is then immobilized on an amorphous SiO₂ surface ($\rightarrow$ dmtacn-gp-Sil). These procedures lead to surface loadings of the ligand between 0.2 and 0.8 mmol g⁻¹. The identity of the surface groups was checked for dmtacn-gp-Sil with solid-state ¹³C NMR spectroscopy. Magic-angle spinning spectra were recorded in the cross-polarization mode for a glycidylated material and for a material with anchored dmtacn. In addition to the signals of the (ring-opened) glycidyloxypropyl chain (δ = 9, 22, 65, 71, 73), other strong signals appear at δ = 52 and 43 which are assigned to the CH₂ and the CH₃ groups of dmtacn. The metal-binding properties of this surface were examined by doping with Cu²⁺ ions. The EPR data indicate that each metal ion interacts with only a single heterogenized ligand.^[12] This is a strong indication that in dmtacn-gp-Sil formation of dinuclear metal complexes is unlikely.

The heterogenized ligand was metalated by stirring in a solution of MnSO₄·H₂O in methanol and thoroughly removing the excess metal sulfate. Epoxidations were started by consecutive addition of α -olefin, solvent, and H₂O₂. Table 1 first reveals that the best catalyst (dmtacn-gp-Sil) was prepared by "reversed" synthesis. While yields based on peroxide were around 50%, an 82% yield based on the substrate was obtained for styrene in acetone (93% conversion, 280 Mn turnovers; entry 5). These activities are far higher than those of analogous systems in which the ligand has, for example, 2-hydroxyalkyl pendant arms instead of the two methyl substituents.^[6c] Secondly, whereas the homogeneous Mn-dmtacn catalyst was inactive in the epoxidation of 1-hexene, the yields of 1,2-epoxyhexane achieved with the heterogenized catalysts were gratifying. Thus, even this weakly activated olefin successfully competes against peroxide disproportionation. This supports the idea that with the present catalyst binuclear pathways like that in H₂O₂ decomposition are unimportant.

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Explicit attention was devoted to the issue of catalyst heterogeneity by removing the catalyst from half of the reaction mixture (by centrifugation or filtration) and monitoring the progress of the reaction in suspension and in the clear solution. These tests were particularly satisfactory for the catalyst prepared by “reversed” synthesis (dmtacn-gp-Sil). When the reaction mixture was split at a styrene oxide yield of 30 %, negligible further activity resided in solution (80 vs 32 % yields).

Although conversions are satisfactory for the reactions of disubstituted olefins (Table 2), epoxide selectivities were more variable than for the reactions of α -olefins (see Table 1). For cyclohexene and *trans*-2-hexene limited allylic oxidation was observed (less than 8 % of the products); the rest of the products were diols. Attempts to suppress diol formation by addition of base were unsuccessful. When the substrate in the reaction was replaced by 1,2-epoxyhexane or epoxycyclohexane, hardly any diols formed (< 2 mol % after 24 h at 298 K). This proves that epoxide solvolysis is not the source of the observed diols.^[13] Careful analysis of the products proves that *cis*-diols formed almost exclusively. Thus the reactions of Table 2 (entries 1–8) systematically produced the same diol that is formed in a stoichiometric oxidation with MnO_4^- and hardly gave the compounds obtained in strongly acidic medium from CH_3ReO_3 and H_2O_2 .^[4f]

The catalytic formation of *cis*-diols with H_2O_2 (up to 60 Mn turnovers) is a unique observation. Hitherto, such catalytic activity was known only for Os and Ru, in complexes containing two *cis* oxo ligands to form cyclic osmate or ruthenate esters.^[14] With other d^0 catalysts such as CH_3ReO_3 proton acidity leads to the formation of *trans*-diols.^[15] Note that permanganate cannot be formed for thermodynamic reasons from Mn^{2+} ions and H_2O_2 . Moreover, dark brown precipitates (as with MnO_4^- under nonacidic conditions) were not observed with the heterogenized Mn catalyst. In the MnO_4^- oxidation, and in many catalytic Ru reactions, overoxidation is a major problem even at low conversion;^[14] however, with the present Mn catalyst such side reactions are limited (e.g. for *cis*-2-hexene, the diol:dione ratio is 10:1). In both the epoxide and the diol fraction there is a limited loss of configuration. Stereoretention for the diol is always a little higher than for the epoxide (94 vs 91 % for *cis*-2-hexene). A tentative explanation may be that epoxide and diol are formed from a single intermediate that is sufficiently long-lived to allow either some bond rotation or a *cis* insertion of a second, manganese-coordinated oxygen atom to form the *cis*-diol.

Scheme 1 shows models for a) homogeneous Mn-tmtacn in the absence of another ligand, b) Mn-tmtacn in the presence of an oxalato coligand, and c) Mn-dmtacn heterogenized by means of covalent linking. In solution binuclear structures

Table 1. Epoxidation of α -olefins with H_2O_2 and complexes of Mn and dmtacn (dmtacn was used in solution^[a] or heterogenized in different ways on support materials).^[b]

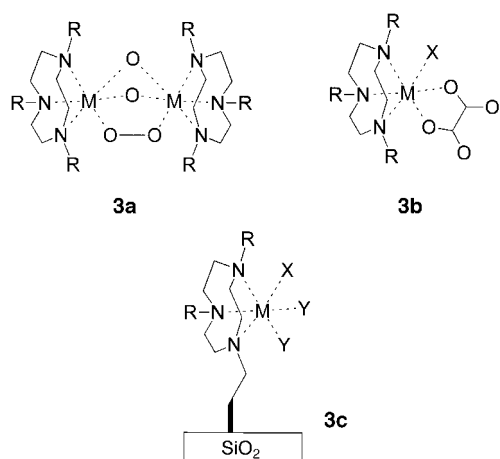
Entry	Catalyst	Loading [mmol g ⁻¹]	Epoxide yield [%] (olefin conversion [%]; time [h]) ^[c]		
			olefin: styrene solvent: acetone	styrene CH ₃ CN	1-hexene CH ₃ CN
1	Mn-dmtacn, homogeneous ^[a]	–	59 (63; 2)	9 (11; 2)	0.1 (0.1; 2)
2	Sil-p-dmtacn	0.21	11 (12; 12)	4.5 (5.5; 12)	3 (4; 12)
3	Sil-gp-dmtacn	0.45	55 (69; 3)	50 (60; 1)	64 (75; 3)
4	MCM-41-gp-dmtacn	0.77	57 (67; 3)	23 (25; 3)	20 (29; 3)
5	dmtacn-gp-Sil “reversed”	0.57	82 (93; 3)	77 (85; 1) ^[d]	70 (92; 3)

[a] Conditions: H_2O_2 :olefin:dmtacn:Mn = 1000:500:1.5:1; 1 mmol of substrate in 1 mL of solvent, 273 K. [b] Conditions: 10 mg of heterogenized catalyst, 1.72 mmol of olefin, 3.44 mmol of H_2O_2 , 1 mL of solvent, 273 K. [c] Other products: 1,2-hexanediol from 1-hexene, and phenylethane-1,2-diol and phenylacetaldehyde from styrene. [d] First run: 255 turnovers. Second run: 150 turnovers. With an olefin:peroxide ratio of 1:1 the conversion is 52 %.

Table 2. Epoxidation and vicinal dihydroxylation of disubstituted olefins with the heterogenized Mn-dmtacn catalyst and H_2O_2 ,^[a] and homogeneous epoxidation with Mn-tmtacn, H_2O_2 , and an oxalate cocatalyst.^[b]

Entry	Substrate	<i>t</i> [h]	Conversion [%]	Selectivity [%]				
				epoxide	(<i>c</i> : <i>t</i>)	diol	(<i>c</i> : <i>t</i>)	overox. ^[c]
dmtacn-gp-Sil + Mn (heterogenized)								
1	<i>cis</i> -2-hexene	2	70	58	(91:9)	37	(94:6)	4
2	<i>cis</i> -2-hexene ^[d]	2	79	67	(86:14)	24	(88:12)	9
3	<i>trans</i> -2-hexene ^[e]	1	24	57	(11:89)	30	(6:94)	7
4	4-CH ₃ - <i>cis</i> -2-pentene	2	74	60	(86:14)	32	(87:13)	7
5	cyclohexene ^[f]	0.3	43	78		12	(99:1)	1
6	cyclohexene ^[f]	2	76	76		13	(97:3)	2
7	indene	3.5	43	57		35	(75:25)	4
8	norbornene	5	85	64		33	(99:1)	3
Mn-tmtacn + Na-oxalate/oxalic acid (homogeneous)								
9	<i>cis</i> -2-hexene	1	72	99	(98:2)	< 1		< 1
10	<i>trans</i> -2-hexene	1	35	99	(2:98)	< 1		< 1
11	cyclohexene ^[g]	1	90	92		< 1		< 1

[a] Conditions: 10 mg of metalated catalyst (dmtacn-gp-Sil; estimated 5 μmol of dmtacn), 1 mmol of olefin, 2 mmol of H_2O_2 , 1 mL of CH_3CN , 273 K. [b] Conditions: 1 μmol of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.5 μmol of tmtacn, 0.67 mmol of olefin, 1.33 mmol of H_2O_2 , 1 mL of CH_3CN ; 3 μmol of cocatalyst (1.5 μmol of oxalic acid and 1.5 μmol of sodium oxalate in 0.1 mL of H_2O). [c] Overoxidation of the diols (mainly diones). [d] Solvent: acetone. [e, f, g] Fraction of allylic oxidation products in total products: 2 % (e), 8 % (f), 8 % (g).



Scheme 1. Proposed structures for Mn-tmtacn oxidation catalysts (R = CH₃, M = Mn, X = activated "O" to be transferred; Y = nucleophile, e.g. H₂O). **3a**: In solution, dimers with catalase-type activity are formed. **3b**: In the presence of oxalate, catalase-type activity is suppressed and the transfer of X to an olefin is promoted. **3c**: With the heterogenized catalyst, both X and Y may be inserted into the olefin.

such as **3a** are readily formed; these are responsible for the catalase-type activity of the complexes.^[10] Addition of oxalate to Mn-tmtacn may lead to a complex such as **3b**. This model is based on analogous structures known for Cu²⁺ and Cr³⁺ complexes.^[16] Apparently, binding of the bidentate oxalate impedes formation of μ -peroxo-bridged dimers. Thus a pathway for peroxide disproportionation is blocked, and clean transfer of a single "O" (labeled X) is observed (Table 2, entries 9–11). Finally, in the heterogenized version **3c**, binuclear complexes are not formed due to the isolation of the ligands over the support. As a result, peroxide disproportionation is much less pronounced than with **3a**. Two labile coordination sites are available in positions adjacent to the transferred active oxygen (X); nucleophiles (e.g., Y = H₂O) on these sites might be inserted into the olefin, together with the "active" oxygen.^[17] This model is the most plausible hypothesis for the coupled catalytic production of epoxides and *cis*-diols.

Experimental Section

The synthesis of dmtacn was based on literature procedures (¹H NMR: δ = 2.802 (m, 4H), 2.72 (m, 4H), 2.70 (s, 4H), 2.46 (s, 6H)).^[9] As an example of heterogenization, the synthesis of dmtacn-gp-Sil is described. A solution of dmtacn (0.118 g, 0.75 mmol) and 3-(glycidyoxy)propyltrimethoxysilane (0.236 g, 1 mmol) in toluene (30 mL) was stirred at 60 °C for 12 h. To this solution was added silica gel 60 (0.75 g, Fluka, 70–230 mesh; previously heated at 120 °C under vacuum). The mixture was stirred for 24 h at 90 °C. Then the powder was subjected to soxhlet extraction with CH₂Cl₂/EtOEt for 24 h, air-dried, and stored in a desiccator. Ligand loadings on the support were determined by thermogravimetric analysis (heating at 120 K h⁻¹; weight loss recorded between 473 and 873 K). Solid-state MAS ¹³C NMR spectra were recorded with a Bruker AMX-300 instrument. EPR spectra were measured on a Bruker ESP 300 instrument.

A portion of the immobilized ligand (10 mg) was metalated by stirring it for 1 h in a solution of MeOH (1 mL), H₂O (250 μ L), and MnSO₄·H₂O (15 μ mol). After centrifugation and removal of the excess metal sulfate, the olefin (1 or 1.72 mmol) and the solvent (1 mL) were added to the catalyst, and the reaction was started by addition over 10 min of a twofold excess of 35 % aqueous H₂O₂ dissolved in 0.5 mL of the solvent. Analysis was performed by gas chromatography (GC-MS). The absence of hydro-

peroxides was systematically proved by injection with or without preliminary addition of PPh₃. The *cis*- and *trans*-diols were separated on GC columns with different polarities (CP-Sil 5 and CP-Sil 88). Comparison was made with authentic products (for the cyclohexane-1,2-diols, Akros), or with products synthesized with KMnO₄ (*cis*-diols) or CH₃ReO₃/H₂O₂ in aqueous HClO₄ (*trans*-diols).^[14]

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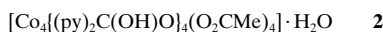
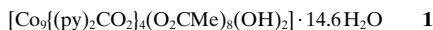
The *gem*-Diol Form of (py)₂CO as a Ligand in Cobalt(II) Carboxylate Clusters: A Cubane Complex and a Novel Nonanuclear Species with a Vertex-Sharing Double Square Pyramidal Structure

Alexandros Tsohos, Stavroula Dionyssopoulou, Catherine P. Raptopoulou, Aris Terzis,* Evangelos G. Bakalbassis,* and Spyros P. Perlepes*

The study of polynuclear complexes of metals in moderate oxidation states has recently begun to lead not only to novel metal polyhedra but also to interesting physical properties. For example, nanosized polyiron and polymanganese complexes with high-spin ground states afford single-domain magnetic particles, which may display hysteresis effects of purely molecular origin.^[1] To understand and exploit such phenomena, further examples of high-nuclearity clusters are required, and this is a challenge for the synthetic chemist. Several groups are pursuing routes to such complexes.^[2]

We have developed the reaction of metal carboxylates with di-2-pyridyl ketone, (py)₂CO, which leads to incomplete replacement of the carboxylate ligands and the formation of large polynuclear arrays of copper^[3] and manganese.^[4] The structural diversity of these complexes stems from the ability of the singly and doubly deprotonated anions of the *gem*-diol

form of (py)₂CO and its derivatives to exhibit a variety of coordination modes, and sometimes two different modes occur in the same complex. We hoped that this strategy would prove to be generally applicable to the 3d metals, and this proved to be the case for cobalt(II). Here we report the cobalt(II) complexes **1** and **2**, which have unusual structures and interesting magnetic properties.



The course of the reaction between Co(O₂CMe)₂ · 4H₂O and (py)₂CO in MeCN under reflux depends on the molar ratio. Treatment of Co(O₂CMe)₂ · 4H₂O with (py)₂CO in a 2:1 molar ratio led to a pale purple solution and subsequent isolation of the nonanuclear complex **1** with doubly deprotonated ions of (py)₂C(OH)₂ as ligands; this is a consequence of the high MeCO₂[−] to (py)₂C(OH)₂ ratio (4:1) used in the reaction. Treatment of cobalt(II) acetate with one equivalent of (py)₂CO led to crystals of the tetranuclear complex **2**, which contains singly deprotonated ions of (py)₂C(OH)₂ as ligands.

Complex **1** (Figure 1)^[5] has a twofold axis passing through Co1 and is held together by two μ₄-OH[−] ions, eight *syn,syn*-μ₂:η¹:η¹-MeCO₂[−] groups, and four (py)₂CO₂^{2−} ligands, which

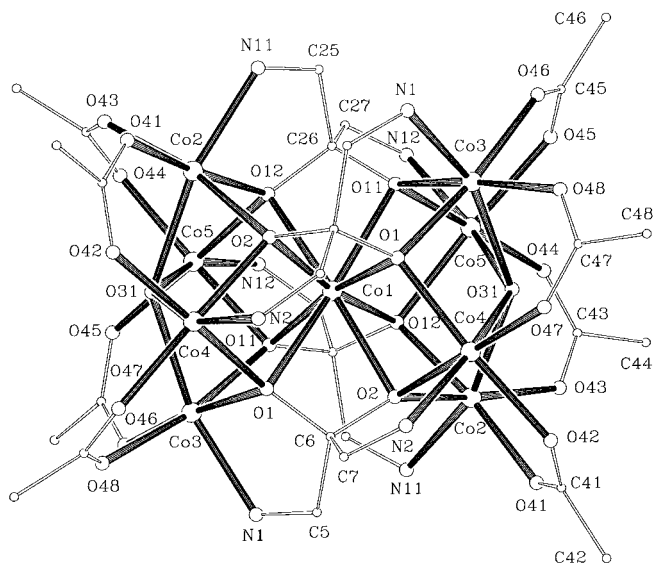
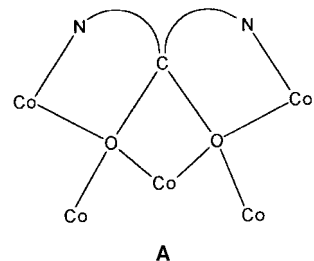


Figure 1. Crystal structure of **1**. Water molecules are omitted for clarity. Most carbon atoms of the pyridine rings have been omitted. Distances [Å]: Co–Co 3.10–6.52, Co1–O 2.24–2.27, Co2,3,4,5–O 2.02–2.31, Co–N 2.12–2.13.

adopt the unprecedented μ₅:η¹:η³:η³:η¹ coordination mode (**A**). The central Co site (Co1) is the shared apex of two square pyramids. An alternative description of **1** is as a square prism centered by an additional interstitial cobalt(II) ion (Co1). Each metal...metal edge of



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the bases is bridged by one acetate, one hydroxo oxygen atom, and one oxygen atom of a $(\text{py})_2\text{CO}_2^{2-}$ ligand. Of the five crystallographically independent Co sites, four (Co2–Co5) have a coordination geometry close to a distorted octahedron (CoNO_5 chromophore). The most salient feature of the structure is the coordination number of eight around Co1, which is extremely rare.^[6] The average Co1–O bond length to the $(\text{py})_2\text{CO}_2^{2-}$ ligands is 2.26 Å, which is about 0.2 Å larger than the average Co2,3,4,5–O bond length (2.07 Å), as expected for a higher coordination number. The coordination geometry around Co1 is best described as a square antiprism; the O1–Co1–O11 and O2–Co1–O12 angles are 47.3 and 46.4°, respectively (the value for a perfect square antiprism is 45°). Another unexpected feature of **1** is the two $\mu_4\text{-OH}^-$ ligands at the center of each base of the double square pyramid. Each hydroxo group is displaced by 0.70 Å out of the best mean plane described by the four metal atoms to which it is bound on the side opposite to Co1. Such μ_4 -hydroxo units are very unusual in 3d metal chemistry.^[7] To the best of our knowledge, only one other nonorganometallic, discrete homometallic nonanuclear Co^{II} aggregate with O and/or N ligation is known.^[8a] Of relevance also is the structure of the anion $[\text{Co}_9(\text{OH})_3(\text{H}_2\text{O})_6(\text{HPO}_4)_2(\text{PW}_9\text{O}_{34})_3]^{16-}$, in which a nonanuclear Co^{II} cluster is encapsulated in a polyoxometalate;^[8b] the large anion contains nine CoO_6 octahedra, which share oxygen atoms with one another, three $\alpha\text{-B PW}_9\text{O}_{34}$ moieties, and two PO_4 groups.

X-ray analysis of **2**^[5] revealed a tetranuclear cubane assembly (Figure 2). The alternating nonmetal vertices of the cube are occupied by four deprotonated oxygen atoms of

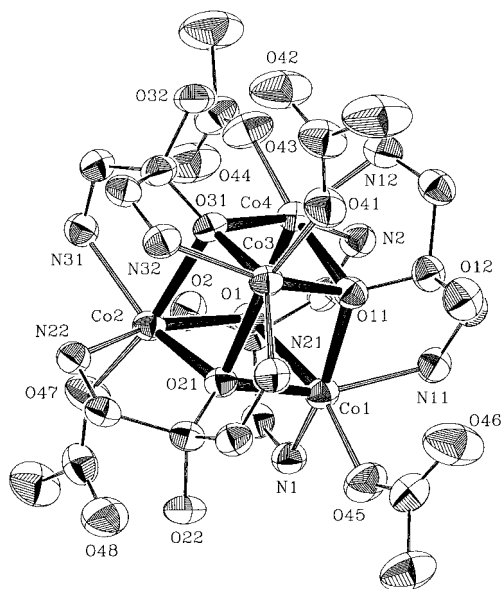


Figure 2. Crystal structure of **2**. The water molecule is omitted. Most carbon atoms of the pyridine rings have been omitted for clarity. Distances [Å]: Co–Co 3.22–3.25, Co–O 1.99–2.27, Co–N 2.10–2.18.

the $\mu_3:\eta^1:\eta^3:\eta^1\text{-(py)}_2\text{C(OH)O}^-$ ligands (**B**). A monodentate acetate group completes a distorted octahedral N_2O_4 coordination environment at each metal center. Complex **2** joins a very small family of Co^{II} cubane complexes.^[9]

Variable-temperature magnetic susceptibility data were collected for **1** and **2** (SQUID magnetometer, 1.0 and 6.0 kG field, Figure 3). The decrease in the product $\chi_{\text{M}}T$ of **1** (χ_{M} is the molar magnetic susceptibility per formula unit) from 27.0 $\text{emu mol}^{-1}\text{K}$ at 300 K to 6.0 $\text{emu mol}^{-1}\text{K}$ at 5 K indicates antiferromagnetic interactions. It is not possible to fit the behavior of an array of nine or four (vide infra) orbitally degenerate Co^{II} centers given current theory.

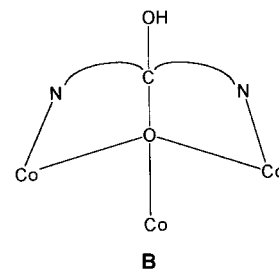


Figure 3. Plots of $\chi_{\text{M}}T$ versus T for **1** (○) and **2** (●).

For **2** $\chi_{\text{M}}T$ increases from 11.2 $\text{emu mol}^{-1}\text{K}$ at 300 K to 13.1 $\text{emu mol}^{-1}\text{K}$ at 7.0 K and then falls to 11.6 $\text{emu mol}^{-1}\text{K}$ at 3 K, consistent with a ferrimagnetically coupled $S = 3$ ground state. Smit et al.^[10] reported high-field magnetization data on the cubane complex $[\text{Co}_4(\text{OMe})_4(\text{acac})_4(\text{MeOH})_4]$ which were consistent with a ferromagnetically coupled Co^{II} cluster.

The results on cobalt(II) complexes reported here again illustrate the extraordinary structural flexibility of mixed $\text{RCO}_2^-/(\text{py})_2\text{CO}$ systems.

Experimental Section

1: MeCN (45 mL) was added to a mixture of $\text{Co}(\text{O}_2\text{CMe})_2 \cdot 4\text{H}_2\text{O}$ (0.27 g, 1.08 mmol) and $(\text{py})_2\text{CO}$ (0.10 g, 0.54 mmol). The solids dissolved on stirring under reflux for 2 h to give a pale purple solution, which was allowed to stand undisturbed at room temperature overnight. Well-formed, X-ray quality crystals of **1** appeared slowly. The purple crystals were collected by filtration, washed with a little cold EtOH, and dried in air. Yield 30%. Elemental analysis calcd for $\text{C}_{60}\text{H}_{87.2}\text{N}_8\text{O}_{40.6}\text{Co}_9$ (%): C 34.3, H 4.2, N 5.3, Co 25.3; found: C 34.4, H 4.1, N 5.5, Co 25.7.

2: Solid $\text{Co}(\text{O}_2\text{CMe})_2 \cdot 4\text{H}_2\text{O}$ (0.24 g, 0.95 mmol) was dissolved with stirring in a solution of $(\text{py})_2\text{CO}$ (0.17 g, 0.92 mmol) in MeCN (40 mL) under reflux. The resulting purple solution was layered with $\text{Et}_2\text{O}/n\text{-hexane}$ (1/1, 45 mL). Slow mixing yielded large red prismatic crystals of **2**, which were collected, washed with a little cold EtOH and Et_2O , and dried in air. Yield 35%. Elemental analysis calcd for $\text{C}_{52}\text{H}_{50}\text{N}_8\text{O}_{17}\text{Co}_4$ (%): C 48.2, H 3.9, N 8.7, Co 18.2; found: C 48.0, H 4.0, N 8.5, Co 18.4.

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Isomerism and Blue Electroluminescence of a Novel Organoboron Compound: $B_2^{III}(O)(7\text{-azain})_2Ph_2^{**}$

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Organic electroluminescent (EL) devices based on organic or organometallic materials have attracted much attention because of their high luminance, low driving voltage, and easy fabrication.^[1-4] To achieve a full color display, the three basic color components red, green, and blue are required. Red and green emitters for organic EL devices have become readily available. Useful and efficient organic or organometallic blue emitters are however still very rare. Previously reported blue emitters of organometallic or coordination compounds in organic EL devices employed either derivatives of 8-hydroxyquinoline or azomethine as the emitting ligands.^[3,4] The common feature of those previously reported blue emitting ligands is that they are all chelating ligands and bind to the central atom through both nitrogen and oxygen donor atoms. We have been interested in the application of organometallic and coordination compounds in EL devices because the properties of this class of compounds such as volatility and stability can be modified readily by manipulating the coordination environment around the central atom.^[5] We have discovered recently that 7-azaindole and di-2-pyridylamine ligands yield a strong blue luminescence when bound to an aluminum or boron center.^[6] The 7-azaindole or di-2-pyridyl amine ligands contain nitrogen donor atoms only and can bind to the central atom by either a bridging mode or a chelating mode. They are therefore very different from the previously

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7-azain = 7-azaindole anion.

reported blue emitting ligands. Furthermore, the commonly used central ions in previously known organometallic or coordination blue emitters^[3, 4] are Al^{III}, Zn^{II}, and Be^{II}. Blue emitters using boron as the central atom are previously unknown, although a few blue photoluminescent boron-containing polymers and small molecules and electron-transferring organoboron compounds have been reported recently.^[7] The main advantage of boron over aluminum is that boron–ligand bonds are in general much more covalent than the corresponding aluminum–ligand bonds.^[8] As a consequence, boron compounds, in general, are much more stable than the corresponding aluminum compounds,^[8] making them an attractive and potentially useful class of compounds for electroluminescence. Recently, we reported the synthesis and structure of B₂^{III}(O)(7-azain)₂(C₂H₅)₂.^[6a] Although this compound has a bright blue photoluminescence, it is however not stable enough for EL applications. To improve the stability of the boron compound so that electroluminescence can be achieved, we investigated the syntheses of new 7-azaindole boron compounds, B₂^{III}(O)(7-azain)₂R₂, where R is an aryl group. We have successfully synthesized the new boron compound, B₂^{III}(O)(7-azain)₂Ph₂ (**1**) which is not only stable and emits blue electroluminescence, but also displays an unusual structural isomerism. We report herein the synthesis, structures, and blue electroluminescence of **1**.

Compound **1** can be obtained by several synthetic routes, of which the best (50 % yield) is to react PhBCl₂ with 7-azaindole and H₂O in a 2:2:1 ratio in the presence of a base such as triethylamine. Compound **1** was found to exist in two isomeric forms, one with an approximate twofold rotation symmetry (**A**) and the other with an approximate mirror plane symmetry (**B**) (Figure 1), as established by NMR spectroscopy, elemental analysis, and single-crystal X-ray diffraction.^[9] These two isomers can be separated by repeated crystallization from CH₂Cl₂/hexane (**A** is less soluble than **B**). Both isomers have an oxo ligand bridging two boron atoms. A similar oxo bridge has been observed in the compound B₄^{III}(2,2'-biimidazole)₂(O)(C₂H₅)₆ reported by Niedenzu et al.^[10a] The B–O, B–N, and B–C bond lengths in **A** and **B** are similar and typical.^[10, 11] The structure of **A** is similar to that of B₂^{III}(O)(7-azain)₂(C₂H₅)₂, where the environment around both boron centers is identical. In ¹H and ¹³C NMR spectra of **A**, only one set of resonances due to the phenyl group are observed, while one resonance is present in ¹¹B NMR spectrum of **A**, indicating that the structure of **A** in solution is the same as that in the solid state. The structure of **B** is very unusual—one of the boron centers is bound by two nitrogen atoms of the six-membered rings, while the other boron center is bound by two negatively charged nitrogen atoms of the five-membered rings. Thus, the two boron centers and the two phenyl groups in **B** are chemically inequivalent. Indeed, two distinct boron chemical shifts are present in the ¹¹B NMR spectrum of **B**. There are also two sets of distinct chemical shifts for the protons and carbon atoms at 2,6 positions of the two phenyl rings in ¹H NMR and ¹³C NMR spectra of **B**, respectively, again indicating that **B** retains its structure in solution. There is no interconversion between **A** and **B** in solution as established by NMR studies. The unusual geometric structural isomerism exhibited by B₂^{III}(O)(7-azain)₂Ph₂

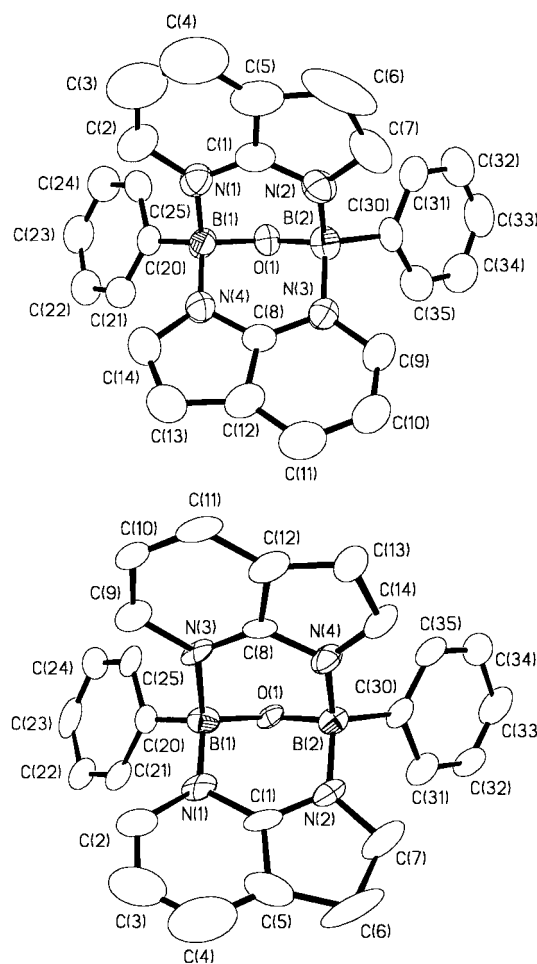


Figure 1. Molecular structures of **A** (top) and **B** (bottom) in the crystal (50 % thermal ellipsoids). Hydrogen atoms are omitted for clarity. Important bond lengths [Å] and angles [°]: **A**: B(1)–O(1) 1.429(6), B(2)–O(1) 1.414(6), B(1)–N(1) 1.619(6), B(1)–N(4) 1.582(7), B(2)–N(2) 1.591(7), B(2)–N(3) 1.628(7), B(1)–C(20) 1.584(7), B(2)–C(30) 1.625(7); B(1)–O(1)–B(2) 120.4(4). **B** (There are two similar and independent molecules of **B** in the asymmetric unit. The structure for one of them is shown here.): B(1)–O(1) 1.399(12), B(2)–O(1) 1.422(12), B(1)–N(1) 1.611(11), B(1)–N(3) 1.648(12), B(2)–N(2) 1.578(12), B(2)–N(4) 1.613(12), B(1)–C(20) 1.591(14), B(2)–C(30) 1.589(14); B(1)–O(1)–B(2) 120.4(7).

has not been observed previously in either boron or aluminum 7-azaindole complexes.

Isomers **A** and **B** are stable in the solid state upon exposure to air, which can be attributed to the oxo bridge and the covalent B–N and B–C bonds. The thermal stability of these two isomers is however very different. Compound **A** has a melting point of 274 °C and can be sublimed readily. In contrast, compound **B** decomposes at temperature greater than 150 °C in the solid state, as established by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Compound **A** and^[12] B₃^{III}O₃Ph₃(7-azainH) were found among the decomposition products. The details of the thermal decomposition mechanism of **B** are not yet fully understood.

The other exciting property of compounds **A** and **B** is that they produce an intense photoluminescence (PL) when irradiated by UV light in solution and the solid state. The PL spectra of both isomers are very similar with an emission

maximum at $\lambda = 450$ nm, attributable to a $\pi^* \rightarrow \pi$ transition of the 7-azaindole anion. The role of the boron atoms in **1** is believed to stabilize the 7-azaindole anion, as the aluminum ion does in related 7-azaindole compounds reported previously.^[6b,c] The high air and thermal stability of compound **A** along with its intense blue photoluminescence makes it an ideal candidate as a blue emitter in EL devices.

To investigate the electroluminescent properties of compound **A**, an EL device using **A** as the light-emitting layer and indium-tin-oxide (ITO) as the substrate was constructed by using vacuum deposition methods. Tris(8-hydroxyquinolino)aluminum (Alq₃) and *N,N'*-di-1-naphthyl-*N,N'*-diphenylbenzidine (NPB) doped with 1 % of 9,10-diphenylanthracene were used as the electron-transporting and hole-transporting layer, respectively. A magnesium silver alloy (Mg_{0.9}Ag_{0.1}) was used as the cathode. When a positive bias was applied to the ITO electrode, the EL device produced a bright blue emission with a peak at 450 nm. The EL spectrum shown in Figure 2 is

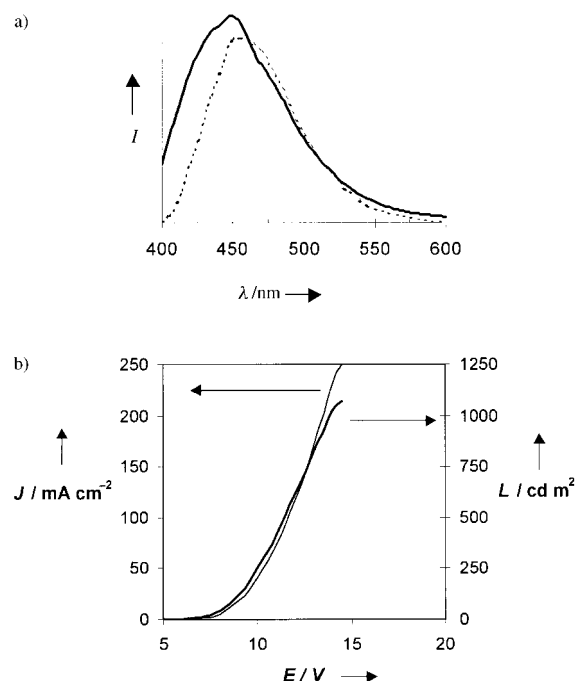


Figure 2. a) The PL (solid line) and EL (dashed line) spectra of **A**. *I* = intensity. b) The current density (*J*), voltage (*E*), and luminance (*L*) characteristics of the EL device.

very similar to the PL spectrum of **A**, indicating that the light emission originates from compound **A**. As shown in Figure 2, the device has a turn-on voltage of approximately 7 V and provides a luminance of 1024 cd m⁻² at 14 V. These preliminary results indicate that compound **A** is very promising for EL applications. Further investigation on the chemistry and EL device optimization of new organoboron compounds is also in progress.

Experimental Section

1: 7-Azaindole (0.474 g, 4 mmol) was added to phenylboron dichloride (0.635 g, 4 mmol) in THF (20 mL) at 23 °C under N₂. After the mixture was stirred for 10 min, water (0.036 g, 2 mmol) was added, and the mixture was stirred for another 10 min. Finally, triethylamine (0.8096 g, 8 mmol) was

added to the mixture slowly and the solution was heated at 60 °C for 5 h. After the solution was allowed to cool to room temperature, THF (40 mL) was added to the solution and then the mixture was filtered. The filtrate was collected and evaporated to dryness in vacuum. Recrystallization of the resulting white powder from CH₂Cl₂/hexane yielded colorless crystalline **1** in 50 % yield (isomer **A** dominant). Pure isomer **A** can be obtained by repeated recrystallization from CH₂Cl₂/hexane. Pure isomer **B** can be obtained from the concentrated mother liquor after the removal of **A**. Single crystals suitable for X-ray diffraction analyses were obtained from THF/hexane for **A** and CH₂Cl₂/hexane for **B**. **A:** ¹H NMR (CDCl₃, 25 °C): δ = 8.13 (d, ³*J* = 6.0 Hz, 2H, 7-azain), 8.05 (d, ³*J* = 7.8 Hz, 2H, 7-azain), 7.78 (d, ³*J* = 6.8 Hz, 4H at 2,6 positions of Ph), 7.69 (d, ³*J* = 3.3 Hz, 2H, 7-azain), 7.44–7.35 (m, 6H at 3,4,5 positions of Ph), 7.03 (dd, ³*J* = 6.0 Hz, ³*J* = 7.8 Hz, 2H, 7-azain), 6.51 (d, ³*J* = 3.3 Hz, 2H, 7-azain); ¹³C NMR (CDCl₃, 25 °C): δ = 134.28 (7-azain), 134.19 (3,5-Ph (the prefix indicates the positions of the carbon atoms)), 133.54 (7-azain), 132.33 (4-Ph), 127.71 (2,6-Ph), 127.31 (7-azain), 113.64 (7-azain), 100.88 (7-azain); ¹¹B NMR (CDCl₃, 25 °C): δ = -42.75; m.p. 274 °C. **B:** ¹H NMR (CDCl₃, 25 °C): δ = 8.17 (d, ³*J* = 6.0 Hz, 2H, 7-azain), 8.08 (d, ³*J* = 7.8 Hz, 2H, 7-azain), 7.83 (d, ³*J* = 6.9 Hz, 2H at 2,6 positions of Ph), 7.67 (d, ³*J* = 3.3 Hz, 2H, 7-azain), 7.60 (d, ³*J* = 6.0 Hz, 2H at 2,6 positions of Ph), 7.40–7.33 (m, 6H at 3,4,5 positions of Ph and Ph'), 7.05 (dd, ³*J* = 6.0, ³*J* = 7.8 Hz, 2H, 7-azain), 6.49 (d, ³*J* = 3.3 Hz, 2H, 7-azain); ¹³C NMR (CDCl₃, 25 °C): δ = 134.33 (7-azain), 133.82 (3,5-Ph, Ph'), 133.53 (7-azain), 132.66 (4-Ph, Ph'), 127.77 (2,6-Ph), 127.58 (2,6-Ph'), 126.96 (7-azain), 113.08 (7-azain), 100.10 (7-azain); ¹¹B NMR (CDCl₃, relative to BCl₃, 25 °C): δ = -40.70, -45.15; m.p. $\approx$ 150 °C, decompose. Elemental analysis calcd. for the mixture of **A** (60 %) and **B** (40 %), C₂₆H₂₀N₄O₂ · 0.4 CH₂Cl₂ (%): C 66.60, H 4.40, N 11.77; found: C 60.71, H 4.57, N 11.82.

EL device: The device was fabricated on an indium-tin-oxide (ITO) substrate which was cleaned by an ultraviolet ozone cleaner immediately before use. Organic layers and a metal cathode composed of magnesium silver alloy (Mg_{0.9}Ag_{0.1}) were deposited on the substrate by conventional vapor vacuum deposition. Prior to the deposition, all the organic materials were purified by a train sublimation method.^[13] The device structure used in this experiment is as follows: ITO / hole transport layer / light-emitting layer / electron-transport layer / Mg/Ag, in which NPB-doped with 1 % of 9,10-diphenylanthracene was employed as the hole-transport layer and Alq₃ as the electron transport layer. To obtain the photoluminescence spectrum of **A**, a thin film (100 nm) deposited on a quartz substrate was measured with a fluorescence spectrophotometer. The current-voltage characteristics were measured by using a Keithley 238 current/voltage unit. The light intensity was measured by a Minolta Chroma Meter CS100. The EL spectrum was obtained by an in-house setup made up of a series of electronic components including a monochromator (Instruments SA Inc), a photomultiplier tube, and a photon counter.

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- [9] Crystal data for **A**: $C_{26}H_{20}N_4O \cdot 0.5 THF$, monoclinic, space group $P2_1/n$, $a = 11.184(6)$, $b = 13.891(8)$, $c = 16.281(8)$ Å, $\beta = 108.72(3)^\circ$, $V = 2395(2)$ Å³, $Z = 4$, GOF on $F^2 = 1.048$, R_1 ($I > 2\sigma(I)$) = 0.0693, $wR_2 = 0.1540$. Crystal data for **B**: $C_{26}H_{20}N_4O \cdot CH_2Cl_2$, triclinic, space group $P\bar{1}$, $a = 12.5473(13)$, $b = 13.4960(13)$, $c = 16.7659(17)$ Å, $\alpha = 90.345(10)$, $\beta = 102.211(10)$, $\gamma = 116.055(10)^\circ$, $V = 2476.5(4)$ Å³, $Z = 4$, GOF on $F = 3.67$, R_1 ($I > 2.5\sigma(I)$) = 0.093, $wR_2 = 0.076$. For **A**, data were collected on a Siemens P4 X-ray diffractometer operated at 50 kV and 40 mA at ambient temperature. Data for **B** were collected on a Siemens SMART CCD diffractometer operated at 50 kV and 35 mA at $-100^\circ C$. The structural solution and refinement of **A** were performed on a PC using Siemens SHELXTL software package while the structural solution and refinement of **B** were performed on a workstation using NRCVAX software package. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-113985 and CCDC-113986. 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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The Melting Point Alternation in the Short-Chain *n*-Alkanes: Single-Crystal X-Ray Analyses of Propane at 30 K and of *n*-Butane to *n*-Nonane at 90 K**

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Dedicated to Professor Reiner Sustmann on the occasion of his 60th birthday

It was probably A. Baeyer who first stated in 1877 that the melting points of the fatty acids do not show a monotonic increase with increasing chain length as do their boiling points. Instead the melting points of the members with even numbers of C atoms are relatively higher than those of the members with odd numbers.^[1] The longer the chain length, the smaller are the relative differences. This is true for the *n*-alkanes as well as for most^[2] of the α -substituted and α,ω -disubstituted *n*-alkane derivatives; a fact that is mentioned in almost all standard text books of organic chemistry. Mostly, "packing effects" are given as an explanation for such an alternation in melting points. This is justified insofar as physical properties, such as sublimation enthalpy and solubility, that are related to the solid state display a similar alternation whereas the properties of the liquid phase have a monotonic dependency on increasing chain length.^[3]

For the *n*-alkanes with $n = 6-24$ (n = number of C atoms) the even numbered members crystallize at low temperatures in a triclinic space group, while for $n = 26$ monoclinic packing is observed. It is possible to define an orthorhombic sub-cell for the latter,^[3a] which is also found as the real cell for polyethylene^[4] and mixed paraffins.^[5] When $n = 7$ the odd-numbered *n*-alkanes also crystallize in a triclinic, ordered modification with an orthorhombic sub-cell. Glassy crystalline "rotator phases" with hexagonal symmetry are found below the melting points for the odd-numbered *n*-alkanes with $n \geq 9$, and the difference between the phase transition and melting point increases with an increase in the chain length. From $n = 22$ hexagonal modifications are also observed for the even-numbered *n*-alkanes.

Although the phenomenon of the even/odd alternation has been known for a very long time, there does not exist a plausible explanation pattern, not even for the short chain ($n < 10$) *n*-alkanes with a triclinic crystal system. Kitaigorodski^[6a] analyzed the arrangements of sections through the long axes of the aliphatic chains, and distinguished between triclinic, monoclinic, and hexagonal subcells. At longer chain lengths these represent the real cells. Further considerations referred to parallel arrangements of cylinderlike units, in

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which the main molecular axis inclines with the cylinder axis. These cylindrical units form layers and the difference in the arrangements of the layers result in a plausible explanation for the polymorphism of the long-chain systems. The reason why the short-chain, triclinic *n*-alkanes display the melting point alternation is still unclear. An explanation seems to be only possible if precise crystal structure investigations are carried out at the same temperature. The subtle differences that are possibly present in the packing patterns may then be identified. With such structural investigations the densities in the solid state could be correlated with the melting points, because the density depends on the number of the van der Waals contacts and the lattice energies. These are again correlated with the sublimation enthalpies, which have an effect on the melting point temperature. For short chain *n*-alkanes, unfortunately, there are neither experimental densities of sufficient precision available, nor can they be obtained by existing X-ray results.

The crystal structures of an ordered and a disordered phase of ethane at -188 and -183 °C, respectively, are known with sufficient precision.^[7] *n*-Butane has a melting point of -138.6 °C and exists in three modifications, a disordered high temperature phase and two coexisting low temperature phases below -165.2 °C, as shown by neutron powder diffraction experiments at -268 , -208 , -183 , and -153 °C.^[8] To the best of our knowledge solid-state diffraction experiments of propane have not been reported. The X-ray measurement temperature is not given for *n*-pentane.^[9] *n*-Hexane was investigated at -115 °C,^[10] *n*-heptane at -173 °C,^[11] and *n*-octane at approximately -60 °C.^[9, 12] The structure determinations for $n=5, 6, 8$ were undertaken with Weissenberg techniques and for $n=7$ with a two circle diffractometer. Single-crystal investigations of the ordered phase of *n*-nonane do not exist, obviously because of the experimental difficulties described below. From the existing investigations it is also unknown if there are differences not only in the packing patterns but also in the molecular structures in respect of their torsion angles along the C–C axes.

We investigated the crystal structures of the series of *n*-alkanes from propane to nonane by X-ray diffraction. The gaseous compounds were first condensed in high vacuum, and thereby purified, and then transferred into an attached Lindemann capillary at -196 °C. The sealed capillaries were transferred to the diffractometer with a detachable cooling device,^[13] where the low temperature device of our own design with an open cold gas stream provided the permanent cooling

process. Single crystals suitable for X-ray diffraction were grown with the help of the in situ method by using a computer controlled device that applied a focused CO₂-laser beam along the capillary.^[14]

Propane has a melting point of -190 °C, but crystallizes at even lower temperatures and therefore it was necessary to apply liquid helium as the coolant. Helium was also used as the surrounding gas steam to avoid icing at the capillary, and the measurement was performed at -243 °C.

n-Nonane shows a solid-state phase transition at -48.4 °C, about 3 °C below the melting point, from the “rotator phase” into the desired, ordered low temperature phase. Single crystals, grown in the rotator phase, shattered to polycrystalline material on further cooling. For a direct crystallization into the ordered phase and thus circumventing the high temperature phase, an approximately 40:60 mixture of *n*-pentane and *n*-nonane was used and crystallized at -100 °C. Such a strategy has also been applied for circumventing plastic phases.^[15] Similar experiments to crystallize *n*-decane, however, were not successful thus far. The disorder in the high temperature phase of *n*-butane could not be resolved during the structure refinement and led to high anisotropic displacement parameters, high residual electron densities, too short C–C bond distances, and a relatively high *R* value.^[16] All experiments to obtain a usable single crystal in the ordered low temperature phase by annealing for several days at 183 °C were unsuccessful. All the other crystal structure investigations were relatively simple and follow the reported procedure.^[14]

A summary of the results is given in Table 1, and details of the crystal structure investigations are listed in reference [16]. The molecular structures of the *n*-alkanes have, within the precision of the experiments, a planar arrangement of the carbon skeleton. The mean C(H₃)–C(H₂) and C(H₂)–C(H₂) distances of the investigated *n*-alkanes (except disordered *n*-butane) are both $1.521(1)$ Å. The mean angles for C(H₃)–C(H₂)–C and C(H₂)–C(H₂)–C(H₂) are $112.8(1)$ and $113.5(1)$, respectively. These values represent the X-ray standard values of undistorted *n*-alkanes.

Figure 1 shows the melting points of the *n*-alkanes together with the densities obtained from X-ray data at -183 °C, the value for propane was extrapolated from -243 °C to -183 °C. It is clear that the experimental X-ray densities follow the trend of melting points if the density calculated from the cell dimensions of the ordered phase of deuterated *n*-butane at -183 °C is taken. As expected, the density of the disordered phase of *n*-butane is lower.^[16]

Table 1. Crystallographic data of the investigated compounds.

No. of C atoms	Space group	<i>a</i> [Å]	<i>b</i> [Å]	<i>c</i> [Å]	α [°]	β [°]	γ [°]	<i>V</i> [Å ³]	<i>Z</i>	<i>T</i> [°C]	ρ [g cm ⁻³]
2 ^[a]	<i>P2₁/n</i>	4.226(3)	5.623(4)	5.845(4)	90	90.41(6)	90	139	2	-188	0.719
3	<i>P2₁/n</i>	4.1480(14)	12.612(5)	6.977(3)	90	91.28(3)	90	364.9(2)	4	-243	0.743 ^[b]
4	<i>P2₁/c</i>	5.7027(15)	5.5247(15)	8.389(2)	90	115.22(2)	90	239.11(10)	2	-183	0.816
5	<i>Pbcn</i>	4.1357(8)	9.025(3)	14.815(5)	90	90	90	553.0(2)	4	-183	0.867
6	<i>P1</i>	4.1309(7)	4.6963(10)	8.5392(17)	83.398(17)	87.265(15)	75.172(15)	159.05(5)	1	-183	0.900
7	<i>P1</i>	4.116(2)	4.686(3)	20.348(13)	78.11(5)	81.79(5)	74.25(4)	368.0(4)	2	-183	0.904
8	<i>P1</i>	4.1230(9)	4.6860(13)	10.974(3)	85.063(22)	83.72(3)	75.10(2)	203.30(9)	1	-183	0.933
9	<i>P1</i>	4.1180(12)	4.6739(17)	24.693(9)	86.27(3)	87.80(3)	74.52(3)	457.0(3)	1	-183	0.932

[a] Data for the ordered phase from reference [7]. [b] Density extrapolated to -183 °C

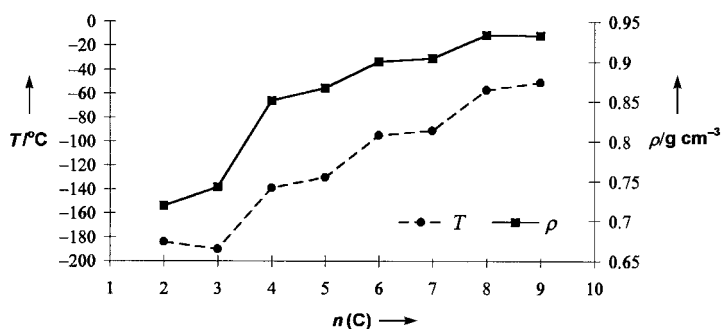


Figure 1. Trends in the melting points [$T/^{\circ}\text{C}$] and densities [$\rho/\text{g cm}^{-3}$] of the n -alkanes with increasing number of C atoms [$n(\text{C})$].

The n -alkanes from n -hexane crystallize in the space group $P\bar{1}$ and show some regularities. The centrosymmetric, even-numbered members of the n -alkanes have one molecule in the unit cell whereas the C_2 symmetric, odd-numbered members have two molecules in the unit cell (Table 1). The lengths of the a and b axes remain similar with increasing chain length. In fact they are slightly shorter for the odd-numbered members relative to the even ones, which contradict the trend that the odd-numbered members have a lower density than the even ones. However, this effect is a consequence of the pronounced differences in the c axes, which compensate for the minor differences in the other axes. The crystal packing of n -hexane and n -heptane is shown along the a axes in Figures 2a and 2b. It is clear that the CH_2 groups fit into the

hollows of the neighboring chains, which causes a shift along the c axis. In a projection along the main axis of the molecules (not shown here) it is clear that the interchain arrangement is similar along the axis for even- and odd-numbered n -alkanes.

The c axes, which are responsible for the differences in the cell volumes and the densities (Table 1), are directed along the main axis of the molecules (Figure 2a and 2b). Therefore the differences in the packing (even/odd) are sought in this direction. Consequently, the way in which the molecules are packed laterally does not play any role in the differences in the packing density of the even- and odd-numbered triclinic n -alkanes. The length of the main molecular axis increases by a constant value of $1.268 \text{ \AA}$ with each additional CH_2 group. Therefore the intermolecular distances between the end groups, namely, the CH_3 groups, are responsible for the alternation in the densities. But the decisive question is, why are the end groups of the even- and odd-numbered n -alkanes are not able to approach to the same distances?

A simplified, geometrical description in a planar view reveals the background (Figure 3): The shape of an even-

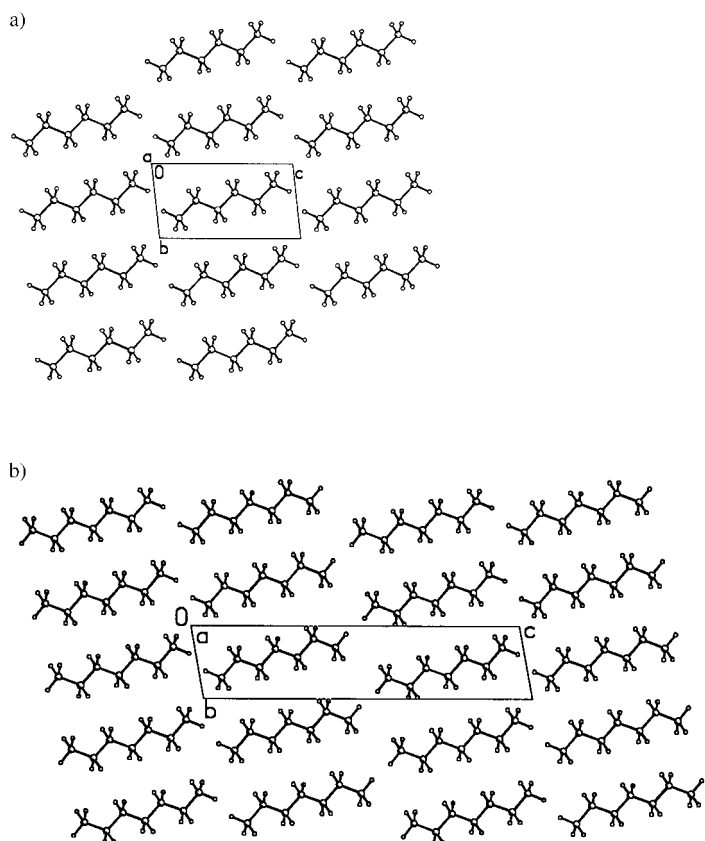


Figure 2. Packing of n -hexane (a) and of n -heptane (b) viewed along the a axis.

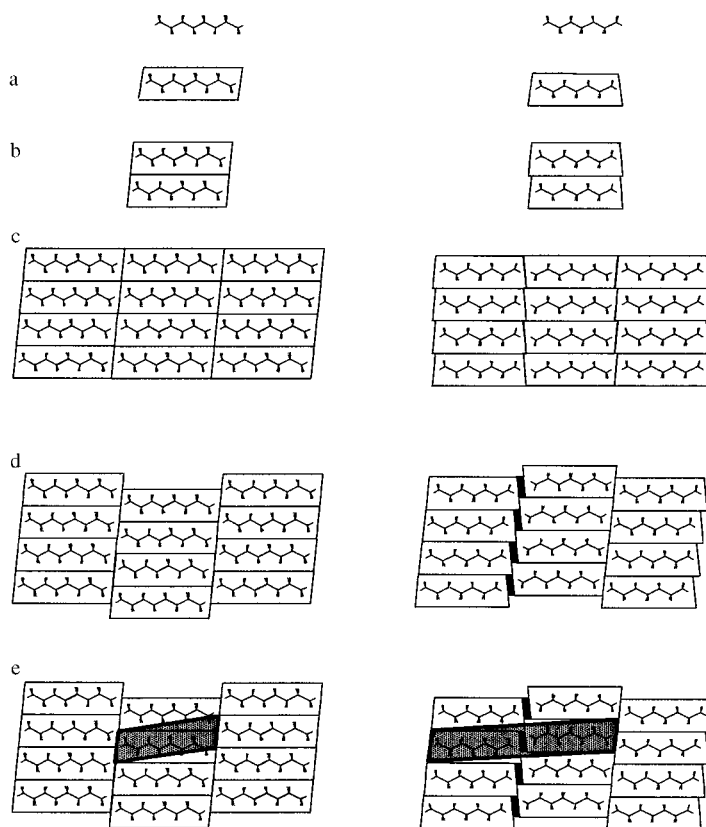


Figure 3. a)–e) Schematic representation of the packing of even-numbered (left) and odd-numbered n -alkanes (right) in a two-dimensional projection. For further information see the text.

numbered n -alkane (C_i symmetry) can be described in the plane of the carbon skeleton as a parallelogram, that of an odd-numbered n -alkane (C_2 symmetry) as a trapezoid (Figure 3a). If a dense packing of parallelograms is required, a pattern may result as shown in Figure 3b. However, an analogous packing is also possible for the trapezoids (Figure 3c). If the main axis of the molecules is increased by an

increment of 1.268 Å, as it is in the case when going from *n*-heptane to *n*-octane, it is expected that the area (in the three-dimensional case the volume) increases by similar proportions (Figure 3c). Such a packing would lead to a monotonic increase in the density, because a parallelogram and a trapezoid have the same area with the same length of the center line and height.

However, the methyl groups situated opposite to each other try to adopt a staggered conformation as may be seen from Figure 2a and 2b. Thus the columns of the parallelograms have to be shifted with respect to each other so as to achieve the staggered arrangement of methyl groups (Figure 3d, left). Such an arrangement is indeed found for the triclinic even-numbered *n*-alkanes. A similar shift is possible in the trapezoid pattern only on one side, on the other side the shift results in very long distances and the methyl groups are not really situated opposite to each other (Figure 3d, right). However, this arrangement has larger gaps (marked in black), compared to the right and center layer. The triclinic unit cells in a two-dimensional projection are shown in Figure 3e.

Thus, the even-numbered *n*-alkanes have optimal intermolecular contacts at both ends, whereas the odd ones possess these at one end, and at the other end the distances are longer. This leads to a less dense packing for the odd *n*-alkanes and as a consequence, as shown above, to relatively lower melting points.

In this description we disregarded the third dimension and indeed, the parallelograms and trapezoids are shifted in height with respect to each other. But this shift is about the same in both even and in odd *n*-alkanes, which allows the simplification to a two-dimensional presentation.

In Table 2 the distances between the methyl C atoms of adjacent columns are listed. For the even-numbered *n*-alkanes

Table 2. Intermolecular distances of the terminal C atoms between the layers.

No. of C atoms	Distance 1 [Å]	Distance 2 [Å]
6	3.623	3.623
7	3.605	3.734
8	3.617	3.617
9	3.606	3.737

they are equal because of the crystallographic centrosymmetry. For the odd *n*-alkanes two different distances are found, one of them corresponds to those of the even-numbered members, and on the other side, where the gaps exist, the distances are longer. Thus the melting point alternation of the *n*-alkanes can be explained from *n*-hexane and higher on the basis of a simple and geometrical model, by the interaction of methyl groups. The same or similar model does not apply to the shorter *n*-alkanes and also not to the α -substituted and α,ω -disubstituted *n*-alkane derivatives, some of which do not display the same alternating pattern.^[2] For this it is necessary to have further structural investigations and packing calculations,^[17] which are currently in progress.

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- [1] A. Baeyer wrote in *Ber. Chem. Ges.* **1877**, *10*, 1286: "From this compilation it can be seen that without any exception a member with an odd number of carbon atoms has a lower melting point than that with an additional carbon atom, while in both series the melting points increase with the exception of the first members. If there is a general law in this pattern it would be the easiest to study the melting points of the anilides and normal fatty acids and it would be desirable that colleagues who have the necessary material available would expand our knowledge in this direction. A law which would say that in homologous series a odd number of carbon atoms has a relatively lower melting point than those with an even number would have a considerable interest in molecular physics and ask for investigations if the crystal form, solubility etc. are correlated with the nature of the number, which is expressed by the quantity of carbon atoms."
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- [16] Crystal structure analyses: $Mo_{K\alpha}$ -radiation, graphite monochromator, four circle diffractometer Nicolet R3, structure solution by direct methods (Siemens SHELXTL-Plus, Version 5.03). All non-hydrogen atoms were refined anisotropically on F^2 . The hydrogen atom positions were taken from a difference Fourier map and refined without constraints and isotropically. Except for propane, which was measured at -243°C , all other measurements were performed at -183°C . Mean C–C distances [Å] and C–C–C angles [$^\circ$] for the measured *n*-alkanes except *n*-butane: C(H₃)–C(H₂) 1.521(1), C(H₂)–C(H₂) 1.521(1), C(H₃)–C(H₂)–C 112.8(1), C(H₂)–C(H₂)–C(H₂) 113.5(1); for the disordered *n*-butane: C(H₃)–C(H₂) 1.517(4), C(H₂)–C(H₂) 1.470(7), C(H₃)–C(H₂)–C(H₂) 113.6(4). a) Propane: growing temperature -243°C , space group $P2_1/n$ (no. 14), $2\theta_{\text{max}} = 60^\circ$, reflections measured: 1420, independent: 971, of which 794 had $I > 2\sigma(I)$, 60 parameters, $R1 = 0.0468$, $wR2 = 0.1206$, residual electron density $+0.34/-0.14 \text{ e \AA}^{-3}$ (CCDC-103186). b) *n*-Butane: growing temperature -158°C , space group $P2_1/c$ (no. 14), $2\theta_{\text{max}} = 60^\circ$, reflections measured: 2358, independent: 693, of which 556 had $I > 2\sigma(I)$, 19 parameters, $R1 = 0.1922$, $wR2 = 0.5413$, residual electron density $+1.03/-0.26 \text{ e \AA}^{-3}$ (CCDC-103187). c) *n*-Pentane: growing temperature -158°C , space group $Pbcn$ (no. 60), $2\theta_{\text{max}} = 60^\circ$, reflections measured: 1774, independent: 804, of which 644 had $I > 2\sigma(I)$, 48 parameters, $R1 = 0.0402$, $wR2 = 0.1238$, residual electron density $+0.38/-0.10 \text{ e \AA}^{-3}$ (CCDC-103188). d) *n*-hexane: growing temperature -113°C , space group $P\bar{1}$ (no. 2), $2\theta_{\text{max}} = 60^\circ$, reflections

measured: 1400, independent: 920, of which 789 had $I > 2\sigma(I)$, 56 parameters, $R1 = 0.0342$, $wR2 = 0.1076$, residual electron density $+0.33/-0.15 \text{ e } \text{\AA}^{-3}$ (CCDC-103189). e) *n*-Heptane: growing temperature -110°C , space group $P\bar{1}$ (no. 2), $2\theta_{\text{max}} = 60^\circ$, reflections measured: 1750, independent: 1674, of which 1388 had $I > 2\sigma(I)$, 128 parameters, $R1 = 0.0499$, $wR2 = 0.3756$, residual electron density $+0.39/-0.19 \text{ e } \text{\AA}^{-3}$ (CCDC-103190). f) *n*-Octane: growing temperature -78°C , space group $P\bar{1}$ (no. 2), $2\theta_{\text{max}} = 60^\circ$, reflections measured: 1356, independent: 1187, of which 991 had $I > 2\sigma(I)$, 73 parameters, $R1 = 0.0386$, $wR2 = 0.1285$, residual electron density $+0.48/-0.15 \text{ e } \text{\AA}^{-3}$ (CCDC-103191). g) *n*-Nonane: growing temperature -98°C , space group $P\bar{1}$ (no. 2), $2\theta_{\text{max}} = 55^\circ$, reflections measured: 3288, independent: 2089, of which 1396 had $I > 2\sigma(I)$, 162 parameters, $R1 = 0.0468$, $wR2 = 0.1549$, residual electron density $+0.31/-0.15 \text{ e } \text{\AA}^{-3}$ (CCDC-103192). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publicationnos CCDC-103186–CCDC-103192. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

- [17] The calculated lattice energies, expressed in kcal mol^{-1} per molecule, show a similar alternation as the melting points: propane: -7.504 , *n*-butane: -8.814 , *n*-pentane: -11.922 , *n*-hexane: -14.783 , *n*-heptane: -16.690 , *n*-octane: -19.657 , *n*-nonane: -21.260 . The Cerius² program (Version 3.5) was used for these calculations. The experimental coordinates serve as the starting points and the electrostatic potentials were assigned from the AM1-Hamiltonian in MOPAC6. The "Crystal Packer" module with a built in Dreiding II force field was used to minimize crystal structures and the lattice energies were obtained from the final minimized crystal structure.

Coordination-Ionspray-MS (CIS-MS), a Universal Detection and Characterization Method for Direct Coupling with Separation Techniques**

Ernst Bayer,* Petra Gfrörer, and Claus Rentel

Spray techniques such as atmospheric pressure chemical ionisation (APCI)^[1] or electrospray ionization (ESI)^[2–5] have become important techniques in mass spectrometry. In particular their simple coupling with separation techniques such as HPLC,^[6] capillary electrophoresis (CE),^[7] and capillary electrochromatography (CEC)^[8] is the method of choice for characterization, analysis, and structural elucidation. A characteristic common to these techniques is the formation of droplets from which ions are subsequently liberated. In the

case of APCI the ionization occurs by charge transfer from a reagent gas plasma formed by corona discharge at the tip of a metal needle. With ESI, the sample solution is nebulized by application of a strong electric field. Spray formation is usually assisted pneumatically by means of a coaxial gas stream. Ionization is achieved by either protonation of basic groups or deprotonation of acidic groups of the analyte molecules depending upon the polarity of the field employed. While electrospray mass spectrometry has found widespread application, for example in the analysis of peptides and proteins, with their readily protonated amino groups, and of the easily deprotonated oligonucleotides, it fails for a large number of nonpolar substances and is of limited utility for substances with only weakly basic or acidic groups. Numerous classes of natural compounds such as terpenes, sugars, alcohols, aromatic compounds, and vitamins in addition to a large number of synthetic organic compounds such as produced in combinatorial chemistry are either inaccessible to analysis by spray techniques or can be detected only with poor sensitivity.

We have developed a new method of chemical ionization in which positively or negatively charged complexes are formed by the addition of a suitable central atom to the analytes, and these complexes can be detected by mass spectrometry. Since both polar and nonpolar organic compounds can form coordination compounds with an appropriate central atom, this form of ionization is highly versatile. The wealth of experience available in the area of coordination chemistry can be drawn upon in this technique and thus new classes of compounds can be analyzed with the spray technique. We therefore refer to this new technique as coordination-ionspray mass spectrometry (CIS-MS). Neither an electric field nor the formation of a reagent gas plasma by corona discharge is necessary for ionization. Efficient nebulization in the ion source is however mandatory. Since pneumatic nebulization in the absence of an electric field is often insufficient to obtain a suitable spray, a supporting voltage was applied in some cases to stabilize the nebulization process.

In the case of poorly ionized compounds, such as fully protected peptides, the formation of clusters leads to complex spectra that are difficult to interpret and to a reduction in sensitivity. In , The ESI mass spectrum of the fully protected 28-mer peptide of the potassium channel of the transmembrane sequence of *Drosophila melanogaster*^[9, 10] is shown in Figure 1 a. With the exception of the terminal amino group all the basic groups are protected and are thus not available for protonation, and only the clusters with sodium and potassium ions are observed within the mass range of the quadrupole mass spectrometer. The ion yield is only poor and the interpretation of the spectra is problematic.

It is known that lithium salts form complexes with peptides and that these prevent the association of peptide chains. Seebach et al.^[11] have employed lithium salts to increase the solubility and to improve the yield in the solid-phase synthesis of peptides that have a high propensity for aggregation by the formation of β structures. The addition of lithium iodide to the transmembrane peptide leads to specific stable metal complexes that can be detected in high ion yield and which allow simple determination of the molecular mass (Figure 1 b).

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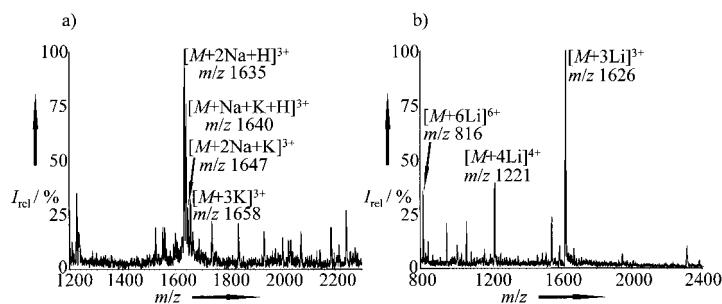


Figure 1. a) ESI mass spectrum of the fully protected transmembrane peptide $[\text{NH}_2]\text{-Lys(BOC)}_{28}\text{-Ile-Val-Gly(Tmob)-Ser(Trt)-Leu-Cys(Trt)-Val-Val-Ala-Gly(Tmob)-Val-Leu-Thr(Trt)-Ile-Ala-Leu-Pro-Val-Pro-Val-Ile-Val-Ser(Trt)-Asn(Trt)-Phe-Asn(Trt)-Tyr(tBu)}_1\text{-[COOH]}$ (m/z 4857) from *Drosophila melanogaster* under normal electrospray conditions; 4.5 kV, flow rate $3.5 \mu\text{L min}^{-1}$. b) CIS mass spectrum after addition of $20 \mu\text{L min}^{-1}$ lithium iodide and with reduced field strength of 1.5 kV; flow rate $10 \mu\text{L min}^{-1}$.

Reduction of the electric field from 4.5 to 1.5 kV leads to an improvement in the signal-to-noise ratio, since other competing mechanisms of ionization and the formation of clusters are suppressed.

Although metal complexes such as metal proteins^[12–16] and alkali and alkaline earth ionic adducts^[17–19] of polar analytes have been measured with electrospray-MS, it has hitherto not been recognized that conversion into charged complexes represents a general method of ionization in solution for mass spectrometric purposes, that it requires neither activation nor conditions of ESI-MS, and that it is therefore applicable to nonpolar analytes. The sample is subjected to less thermal stress than APCI and the sensitivity is significantly improved. Lower detection limits in the range of 300–500 fmol are attained for carotinoids.^[20] The detection limit of palmitoleic acid methyl ester was 370 fmol in the “single ion mode” and 37 fmol with “multiple reaction monitoring”. These substances were detected as their silver complexes.

Since olefins and polyolefins such as terpenes, sesquiterpenes, carotinoids, vitamins, and steroids either cannot be detected at all or only with poor sensitivity with ESI-MS they would appear particularly attractive for CIS-MS. With metals of the first and eighth transition groups of the periodic table, for example, Cu^I , Ni^{II} , Pd^{II} , Pt , and Ag^I , these unsaturated compounds form highly stable π or π -allyl complexes. Figure 2 shows the CIS-MS of the palladium- π -allyl complex of the terpene alcohol isopulegol without the electrical field that is mandatory for ESI-MS. The $[M+\text{Pd}-\text{H}]^+$ ions with the isotopic distribution characteristic of palladium are the prominent species. If silver(I) is used as central atom the π complexes rather than the π -allyl complexes are detected. Here also, avoidance of other mechanisms of ionization leads to clear-cut spectra with low noise levels.

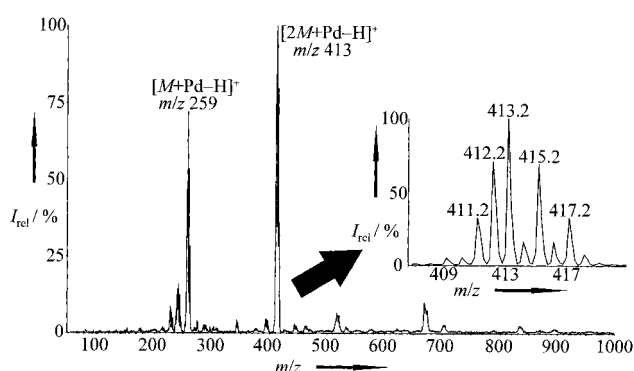
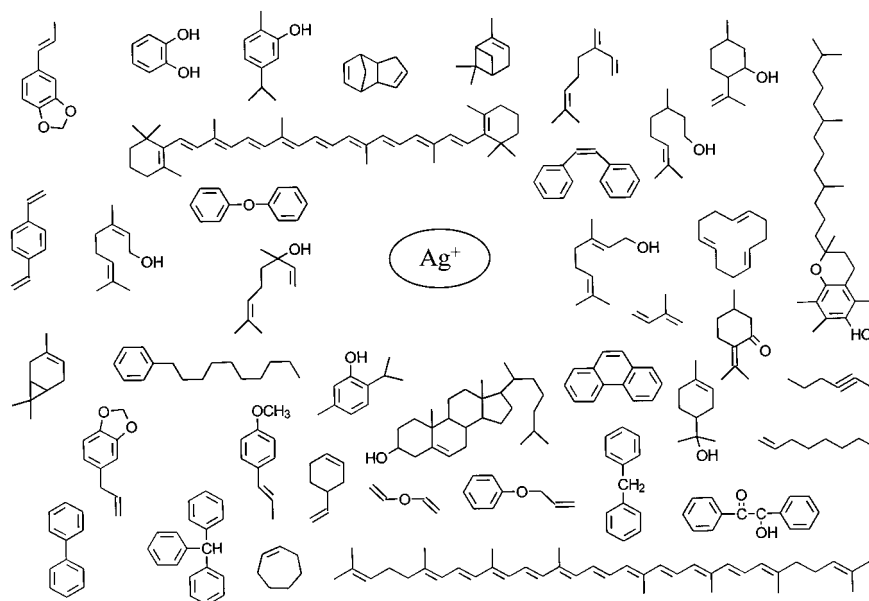


Figure 2. CIS mass spectrum of isopulegol complexed with $100 \mu\text{g mL}^{-1}$ Pd^{II} acetate; no voltage applied, flow rate $10 \mu\text{L min}^{-1}$.

We have investigated a large number of olefins, polyolefins, and aromatic compounds by CIS-MS, including steroids, vitamins of the D and E families, carotinoids, polystyrenes, terpenes, and unsaturated fatty acids. Scheme 1 shows a selection of the analytes measured in the form of their Ag^I complexes. On-line formation of the charged species is carried out after the chromatographic separation since in most cases the chromatographic resolution of even relatively stable complexes formed prior to separation was unsatisfactory.

In the case of polyolefins, $[M]^+$ radicals are often found as by-products in addition to the $[M+\text{Ag}]^+$ ions, as is seen in the HPLC separation and CIS-MS detection of carotinoids in Figure 3.^[20] It is apparent from Figure 3 that on-line complexation and mass spectrometric detection do not lead to peak broadening relative to UV detection. The kinetics of complexation are rapid under the chosen experimental conditions. In addition to elements of the first and eighth transition groups of the periodic table, many other metals are suitable as central atoms and can be selected according to the principles of complex chemistry. Table 1 gives a selection of potential reagents for various ligands.



Scheme 1. Selection of compounds that can be detected by CIS-MS as their positive π complexes with Ag^+ .

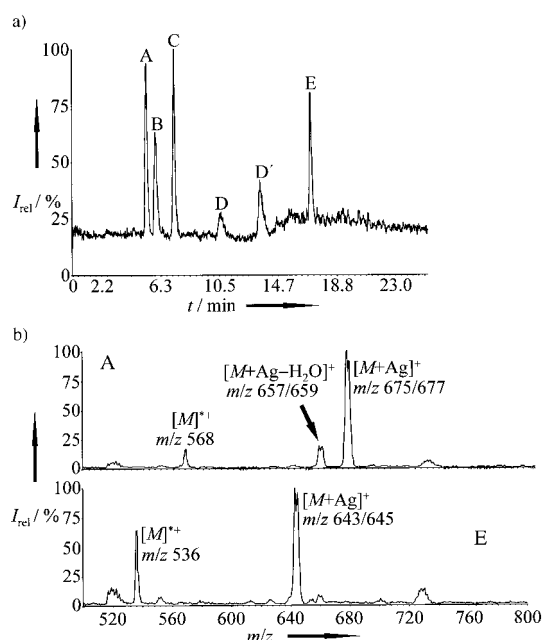


Figure 3. a) Ion chromatogram of the HPLC-MS of lutein (A), zeaxanthin (B), canthaxanthin (C), echinenon (D, D'), and β -carotene (E). b) CIS mass spectra of the separated carotenoids A and E.

Table 1. Reagents for CIS-MS

Central atom	Reagent	Solvent	Analyte
B ^{III}	BF ₃	organic solvent	alcohols, ethers, esters
B ^{III}	H ₃ BO ₃	H ₂ O, alcohol	sugars, polysaccharides, alcohols
B ^{III}	B(OCH ₃) ₃	H ₂ O, alcohol	ethers, alcohols
Ag ^I	AgNO ₃	H ₂ O, alcohol, acetonitrile	arenes, olefins, polyolefins, carotenoids
Pd ^{II}	Pd(OAc) ₂	H ₂ O, alcohol, acetonitrile	vitamins A, D, and E, estrogens
Li ^I	Li halogenides	H ₂ O, alcohol, acetonitrile	peptides, sugars, alcohols

CIS-MS is not restricted to positive ions but can also be used in the negative mode, in particular when the central atom of the complexing reagent has an electron deficiency. Boron(III) is such a central atom. Aliphatic alcohols and sugars are difficult to detect with electrospray MS. However after reaction with boron(III) trifluoride, the negatively charged boron complexes are readily detected in CIS-MS, as demonstrated by the mass spectrum of cholesterol (Figure 4a). Here also, a reduction in the field strength (Figure 4b) causes suppression of other ionization mechanisms, which leads to simpler spectra and lower limits of detection. Boric acid is another reagent that is commonly used for structural investigation of sugars. Adjacent hydroxyl groups form more stable boron complexes than isolated hydroxyl groups. Under the conditions selected for CIS-MS, only the boron complexes with adjacent hydroxyl groups, for example, with fructose (Figure 5), are stable and detectable by MS whereas glucose is not detected. Once again, reduction of the field strength suppresses nonspecific ions and results in more clear cut

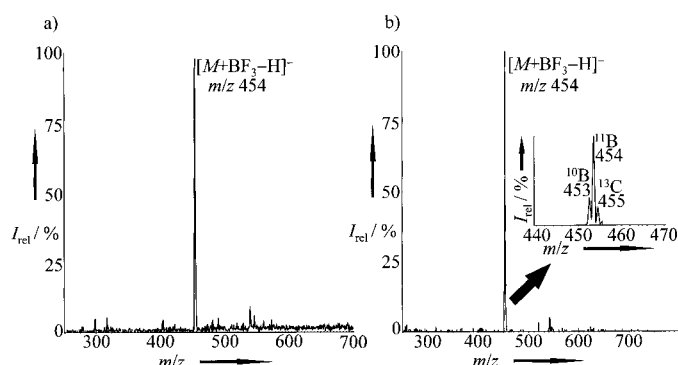


Figure 4. CIS mass spectrum of cholesterol after complexation with BF₃ in diethyl ether a) at -4.5 kV with a flow rate of 3 $\mu\text{L min}^{-1}$ and b) at -1.2 kV and a flow rate of 10 $\mu\text{L min}^{-1}$.

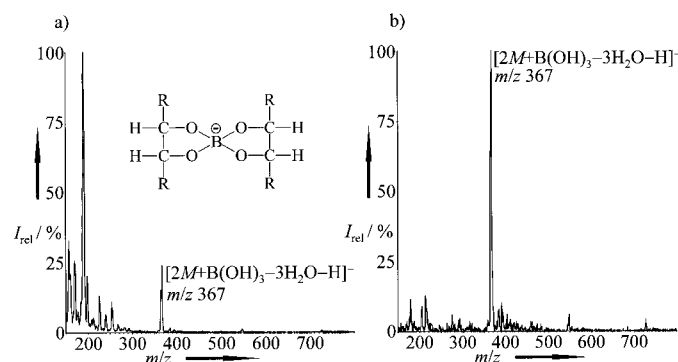


Figure 5. CIS mass spectrum of fructose a) at -3.7 kV, and b) at -1.7 kV. Flow rates are as in Figure 4.

spectra (Figure 5b), since the nonspecific formation of solvent clusters in the lower mass range is hindered. CIS-MS of oligosaccharides represents a relatively simple method for the characterization and structural elucidation of oligosaccharides, especially in complex mixtures in combination with previous separation by HPLC, CEC, or CZE.

The fragmentation of complexes and the analysis of the fragment ions is interesting on account of the fragmentation patterns encountered, as has been demonstrated for conjugates with alkali ions.^[18]

Direct coupling of all common separation methods with CIS-MS is possible, with increasing interest being focussed in recent times upon the miniaturized techniques such as capillary HPLC (CHPLC), CZE, and CEC.

Figure 6 shows the separation of unsaturated fatty acid methyl esters by pressure-assisted CEC, which we developed for gradient elution,^[21-23] with CIS-MS detection of the silver complexes. The silver complexes were formed after the separation and before entry into the ion source. The CIS mass spectra of the fatty acid methyl esters obtained on-line from the CEC separation are shown in Figure 6b.

The combination of separation in the liquid phase and CIS-MS is a new and widely applicable analytical method, the diverse possibilities of which will become fully apparent with the rigorous application of the known principles of coordination chemistry.

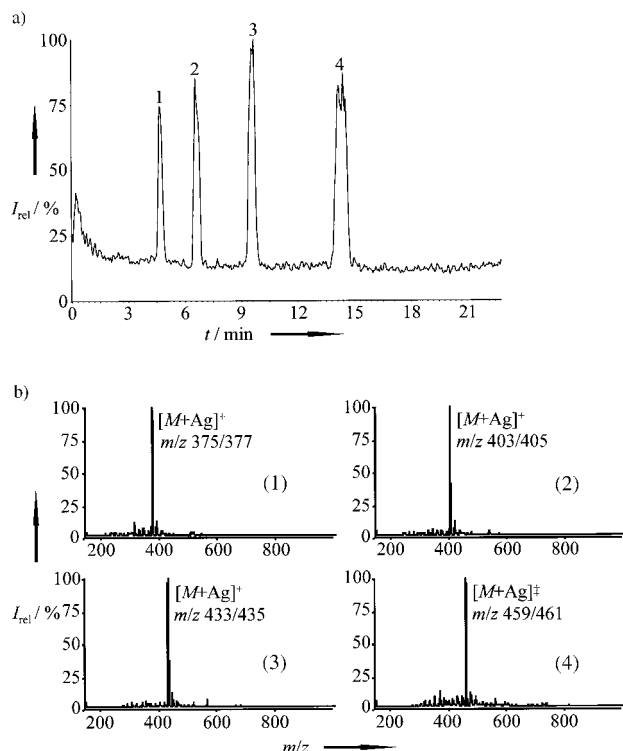


Figure 6. Direct coupling of the separation of fatty acid esters with pressure-assisted electrochromatography (pCEC) with CIS-MS detection. 1) Hexadecenoic acid methyl ester, 2) oleic acid methyl ester, 3) *cis*-eicos(9)enoic acid methyl ester, 4) eruca acid methyl ester. a) Total ion current and b) CIS mass spectra of the silver complexes.

Experimental Section

At present no commercial ion source exists that is optimized for CIS-MS. Since all electrospray ion sources require strong electric fields not only for the ionization but also for the generation of as monodisperse a spray as possible, only ion sources with vigorous mechanical nebulization are able to sustain a satisfactory spray under conditions of reduced electric field. In addition, an increase in the temperature of the curtain gas to up to 80 °C is recommended. These basic requirements are not fulfilled by all commercial electrospray ion sources. The ion source of the API III and API 3000 mass spectrometers from PE SCIEX (Toronto, Canada) were used in our investigations but were not optimized for CIS-MS. Nebulization occurs pneumatically by means of a gas stream.

The experimental setup for coupling a separation technique to the interface for the addition of the coordinating compound is shown schematically in Figure 7. We used the previously described apparatus for capillary HPLC,

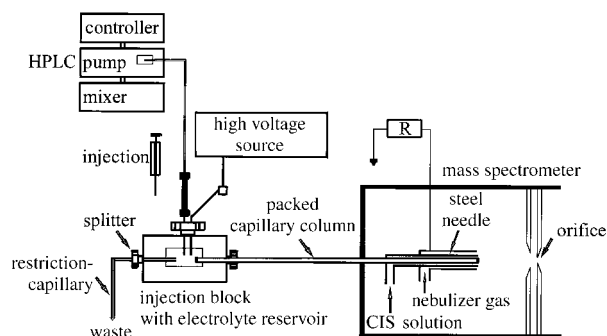


Figure 7. Instrument setup for the direct coupling of CHPLC, CZE, or pCEC with CIS-MS with addition of the coordinating central atom by means of a sheath flow stream.

capillary electrophoresis, and gradient capillary electrochromatography, which can be applied alternatively to all three techniques.^[21–23] Addition of the complexing reagent by the “sheath-flow” method described by Smith et al.^[7] for coupling capillary electrophoresis with ESI-MS (Figure 7) has proved satisfactory. The end of the capillary column is enclosed in a steel capillary (internal diameter 210 μm) through which the reagent solution is pumped by means of a syringe pump. These capillaries are held within a second steel capillary through which the nebulizing gas flows. Alternatively, the complexing reagent may be added through a T-junction. The following coordinating reagent solutions were used: 20 $\mu\text{g mL}^{-1}$ LiI, 100 $\mu\text{g mL}^{-1}$ Pd^{II} acetate, 50 $\mu\text{g mL}^{-1}$ AgNO₃, and 100 $\mu\text{g mL}^{-1}$ boric acid. The flow rate before entry into the ion source was between 3 and 40 $\mu\text{L min}^{-1}$. Voltages of between 80 and 150 V are applied to the orifice. Further experimental details are given in the legends to the figures.

The HPLC separation of carotinoids (Figure 3a) was performed on a C3D-RP phase according to the conditions described in reference [24].

A Gromsil ODS-O AB (particle size = 3 μm) packed, fused silica capillary of 100 μm internal diameter and 25 cm long was used for the CIS-MS coupling. The eluent consisted of 40 mmol ammonium acetate (pH 9) and acetonitrile (5/95). An aqueous solution of 100 $\mu\text{g mL}^{-1}$ silver nitrate was added at a flow rate of 3 $\mu\text{L min}^{-1}$ by means of a syringe pump for the sheath flow. The ratio of sheath flow to CEC flow was approximately 3:1

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On the Mechanism of Long-Range Electron Transfer through DNA**

Bernd Giese,* Stephan Wessely, Martin Spormann, Ute Lindemann, Eric Meggers, and Maria E. Michel-Beyerle

Over the past years the discussion on the mechanism of long-range charge transfer through DNA has been centered around the β value in Equation (1). This equation describes the fast exponential decrease in the rate of charge transfer with increasing distance.^[1] Depending on the experiment, β values of about $1.0 \text{ \AA}^{-1}$ or $0.1 \text{ \AA}^{-1}$ were reported.^[2,3] Such differences in β values have dramatic effects on the rate. Thus, for a charge transfer over $50 \text{ \AA}$ and a β value of $0.1 \text{ \AA}^{-1}$, the rate should be nearly 20 orders of magnitude faster than for a β value of $1.0 \text{ \AA}^{-1}$. It is unlikely that both β values can be realized for long-range charge transport in DNA.

Recently we proposed a hopping mechanism for the long-range charge transfer^[4] in which the charge tunnels between DNA bases of similar redox potentials. Thus, long-range charge transfer in DNA consists of a series of short-range tunneling processes which can be described by Equation (1). Whereas the *positive* charge is transported by oxidation of the guanine bases (G) during hole transfer in mixed DNA sequences, the *negative* charge should be conducted by reduction of the bases thymine (T) and cytosine (C). This conclusion is derived from the redox potentials of the bases, which are different for guanine (G) and adenine (A)^[5,6] and similar for thymine (T) and cytosine (C).^[6,7] In the case that every single hopping step occurs over the same distance, the hopping mechanism is described by Equation (2).^[4a,b] In sharp contrast to Equation (1), a weak distance dependence of the relative rate constants k results from Equation (2).

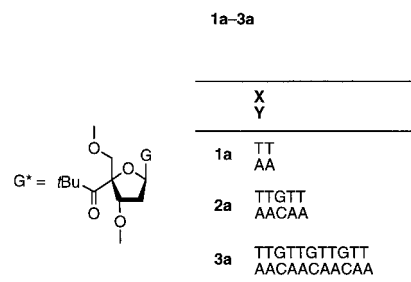
$$\ln k \propto -\beta \Delta r \quad (1)$$

$$\ln k \propto -\eta \ln N \quad (2)$$

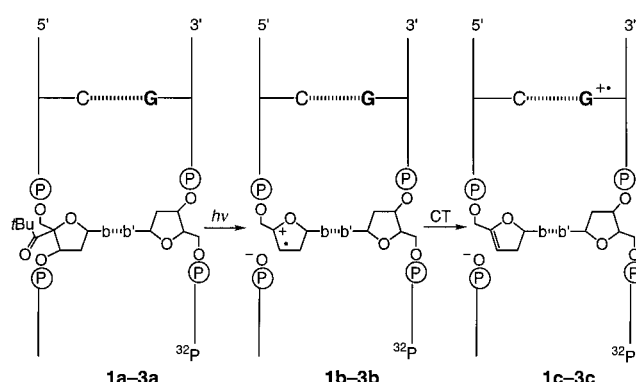
In Equation (2), η is a proportionality factor which in the simplest case should be about 2, and N is the number of hopping steps.^[4a,b] We have now proven the validity of Equation (2) experimentally through charge-transfer reactions over 10 to $40 \text{ \AA}$ in DNA strands **1a–3a**.

The experiments are based on an assay in which a 4'-acylated nucleoside is incorporated into double-stranded DNA.^[4a] Norrish I photocleavage and subsequent heterolysis

3'-CGCATTATATTATGGG X G₂₂CA TTATAATACGCTGCACGCA-³²P
5'-GCGTAATATAATACCC Y C G^{*}TAAATATTATGCGACGTGCGT-3'



leads to the sugar radical cations **1b–3b**, which transfer the positive charge onto the neighboring G base of the ³²P-labeled complementary strand (**1c–3c**; Scheme 1). Starting from



Scheme 1. Generation of the guanine radical cation $G^{+\bullet}$ in the ³²P-labeled strands **1c–3c** by photolysis of the ketone unit in the modified strands **1a–3a**.

here the positive charge is transported through the DNA over the nucleotide pairs **XY** until it reaches a GGG unit, where it is trapped irreversibly. In competition with the charge transfer, the radical cation $G^{+\bullet}$ can be trapped with H_2O . This leads to a damaged G^{ox} position,^[4a] at which the DNA is cleaved after treatment with piperidine. We have shown recently that the relative rate constant of charge transfer (k_{rel}) from $G^{+\bullet}$ over the G bridges to the GGG unit is equal to the ratio of the cleavage products GGG/G; cleavage at all G positions was taken into account.^[4a]

The experiments were carried out with double-stranded 40- to 49-mers **1a–3a**, which have repeating AT,AT double units between the G bases.^[8] Following Equation (2) the number of hopping steps N has the value of 1, 2, or 4 for the strands **1a**, **2a**, and **3a**, respectively. Two independent series of measurements were performed; the mean values are compiled in Table 1.^[9]

Figure 1 shows that a plot of $\ln k_{rel}$ versus $\ln N$ yields a η value of 1.7 ± 0.2 . Thus, hole transfer over 10, 20, and $40 \text{ \AA}$ is well described by a hopping model.^[10] In Scheme 2 the arrows demonstrate how the charge of $G_{22}^{+\bullet}$ hops between the G bases until it reaches the GGG unit.

If Equation (1) was applied and the experimental data were fit to a straight line, a β value of 0.07 would result. This hypothetical β value implies a superexchange mechanism in

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Table 1. Experimental data for the double strands **1a–3a** and **4–6**. The oxidative damage at the G and GGG units was quantified after piperidine treatment and polyacrylamide gel electrophoresis with a phospho-imager.

DNA strand	<i>N</i>	Δr [Å]	GGG/G = k_{rel}
1a	1	10	8.9 ± 1.9
2a	2	20	2.8 ± 0.4
3a	4	40	0.88 ± 0.12
4	—	54	$2.3 \pm 0.7^{[4a]}$
5	—	54	0.04 ± 0.02
6	—	17	$0.03 \pm 0.015^{[4a]}$

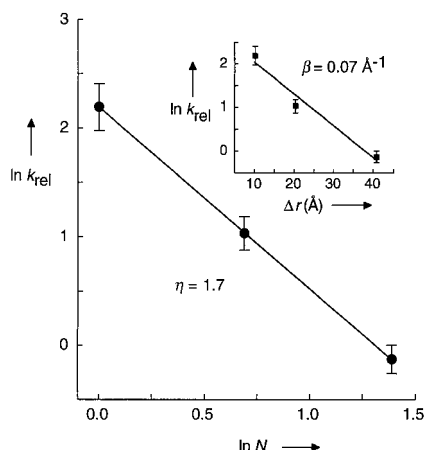
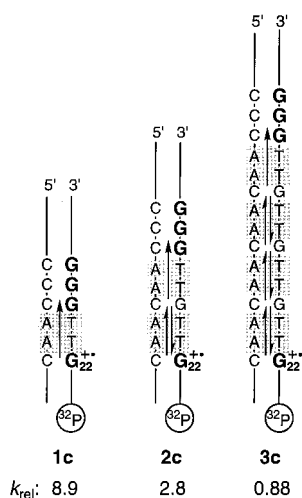
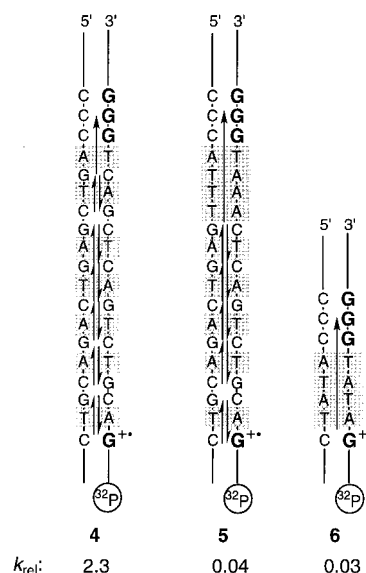


Figure 1. Dependence of the relative rate of charge transport (k_{rel}) on the number of hopping steps N and on the total distance Δr (inset).



Scheme 2. Charge transfer (indicated by the arrows) from G_{22}^{++} to the GGG unit in one (**1c**), two (**2c**), or four hopping steps (**3c**).

which the distance dependence is very small. However we have shown recently that long-range charge transfer through mixed DNA strands cannot be described by this superexchange mechanism.^[4a] This is also demonstrated by experiments with oligonucleotides **4** and **5**.^[11] In both strands the charge was transported over 54 Å, but the transfer is 60 times faster in **4** than in **5**. Following our hopping mechanism,^[4a] the rate-determining step in double strand **5** is the tunneling over a sequence of four AT base pairs. Therefore, in **5** charge transfer over 54 Å occurs at a similar rate as over 17 Å in **6** (Scheme 3).^[4a]



Scheme 3. Charge transfer (indicated by the arrows) from G^{++} to the GGG unit in different double strands **4**, **5**, and **6**.

Our studies show that charge transport through DNA can take place by hopping steps between bases having similar redox potentials. Since guanine (G) is the base of lowest oxidation potential in DNA, transfer of the positive charge occurs by tunneling between the guanine bases. The rate of these tunneling steps depends strongly on the distance between the neighboring G bases. Therefore, the base sequence plays a decisive role on the hole transfer. Following the hopping mechanism, transport of a negative charge takes place by reduction of thymine (T) and cytosine (C), which possess similar reduction potentials. Since one of these pyrimidine bases is present in each base pair, one can expect that electron transfer should not be dependent on the sequence.

A modification of natural DNA changes the redox potential of single bases. Following the hopping mechanism this should exert a strong influence on the efficiency of charge transfer.

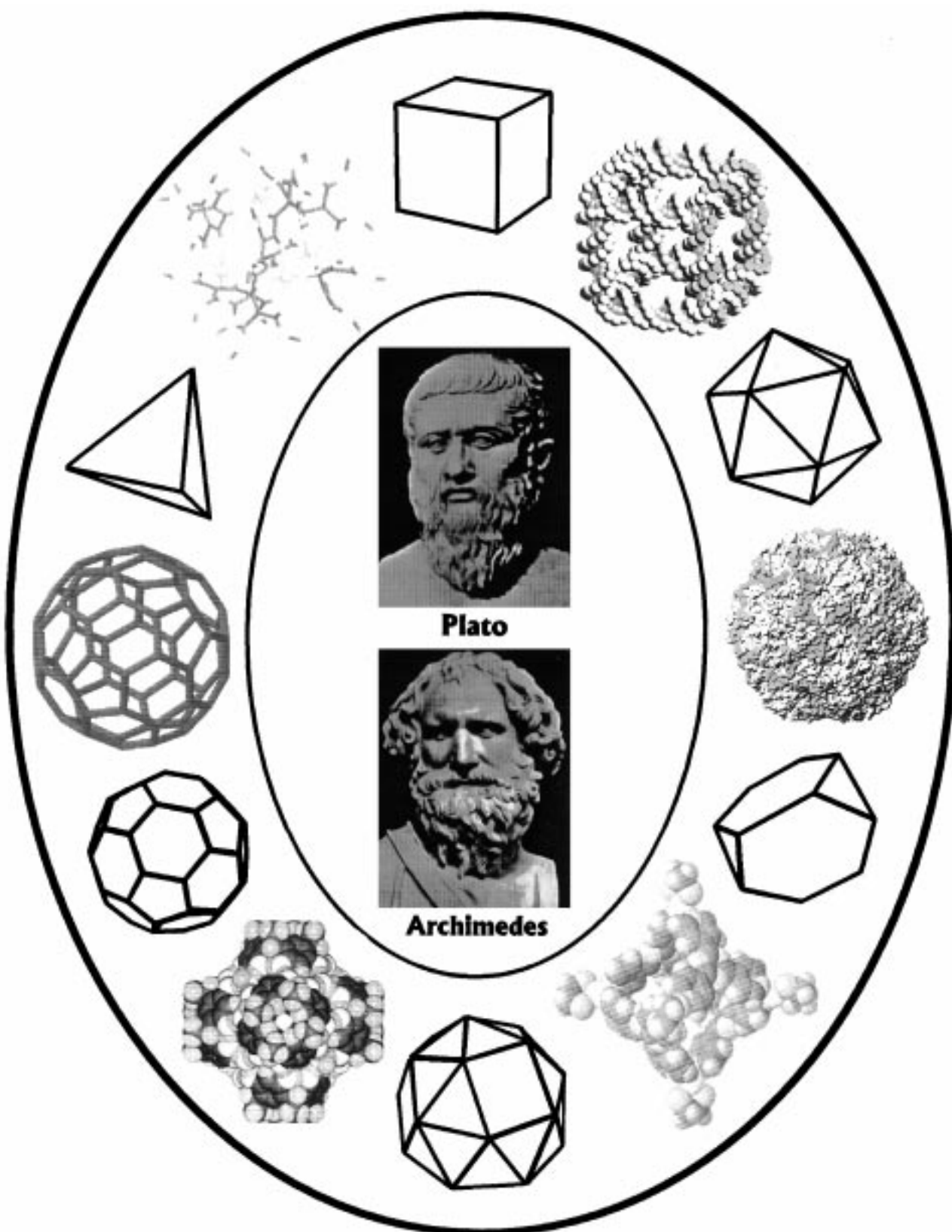
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Keywords: bioorganic chemistry • DNA oxidation • electron transfer • radicals

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- [8] In contrast to the assay that we have used previously,^[4a] the experiments were carried out with 4'-pivaloyl-substituted guanosine. The synthesis of this guanosine derivative was performed in analogy to that for the modified thymidine: A. Marx, P. Erdmann, M. Senn, S. Körner, T. Jungo, M. Petretta, P. Imwinkelried, A. Dussy, K. J. Kulicke, L. Macko, M. Zehnder, B. Giese, *Helv. Chim. Acta* **1996**, *79*, 1989. Photolysis of monomeric 4'-pivaloyl-substituted guanosine that was phosphorylated at the 3'-OH position gave the enol ether in 85% yield. This shows that the charge transfer between the carbohydrate radical cation and the base G is very fast.
- [9] The double strands **1a–3a** contained 3 ± 0.5 pmol of 4'-pivaloyl-modified strands and 1 ± 0.2 pmol of the corresponding 5'-³²P-labeled complementary strands. They were photolyzed for 15 min in phosphate buffer (200 μ L, 20 mM, 100 mM NaCl, pH 7.0) at 15°C with a 500-W Hg high-pressure lamp in the absence of oxygen. An aliquot of the solution was incubated for 30 min with 1M piperidine at 90°C, then lyophilized overnight, and analyzed by denaturated polyacrylamide gel electrophoresis (1500 V, 3.5 h). The modified strands were replaced by unmodified strands in control experiments. The dried gels were autoradiographed with a phospho-imager and analyzed with the software ImageQuant. Quantification of the piperidine-induced bands was performed by subtracting the intensities obtained in the control experiments from the intensities obtained with the modified double strands.
- [10] Experiments with other DNA strands are under way.
- [11] The measurements were carried out under the conditions described in detail in reference [4a]. The sequences of the strands correspond to those given there.

Platonic and Archimedean solids represent the limited number of ways in which more than three identical chemical subunits that correspond to regular polygons may be arranged along the surface of a sphere to approximate a spherical shell.



The underlying geometrical principles can be used for the classification of organic, inorganic, and biological host frameworks (some of which are shown here) and to identify synthetic opportunities for new containers.

Structural Classification and General Principles for the Design of Spherical Molecular Hosts

Leonard R. MacGillivray and Jerry L. Atwood*

Complexes made by hosts that completely surround their guests provide a means to stabilize reactive and transient chemical intermediates, to transfer biologically active molecules to target cells, and to construct molecular scale devices. Whereas nature has utilized amino acids, in the form of polypeptide chains, to construct shell-like hosts of nanoscale dimensions (for example, viruses and ferritin) that exhibit certain functions (for example, packaging of DNA and Fe cores), chemists have a myriad of organic and inorganic building blocks at their disposal. These may be used to synthesize diverse families of shells as demonstrated by the emergence of container molecules, ions, and assemblies (for example, cryptands, carcerands, polyoxometallates, inorganic-organic cages, and molecular

capsules). Synthetic containers of nanoscale dimensions remain rare, however, and with interests in chemical synthesis moving towards the fabrication of nanoscale molecular frameworks and the miniaturization of functional microstructures, it seems likely that the structures of biological shells will continue to inspire the design of synthetic analogues. It is clear that if chemists are to proceed with the endeavor of synthesizing shell-like hosts then a design strategy for the construction of such frameworks would be desirable, with a method that translates features related to the structures of biological shells to synthetic systems. Here, we demonstrate such an approach. In particular, we have structurally classified a variety of organic, inorganic, and biological host frame-

works and show that their structures may be cataloged according to principles of solid geometry in which the five Platonic and 13 Archimedean solids may be used as models for spheroid design. In essence, the method relies on recognizing the relationship between the convex uniform polyhedra and container molecules where, in both cases, identical subunits and bonding arrangements make up each shell. This systematic approach has led us to identify containers that have yet to be synthesized or discovered and we propose such systems as targets for chemical synthesis.

Keywords: host-guest chemistry · macrocycles · nanostructures · non-covalent interactions · topochemistry

1. Introduction

Since the introduction of the cryptands,^[1] chemists have strived to design discrete spherical molecular hosts similar to those found in nature (for example, spherical viruses, fullerenes). Such frameworks possess cavities capable of entrapping atomic and/or molecular sized guests and have applications in chemistry (for example, catalysis),^[2] biology (drug delivery),^[3] and materials science (molecular devices).^[4] Self-assembly,^[5, 6] the building of molecular architecture with identical chemical subunits, has been a central theme and, at the highest level of sophistication, has yielded monomolecular^[7] and supramolecular dimeric capsules^[2] capable of packaging molecular guests.

In such a context, a recent discovery in our laboratory of a self-assembling molecular spheroid^[8] has prompted us to examine the topologies of these and related hosts with a view to understand their structures on the basis of symmetry. In addition to providing a grounds for classification, such an approach allows one to identify similarities at the structural level that may not seem obvious at the chemical level, and may be used to design future systems. Notably, this approach has led us to identify from principles of solid geometry limited designs that are possible for the construction of spherical shells, those being the five Platonic and 13 Archimedean solids.^[9] Herein we now present a general design strategy for the construction of spherical molecular hosts.

2. Spheroid Design

Our strategy stems from ideas developed within host-guest^[10] and supramolecular chemistry.^[11] As is the case of spherical viruses^[3] and fullerenes,^[4] an appropriately sized,

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Figure 1. Hollow spherical shell S .

shaped, and functionalized guest is packaged within the interior of a host such that it is completely surrounded or enclosed. From a geometrical standpoint, this may be achieved by centralizing the guest within a hollow spherical shell S (Figure 1). We note, however, that it is impossible to mimic S from a chemical standpoint since atoms and molecules are discrete entities whereas the surface of S is uniform. Thus, an alternative procedure must be employed to design a spherical molecular host.

2.1. Self-Assembly

In a paper that described the structure of regular viruses,^[12] Caspar and Klug have shown that viral capsids use self-assembly to construct spherical shells up to a hundred nanometers in diameter by utilizing identical copies of proteins as chemical subunits. Such a design strategy is desirable since it employs an economy of information,^[6, 12] which gives rise to a host whose subunits exist in identical chemical environments, the exposed surface area is at a minimum, and strain energy is distributed evenly along its surface.^[4] This process is also amenable for spherical shells at the angstrom level as realized with the discovery of buckminsterfullerene or C_{60} .^[13] Notably, in the extreme case, an infinite number of identical subunits placed along the surface of a sphere will lead to a shell topologically equivalent to S . Thus, to construct a spherical molecular host one must ultimately consider the number of subunits n for spheroid design and their placement along the surface of the shell.

2.2. Subunits for Spheroid Design and Self-Assembly

To construct a spherical shell from a single subunit, $n = 1$, the only structure obtainable is S . It is impossible to construct a spherical molecular host from a single chemical entity (as outlined above) and therefore S does not represent a self-assembled spherical framework.

For $n = 2$, each subunit must cover one half of the surface of the sphere. This can only be achieved if the subunits exhibit curvature and they are placed such that their centroids lie at a maximum distance from each other. These criteria place two points along the surface of a sphere, separated by a distance equal to the diameter of the shell. We assign these positions the north and south poles. As a consequence of this arrangement, there exists two structure types for $n = 2$. The first belongs to the point group $D_{\infty h}$ and consists of two identical subunits attached at the equator (Figure 2a). Since it is

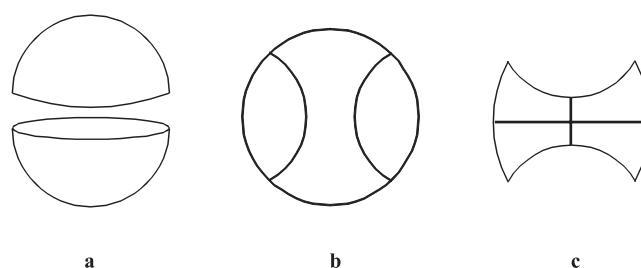


Figure 2. Shells with $n = 2$: a) $D_{\infty h}$ symmetry, b) D_{2d} symmetry (tennis ball), c) subunit of D_{2d} shell depicting the four asymmetric units.

impossible to create a shell-like hemisphere that possesses ∞ -fold rotation symmetry by using atoms and molecules, this structure is not obtainable from a chemical standpoint. The second belongs to the point group D_{nd} and its simplest

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member, $n=2$, is topologically equivalent to a tennis ball (Figure 2b). Each subunit of this system is symmetrical and may be divided into four identical asymmetric units (Figure 2c), which implies that eight asymmetric units are required to design the shell. Thus, $n=2$ represents the minimum number of subunits that may be used to construct a spherical molecular host by self-assembly.

For $n=3$, each subunit must cover one third of the surface of the sphere. Following the design conditions described previously, placing three identical subunits along the surface of a sphere results in an arrangement in which their centroids constitute the vertices of an equilateral triangle (Figure 3a).

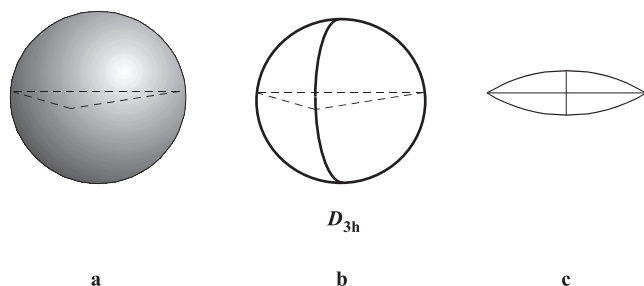


Figure 3. Shell with $n=3$: a) equilateral triangle from S , b) D_{3h} symmetry, c) subunit of D_{3h} shell depicting the four asymmetric units.

As a result there is only one structure type for $n=3$. The structure belongs to the point group D_{3h} and, as for the $n=2$ system, the subunits must exhibit curvature (Figure 3b). Since each “arm” may be divided into four identical asymmetric units (Figure 3c), 12 asymmetric units are required to construct the shell.

For $n=4$, positioning four points along the surface of a sphere such that they lie a maximum distance from each other places the points at the vertices of a tetrahedron (Figure 4a). This is the first case in which joining the points through line segments gives rise to a closed surface container. The container, a tetrahedron, is comprised of four identical subunits in the form of equilateral triangles, where surface curvature is supplied by edge sharing of regular polygons rather than the subunits themselves (Figure 4b). Each triangle may be divided into six asymmetric units as a result of its symmetry, (Figure 4c), which implies that 24 asymmetric units (4×6) are required to generate the shell. The tetrahedron belongs to the point group T_d and its polygons are related by combinations of twofold and threefold rotation axes.

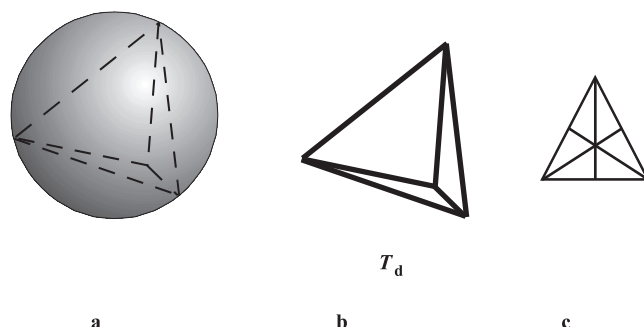


Figure 4. Shell with $n=4$: a) equilateral triangles from S , b) T_d symmetry, c) subunit of T_d shell depicting the six asymmetric units.

2.3. Platonic Solids

The tetrahedron belongs to a family of five convex uniform polyhedra known as the Platonic solids (Figure 5, Table 1).^[9] Each member of this family possesses cubic symmetry (that is, 32, 432, or 532 symmetry) and is made of the same regular

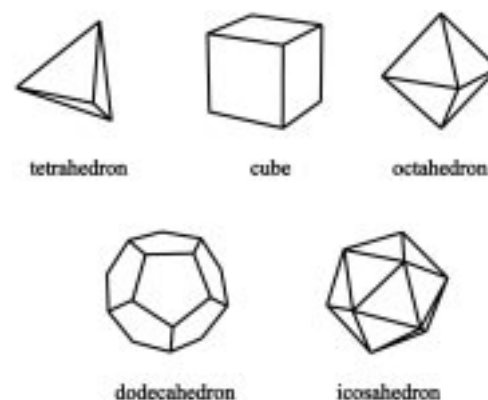


Figure 5. The five Platonic solids (see also Table 1).

Table 1. Platonic solids.

Solid	Vertices	Edges	Face type	Faces
tetrahedron	4	6	triangle	4
cube	8	12	square	6
octahedron	6	12	triangle	8
dodecahedron	20	30	pentagon	20
icosahedron	12	30	triangle	20

polygons (for example, equilateral triangle, square) arranged in space such that its vertices and edges, and three coordinate directions, are equivalent. There is a finite number of such polyhedra as a consequence of the fact that there exists a limited number of ways in which identical regular polygons may be adjoined to construct a convex corner. Equilateral triangles may be adjoined in three ways while squares and pentagons may be adjoined in only a single manner. Moreover, it is impossible to create a convex corner from regular polygons with six or more sides since the sum of the angles around each vertex would be greater than or equal to 360° .^[9] These principles give rise to five isometric polyhedra that are achiral and whose polygons are related by combinations of n -fold rotation axes. The Platonic solids include the tetrahedron, which belongs to the point group T_d , possesses 32 symmetry, and requires a minimum of 12 asymmetric units; the cube and octahedron, which belong to the point group O_h , possess 432 symmetry, and require a minimum of 24 asymmetric units; and the dodecahedron and icosahedron, which belong to the point group I_h , possess 532 symmetry, and require a minimum of 60 asymmetric units.^[12] The number of asymmetric units required to generate each shell doubles if mirror planes are present in these structures.

2.4. Archimedean Solids

In addition to the Platonic solids, there exists a family of 13 convex uniform polyhedra known as the Archimedean solids

(Figure 6, Table 2).^[9] Each member of this family is made up of at least two different regular polygons and may be derived from at least one Platonic solid through either truncation or the twisting of faces. In the case of the latter, two chiral members, the snub cube and the snub dodecahedron, are realized. The

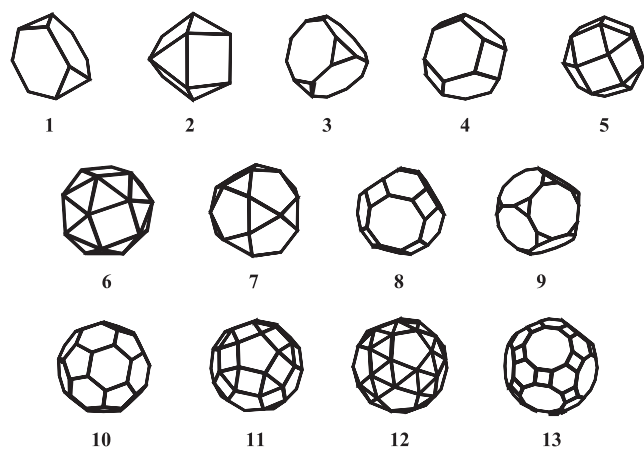


Figure 6. The 13 Archimedean solids, in order of increasing number of vertices. Truncated tetrahedron (1), cuboctahedron (2), truncated cube (3), truncated octahedron (4), rhombicuboctahedron (5), snub cube (6), icosidodecahedron (7), rhombitruncated cuboctahedron (8), truncated dodecahedron (9), truncated icosahedron (10), rhombicosidodecahedron (11), snub dodecahedron (12), rhombitruncated icosidodecahedron (13) (see also Table 2).

remaining Archimedean solids are achiral. Like the Platonic solids, the Archimedean solids possess identical vertices, exhibit either 32, 432, or 532 symmetry, and require a minimum of either 12, 24, or 60 asymmetric units, respectively. The Archimedean solids possess a larger variety of polygons than the Platonic solids. These include the equilateral triangle, square, pentagon, hexagon, octagon, and decagon.

2.5. Models for Spheroid Design

It is our contention here that the Platonic and Archimedean solids represent the limited number of ways in which $n > 3$ identical chemical subunits that correspond to regular polygons may be arranged along the surface of a sphere to approximate *S*. As a result, these solids may be used as models

for the construction of spherical molecular hosts. These polyhedra provide combinations of n -fold rotation axes and subunits in the form of regular polygons for self-assembly, which allow one to determine where along the surface of a sphere chemical subunits should be placed and the bonding arrangements they should adopt. In effect, the Platonic and Archimedean solids simplify the task of constructing spherical hosts by facilitating a priori spheroid design, and serve as targets in chemical synthesis.

2.6. Prisms and Antiprisms

In addition to the Platonic and Archimedean solids, and to complete the convex uniform polyhedra, there exist two infinite families of solids known as prisms and antiprisms (Figure 7, Table 3).^[9] These polyhedra consist of two regular n -gons bisected by a perpendicular n -fold rotation axis and are

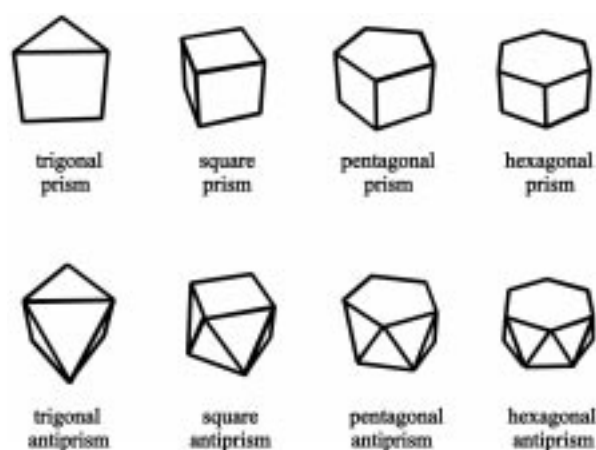


Figure 7. Prisms and antiprisms.

Table 3. Prisms and antiprisms.

Solid	Vertices	Edges	Faces
prism	$2n^{[a]}$	$3n$	n squares (or n rectangles) + 2 n -gons
antiprism	$2n$	$6n$	$2n$ triangles + 2 n -gons

[a] n -fold rotation axis

Table 2. Archimedean solids.

Solid	Vertices	Edges	f(3) ^[a]	f(4)	f(5)	f(6)	f(8)	f(10)
(1) truncated tetrahedron	12	18	4	–	–	4	–	–
(2) cuboctahedron	12	24	8	6	–	–	–	–
(3) truncated cube	24	36	8	–	–	–	6	–
(4) truncated octahedron	24	36	–	6	–	8	–	–
(5) rhombicuboctahedron	24	48	8	18	–	–	–	–
(6) snub cube	24	60	32	6	–	–	–	–
(7) icosidodecahedron	30	60	20	–	12	–	–	–
(8) rhombitruncated cuboctahedron	48	72	–	12	–	8	6	–
(9) truncated dodecahedron	60	90	20	–	–	–	–	12
(10) truncated icosahedron	60	90	–	–	12	20	–	–
(11) rhombicosidodecahedron	60	120	20	30	12	–	–	–
(12) snub dodecahedron	60	150	80	–	12	–	–	–
(13) rhombitruncated icosidodecahedron	120	180	–	30	–	20	–	12

[a] f(n) where f denotes face and n indicates the number of sides of the face.

related by n twofold rotation axes. Prisms differ from antiprisms such that the n -gons of a prism are congruent whereas n -gons of an antiprism are related by a twist. As a result, joining the vertices of a prism through line segments gives rise to squares while the same procedure applied to an antiprism gives rise to alternating up and down triangles. Notably, the distance between the n -gons of prisms and antiprisms may be reduced or increased, which either flattens or elongates the solid, respectively. The minimalist members of both families are based upon the equilateral triangle. As a result, prisms and antiprisms exhibit dihedral D symmetry. Prisms belong to the point group D_{nh} , while antiprisms belong to the point group D_{nd} ($n \geq 3$). With the exception of the cube (square prism) and octahedron (trigonal antiprism), the three coordinate directions of prisms and antiprisms are not equivalent. We therefore refer to these systems as pseudo-spherical.

2.7. Dihedral Symmetry: $n = 2$ and $n = 3$ Shells

Prisms and antiprisms may be used in a similar way to the Platonic and Archimedean solids for the construction of hosts based upon $n = 2$ and $n = 3$ subunits as a result of their dihedral symmetry. As stated, two identical subunits may be arranged to approximate S by using shells with D_{nd} symmetry. Likewise, three identical subunits may be arranged to approximate S by using a shell with D_{3h} symmetry. A common feature of these structures is dihedral symmetry where half of each shell is defined by a twofold rotation axis. Moreover, close inspection of these frameworks reveals that they are topologically equivalent to antiprisms and the trigonal prism, respectively. As a result, these polyhedra may be used as models for the construction of $n = 2$ and $n = 3$ structures. In fact, shells consisting of $n = 3$ subunits belong to the $n = 2$ family since their north and south poles are identical. Like the D_{nd} shells, there exists an infinite number of D_{nh} structures since there exists an infinite number of prisms. It must be stressed, however, that, unlike the Platonic and Archimedean solids, the subunits of these systems must exhibit curvature.

2.8. D_{2h} and D_{2d} Shells

Since the simplest members of prisms and antiprisms possess a threefold rotation axis of symmetry or greater, these polyhedra cannot be used as models for D_{2h} and D_{2d} shells. Moreover, it is impossible to construct a polyhedron without polygons. There exist, however, common objects that possess the symmetries of these frameworks, which may be considered for the construction of these shells. For example, a rugby ball with alternating gray and black patches possesses D_{2h} symmetry (Figure 8). On the other hand, a tennis ball, as pointed out previously, possesses D_{2d} symmetry (Figure 2b). As is the case for prisms and antiprisms, the three coordinate directions of D_{2h} and D_{2d} structures are not equivalent and



Figure 8. Rugby ball shell.

their subunits must exhibit curvature. We therefore also refer to these systems as pseudo-spherical.

3. Examples from the Laboratory and from Nature

Our premise that pseudo-spherical and spherical molecular hosts may be classified into two categories, $n = 2$ and $n > 3$, according to principles of geometry, renders both organic and inorganic components viable for their design and permits the individual subunits to be held together by covalent and/or noncovalent bonds. Indeed, a common feature displayed by organic and inorganic chemists is that they must utilize space for assembling atoms into molecular frameworks. To demonstrate the utility of this approach, we now present selected examples of pseudo-spherical and spherical hosts from the laboratory and nature. We will begin with dihedral systems and work our way to cubic frameworks (Table 4).

Table 4. Summary of symmetries for shell-like hosts.

Solid or shell	Spherical or pseudo-spherical	Symmetry	Point group(s)	Asymmetric units
Platonic/Archimedean	spherical	icosahedral	I_h, I	120, 60
	spherical	octahedral	O_h, O	48, 24
	spherical	tetrahedral	T_h, T_d, T	24, 24, 12
prisms/antiprisms	pseudo-spherical	dihedral	D_{nh}, D_{nd}	$4n, 4n$
rugby ball	pseudo-spherical	dihedral	D_{2h}	8
tennis ball	pseudo-spherical	dihedral	D_{2d}	8

3.1. “Tennis Ball” Shell (D_{2d})

Rebek et al. presented the first example of a supramolecular “tennis ball” (Figure 9a).^[14] This system has been designated a molecular capsule and the subunits are based upon diphenyl glycoluril and durene, which self-assemble in chloroform by way of eight N–H⋯O hydrogen bonds. The methylene linkages between the glycoluril units and the aromatic moiety provide much of the curvature of this system. Since their introduction, Rebek et al. have constructed larger D_{2d} hosts^[15] and revealed their molecular recognition properties.

In addition to organic components, inorganic components have been used for D_{2d} capsules. In particular, Müller et al. have demonstrated the ability of VO_5 square pyramids to assemble from aqueous media to form a D_{2d} polyoxovanadate shell $[HV_{22}O_{54}]^{5-}$, which encapsulates a perchlorate ion (Figure 9b).^[16] Thorn et al. have also demonstrated the ability of such VO_5 units to form a D_{2d} shell by assembly with phosphonate ligands $[(V_2O_3)_2(O_3PPh)_4]^{2-}$, which entraps a fluoride ion (Figure 9c).^[17]

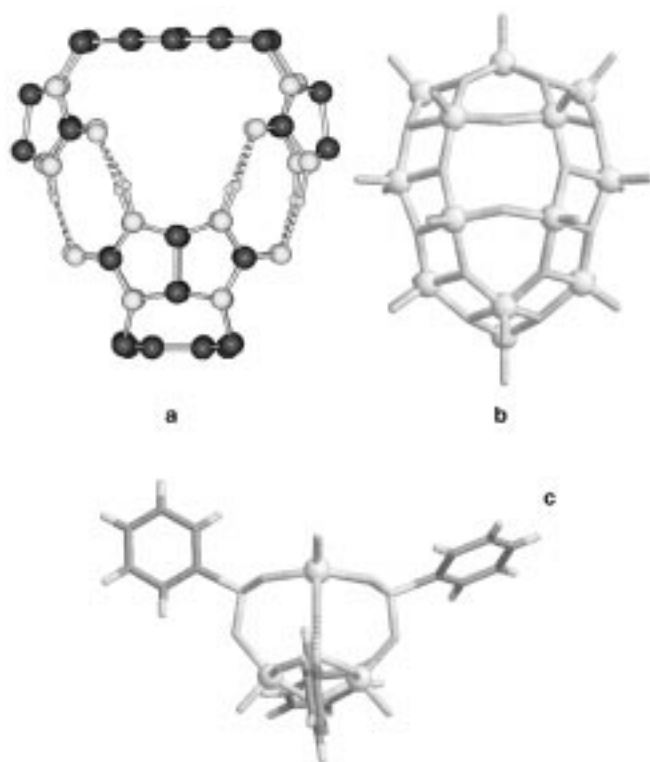


Figure 9. X-ray crystal structures of D_{2d} (tennis ball) hosts: a) a molecular capsule (Rebek et al.), b) the $[\text{HV}_{22}\text{O}_{34}]^{5-}$ ion (carbon atoms have been omitted for clarity) (atomic color scheme: gray = carbon, white = hydrogen, red = oxygen, blue = nitrogen, orange = vanadium, magenta = phosphorus).

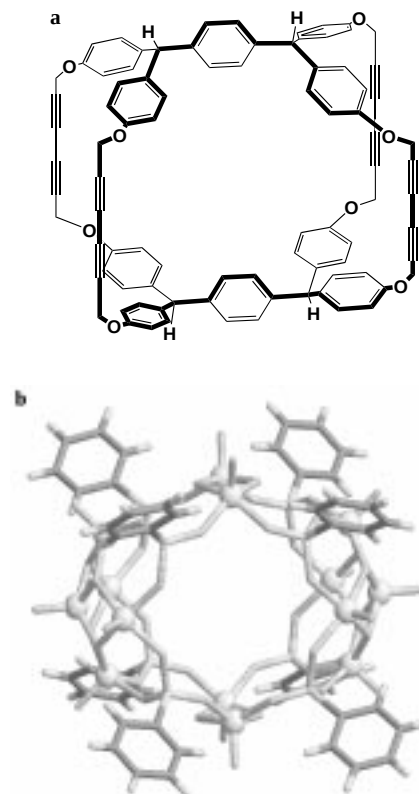


Figure 10. D_{2h} (rugby ball) hosts: a) a system based upon tetrakis(propargylic ether) units (Vögtle et al.), b) the $[\text{V}_{14}\text{O}_{22}(\text{OH})_4(\text{PhPO}_3)_8]^{6-}$ ion.

3.2. “Rugby Ball” Shell (D_{2h})

To our knowledge, the first D_{2h} host was synthesized by Vögtle et al.^[18] The cage is based upon the cyclodimerization of tetrakis(propargylic ether) units that are separated by covalently attached diyne spacers (Figure 10a). This host possesses two chemically distinct “windows” that allow access to its cavity.^[18]

In a similar way to their D_{2d} system, Müller et al. have demonstrated the ability of VO_5 pyramids to form the D_{2h} shells $[\text{H}_2\text{V}_{18}\text{O}_{44}]^{4-}$ and $[\text{V}_{14}\text{O}_{22}(\text{OH})_4(\text{PhPO}_3)_8]^{6-}$, each of which encapsulates either an azide ion, water molecules, or both (Figure 10b).^[16, 19] Zubietta et al. have also reported the ability of such VO_5 pyramids to form the similar D_{2h} shell $[\text{V}_{12}\text{O}_{14}(\text{OH})_4(\text{PhAsO}_3)_{10}]^{4-}$, which entraps two methanol molecules.^[20]

3.3. Prisms (D_{nh} , $n \geq 3$)

The [2.2.2]cryptand synthesized by Lehn et al. was the first $n = 2$ host (Figure 11a).^[1] The subunits of this system possess idealized D_{3h} symmetry and are propagated at the north and south poles by sp^3 hybridized nitrogen atoms and may be divided into the six asymmetric units $[\text{N}(\text{CH}_2\text{CH}_2\text{OCH}_2)_3]/6$.

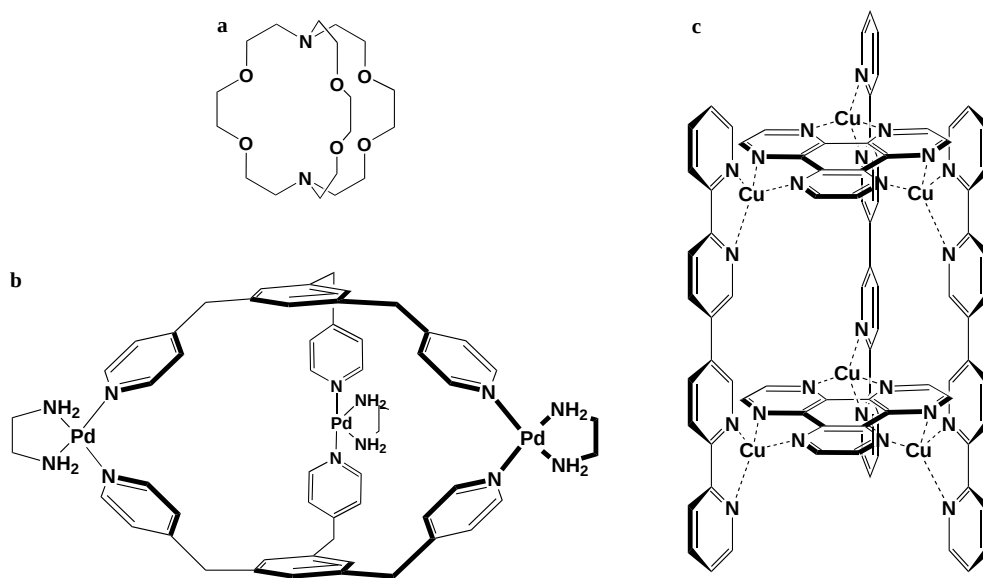


Figure 11. D_{3h} hosts: a) [2.2.2]cryptand, b) a metal–organic palladium host (Fujita et al.), and c) a metal–organic copper host (Lehn et al.).

Owing to the size of the subunits, the [2.2.2]cryptand is capable of complexing atomic cations.^[1]

Following the introduction of the cryptands, a variety of D_{3h} hosts have emerged.^[18, 21] To effect their binding properties, structural changes are often made at their poles and/or their side arms and, in some instances, metal ions are used. Examples include metal–organic cages (Figure 11 b, c).^[22, 23] These systems are capable of binding molecular guests as a consequence of the larger sizes of their subunits.^[18]

The carcerands, introduced by Cram et al., were the first D_{4h} hosts (Figure 12 a).^[8] These molecules consist of cavitands covalently attached in a head-to-head fashion and

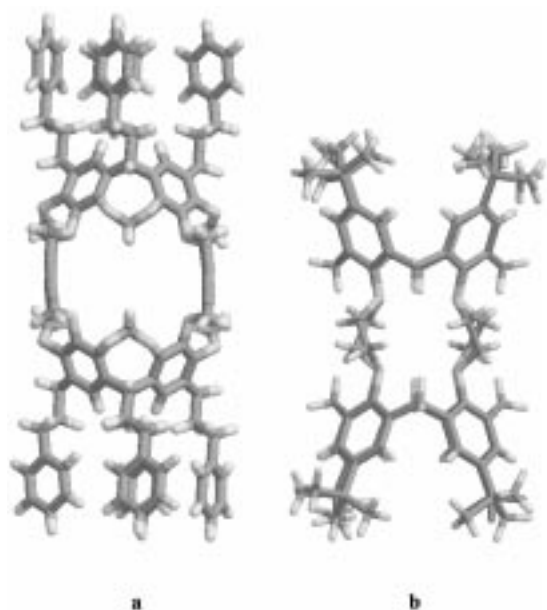


Figure 12. X-ray crystal structure of D_{4h} hosts: a) a carcerand (Cram et al.), b) a calix[4]tube (Beer et al.).

are capable of incarcerating molecular guests such that they are unable to escape. Our lab has recently shown^[24] that a similar host may be realized from calix[4]resorcinarenes through noncovalent bonds. Specifically, two *C*-phenethylcalix[4]resorcinarenes and eight isopropanol molecules were shown to assemble by way of 16 O–H⋯O hydrogen bonds to form a D_{4h} complex in the solid state. Beer et al. have also introduced a D_{4h} host based upon calix[4]arenes, aligned in a tail-to-tail fashion, which binds atomic sized cations (Figure 12 b).^[25] Other D_{4h} systems include metal-based carcerands^[26] and porphyrin dimers.^[27]

The C_{70} fullerene is an example of a D_{5h} shell (Figure 13 a). Each subunit of C_{70} is composed of 35 carbon atoms and is attached at the equator through five covalent bonds.^[4] Pope et al. have described the formation of the shell-like polytungstate $[\text{NaP}_5\text{W}_{30}\text{O}_{110}]^{14-}$, which possesses approximate D_{5h} symmetry (Figure 13 b).^[28] The anion, which is partly made up of five P_5W_6 units, was observed to entrap an exchangeable sodium ion in both solution and the solid state.^[28]

Zimmerman et al. have recently presented an example of a dendrimeric assembly that exhibits D_{6h} symmetry (Figure 14 a).^[29] The subunits are held together by six covalent bonds and contain 24 asymmetric units, each of which

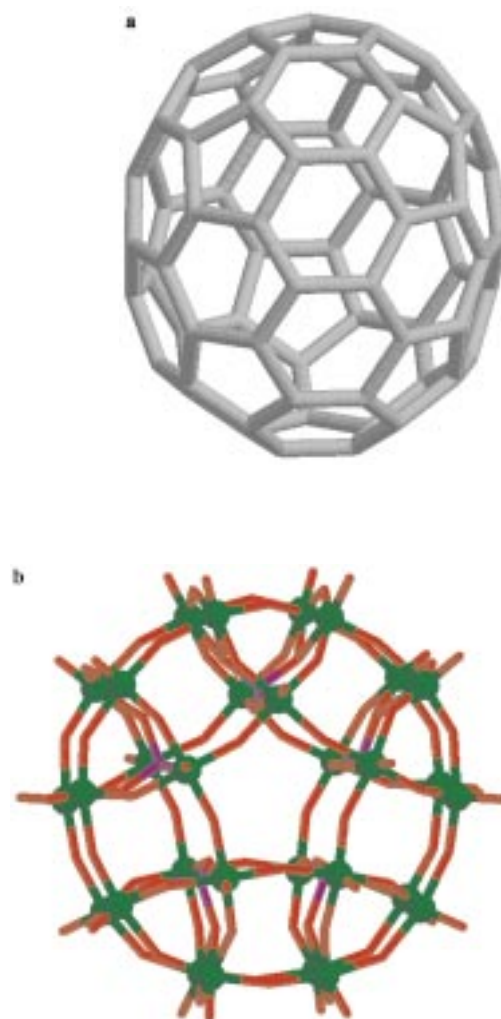


Figure 13. X-ray crystal structure of D_{5h} hosts: a) C_{70} , b) the $[\text{NaP}_5\text{W}_{30}\text{O}_{110}]^{14-}$ ion (atomic color scheme: green = tungsten)

interacts at the north and south poles by way of hydrogen bonded carboxylic acid dimers. Kim et al. have also demonstrated the ability of a D_{6h} host based upon a “lidded” cucurbituril to function as a molecular capsule (Figure 14 b).^[30] while Shinkai et al. have reported the design of a D_{6h} shell by linking two calix[6]arenes through bridging sulfur atoms (Figure 14 c).^[31]

To the best of our knowledge, there are no examples of hosts that possess D_{nh} ($n \geq 7$) symmetry. We note, however, that Klüfers et al. have demonstrated the ability of two γ -cyclodextrin molecules to assemble with 16 lead(II) ions to form a shell-like complex that possesses approximate D_8 symmetry as a result of the *anti* arrangement of the macrocycles.^[32]

3.4. Antiprisms (D_{nd} , $n \geq 3$)

Rebek et al. recently introduced a D_{3d} molecular capsule (Figure 15).^[33] Like its D_{2d} predecessor, the subunits of this capsule, each of which possesses six asymmetric units, are based upon glycoluril and their curvature primarily arises

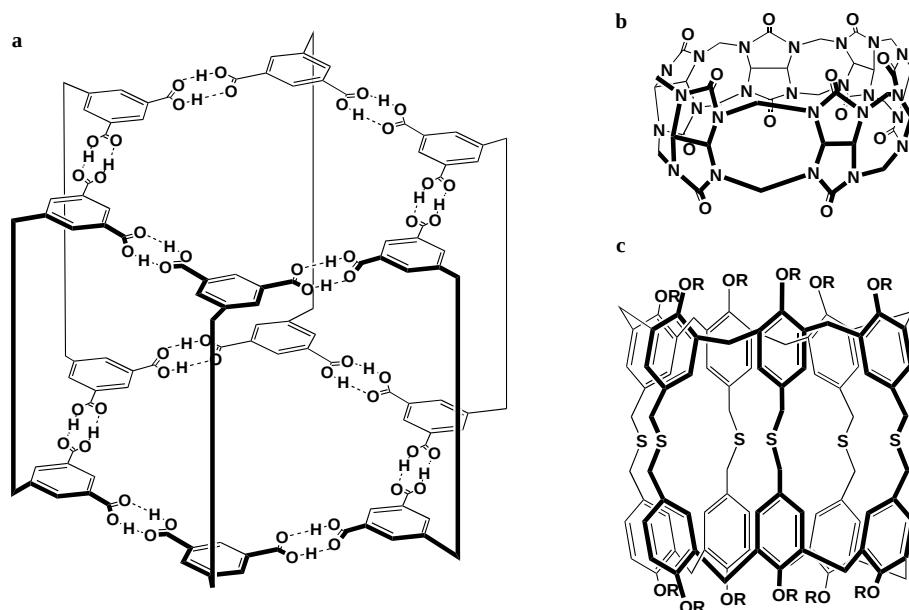


Figure 14. D_{6h} hosts: a) a dendrimeric assembly (Zimmerman et al.), b) cucurbituril (Kim et al.), c) a calix[6]arene shell (Shinkai et al.).

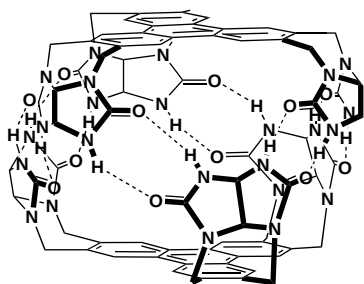


Figure 15. D_{3d} host: the flattened capsule from Rebek et al.

from methylene linkages to an aromatic core. The assembly is held together by 12 $N-H \cdots O$ hydrogen bonds.^[33]

Prior to the D_{3d} capsule, Rebek et al. also introduced a D_{4d} host (Figure 16a).^[34] The host is based upon a head-to-head alignment of two calix[4]arenes, with the subunits of this system being held together by a circular configuration of eight urea moieties that interact by way of 16 $N-H \cdots O$ hydrogen bonds. Notably, the D_{4d} symmetry of this host is only realized in the absence of the hydrogen bonds. According to molecular modeling studies this capsule is roomy enough to encapsulate two benzene molecules.^[34]

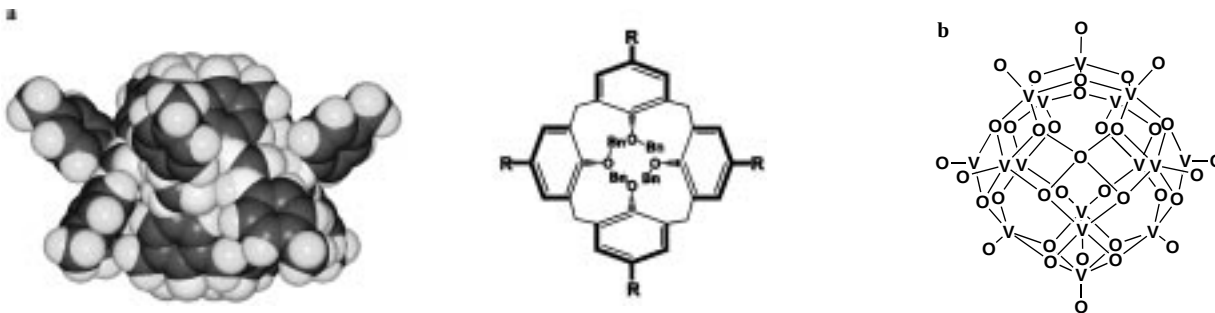


Figure 16. D_{4d} hosts: a) X-ray crystal structure of a calix[4]arene dimer (Rebek et al.), Bn groups are omitted for clarity (inset: ionic representation of the calix[4]arene, $R = NH(CO)NH(C_6H_5)$, Bn = benzyl), b) the $[H_4V_{18}O_{42}]^{8-}$ ion.

Müller et al. have also shown that VO_5 pyramids assemble under selected conditions to give rise to the D_{4d} capsule $[H_4V_{18}O_{42}]^{8-}$ (Figure 16b).^[35] The cavity of this ionic host accommodates a halide ion.^[35] To our knowledge, there are no examples of hosts that possess D_{nd} ($n \geq 5$) symmetry.

3.5. Platonic Solids

The Platonic solids constitute a family of five convex uniform polyhedra made up of the same regular polygons and possess either 32, 432, or 532 symmetry. As a result the three coordinate directions within each solid are equivalent, which makes these polyhedra models for spheroid design.

3.5.1. Tetrahedral Systems (T_d , T_h , T)

The macrotricyclic spherand designed by Lehn et al. was the first tetrahedral host (Figure 17a).^[36] The bridgehead nitrogen atoms, located at the corners of the tetrahedron, and the ethyleneoxy units, the edges, supply the threefold and twofold rotation axes, respectively. As a result, the spherand is composed of 24 asymmetric units, $[(N/3)-(CH_2CH_2)-(O/2)]/2$. Notably, this molecule and its tetraprotonated form has been shown to bind an ammonium and chloride ion, respectively. Schmidtchen et al. have introduced similar tetrahedral cages with edges comprised entirely of methylene bridges^[37] while Vögtle et al. have demonstrated the synthesis of a hollow hydrocarbon called spheriphane (Figure 17b).^[18]

Saalfrank et al. were the first to introduce metal-based tetrahedral cages by using metal ions as corner units and bridging malonate ligands as edges (Figure 17c).^[38] Interestingly, these M_4L_6 cages are adamantane-like as a result of a bend in each ligand.^[38] In terms of host–guest behavior, an Fe-based system has been shown to complex a single ammonium ion.^[39]

Similar tetrahedral cages have emerged following the introduction of these metal-based systems. Raymond et al.^[40]

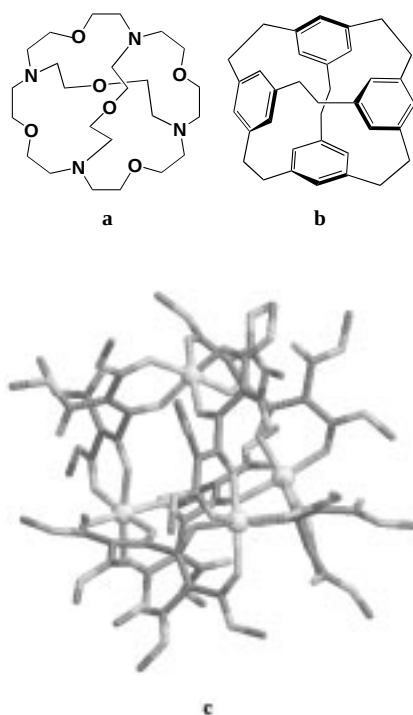


Figure 17. Tetrahedral hosts with T_d symmetry: a) a spherand (Lehn et al.), b) spheraphane (Vögtle et al.), c) a metal-based cage (Saalfrank et al.) (atomic color scheme: orange = iron).

and Huttner et al.,^[41] for example, have revealed that linear, rather than bent, bridging ligands may be used to form such cages. In the case of the former, bidentate ligands were used to completely fill the coordination sites around each metal center while, in the latter, monodentate ligands along with

“stopper” units were employed. These hosts were shown to form complexes with four dimethylformamide molecules and a tetrafluoroborate ion, respectively.^[40, 41]

Huan et al.,^[42] Birker et al.,^[43] and Bürgi et al.^[44] have described the formation of the tetrahedral shell-like hosts $[\text{H}_{12}(\text{VO}_2)_{12}(\text{C}_6\text{H}_5\text{PO}_3)_8]^{4-}$, $[\text{M}_8^{\text{I}}\text{M}_6^{\text{II}}\{\text{SC}(\text{Me})_2\text{CH}(\text{NH}_2)\text{CO}_2\}_{12}\text{Cl}\}^{5-}$ (where $\text{M}_8^{\text{I}}\text{M}_6^{\text{II}} = \text{Cu}_8^{\text{I}}\text{Ni}_6^{\text{II}}$, $\text{Ag}_8^{\text{I}}\text{Ni}_6^{\text{II}}$, $\text{Ag}_8^{\text{I}}\text{Pd}_6^{\text{II}}$), and $[\text{ICd}_8(\text{SCH}_2\text{CH}_2\text{OH})_{12}]^{3+}$, which possess vanadium and sulfur atoms at the vertices of a icosahedron (Figure 18). These structures also possess phosphorus and metal ions at the centroids of triangular faces that correspond to the corners of a cube. As a result, these shells belong to the point group T_h , the point group of a volleyball (Figure 19).

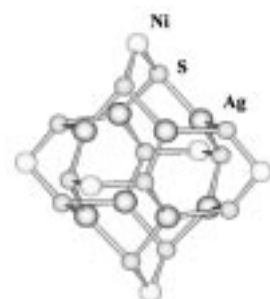


Figure 18. Tetrahedral host with T_h symmetry. X-ray crystal structure of the $[\text{Ag}_8\text{Ni}_6\{\text{SC}(\text{Me})_2\text{CH}(\text{NH}_2)\text{CO}_2\}_{12}\text{Cl}\}^{5-}$ ion showing the positions of the Ag, Ni, and S atoms.

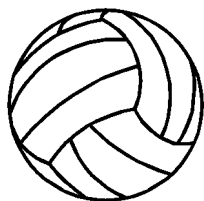


Figure 19. A volleyball, an object that possesses T_h symmetry.

3.5.2. Octahedral Systems (O_h , O)

We now describe four octahedral hosts related to the Platonic solids. Three are based upon the cube while one possesses features of both a cube and octahedron.

The first is a cyclophane-based system reported by Murakami et al. (Figure 20a).^[45] The sides of the host consist of tetraaza[3.3.3]paracyclophane units and its octaprotonated



Figure 20. Octahedral hosts: a) a cyclophane-based cube (Murakami et al.), b) X-ray crystal structure of the $[(\text{VO}_6)(\text{RPO}_3)_8]^+$ ($\text{R} = t\text{Bu}$, OSiMe_3) ion, c) DNA-based cube (Chen and Seeman; printed with permission by Professor Nadrian Seeman, for further details see <http://seemanlab4.chem.nyu.edu>).

cation has been shown to bind anionic guests.^[45] The molecule possesses 48 asymmetric units of $\{(N/3)-(CH_2)-((C_6H_4)/2)/2$.

The second is a polyoxovanadate, $[(\text{VO}_6)(\text{RPO}_3)_8]^+$ ($\text{R} = t\text{Bu}$, OSiMe_3), reported by Zubieta et al.^[46] and Thorn et al.^[17] which consists of VO_5 pyramids and phosphonate ligands (Figure 20b). The vanadium atoms of the shell are located at the vertices of an octahedron while the phosphorus atoms are located at the corners of a cube, thus displaying the

dual relationship of these polyhedra. In both cases, the host has been shown to complex a chloride ion.^[46, 17]

The third is a gold selenide cube $[\text{NaAu}_{12}\text{Se}_8]^{3-}$ reported by Huang and Kanatzidis.^[47] The anion is made up of eight selenium atoms and 12 gold atoms. A sodium cation occupies the center of the complex.^[47]

The fourth is a cube synthesized by Chen and Seeman, the components of which are based upon deoxyribonucleic acid (DNA) (Figure 20c).^[48a] The directionality and ability of the double helix to form branched junctions^[48b] are exploited for the edges and vertices, respectively. Interestingly, each face of this molecule forms a cyclic strand that is catenated with strands of adjacent faces. Molecular modeling experiments indicate the length of each edge to be approximately 68 Å.^[48a]

3.5.3. Icosahedral Systems (I_h , I)

Spherical viruses are icosahedral molecular hosts related to the Platonic solids (Figure 21a).^[3] These hosts consist of identical copies of proteins that assemble by way of non-covalent forces and range from 15 to 90 nm in diameter, and

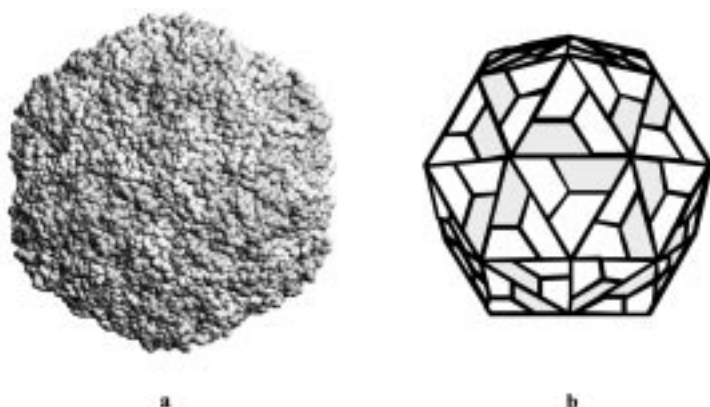


Figure 21. An icosahedral host, a) X-ray crystal structure of the rhinovirus, a spherical virus linked to the common cold, b) a schematic representation of the rhinovirus displaying triangulation (color scheme: red, green, blue spheres = amino acids).

encapsulate strands of ribonucleic acid (RNA). Although spherical viruses require a minimum of 60 subunits, most are made up of $60n$ ($n = 2, 3, 4, \dots$) subunits because of a reduction in the symmetry of their polygons (Figure 21b). This process, known as triangulation, gives rise to quasi-equivalent positions along the surface of the shell that enable the virus particle to cover the RNA with the largest number of subunits. Since only certain triangulations are permitted by symmetry, it is possible to classify virus structures into a coherent system.^[12]

Kretschmer et al. have recently described the ability of 12 CpSmCl_2 units (Cp = cyclopentadienyl) to form the neutral samarium-based shell $[\text{Cp}_{12}\text{Sm}_{12}(\mu_3\text{-Cl})_{24}]$, in which 12 samarium atoms are located at the vertices of an icosahedron and 20 chloride ions are at the vertices of a dodecahedron.^[49] The remaining chloride ions form a tetrahedron at the center of the shell.

3.6. Archimedean Solids

The Archimedean solids constitute a family of 13 convex uniform polyhedra made up of two or more regular polygons and, like the Platonic solids, possess either 32, 432, or 532 symmetry. As a result, the three coordinate directions within each solid are equivalent, which makes these polyhedra, in addition to the Platonic solids, models for spheroid design.

3.6.1. Truncated Tetrahedron (1)

Fujita et al.,^[50] Stang et al.,^[51] and Hartshorn and Steel^[52] have recently described the synthesis of M_6L_4 cages that are topologically analogous to a truncated tetrahedron (Figure 22). These systems, which may be regarded as inverted

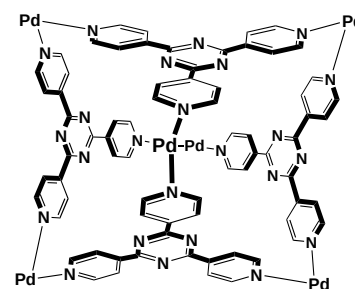


Figure 22. A metal-based cage (Fujita et al.): a host based upon the truncated tetrahedron.

M_4L_6 frameworks, consist of metal ions and aromatic-based bridging ligands that constitute the twofold and threefold rotation axes, respectively. Notably, owing to the presence a chiral stopper unit, the system reported by Stang et al. possesses T symmetry and is therefore chiral.^[51] In terms of host–guest behavior, X-ray crystallographic studies have revealed the assembly reported by Fujita et al. to form a complex with four adamantyl carboxylate ions while that of Hartshorn and Steel to encapsulate a molecule of dimethylsulfoxide.^[50, 52] According to mass spectrometric data, the cage reported by Stang et al. associates with four triflate ions.^[51]

Zubieta et al. have also demonstrated the formation of $[\text{Mo}_{16}(\text{OH})_{12}\text{O}_{40}]^{8-}$, a cage topologically equivalent to a truncated tetrahedron.^[53] This host is composed of four Mo^{VI} and twelve Mo^{V} centers and possesses a central $[\text{Mo}_{12}\text{O}_{40}]^{20-}$ core that encapsulates protons or a sodium ion.^[53]

3.6.2. Cuboctahedron (2)

González-Duarte et al. have recently described the ability of eight cadmium ions and 16 thiolate ligands to assemble from aqueous media to form the highly charged cage $[\text{Cd}_8\{\text{SCH}(\text{CH}_2\text{CH}_2)_2\text{N}(\text{H})\text{Me}\}_{16}]^{15+}$, the sulfur atoms of which sit at the vertices of a cuboctahedron.^[54] The host contains a central chloride ion and an inner tetrahedral array of cadmium ions.^[54] Interestingly, Ross et al. have considered through MM2 molecular model simulations the existence of a molecule containing eight benzene rings and either 12 oxygen or sulfur atoms, which they refer to as heterospherophane (Figure 23).^[55] Although it is not mentioned in the original

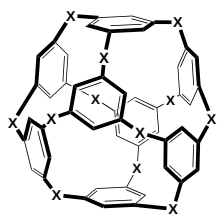


Figure 23. A theoretical organic shell based upon the cuboctahedron (X = O, S).

report, the shell exhibits a topology identical to a cuboctahedron.

3.6.3. Truncated Octahedron (4)

Zhang and Seeman have constructed a DNA-based shell analogous to a truncated octahedron.^[56] The edges of this molecule, each of which contains two turns of the double helix, contain 1440 nucleotides and the molecular weight of

the structure, which is an overall [14]catenane, is 790 000 Daltons. Interestingly, the design strategy relies on a solid-support approach in which a net of squares is ligated to give the polyhedron. It is currently unclear what shape the molecule adopts in various media.^[56]

Kretschmer et al. have described the formation of the lanthanide complex $[\text{Cp}_6\text{Yb}_6\text{Cl}_{13}]^-$, which conforms to a truncated octahedron.^[49] The anion contains six ytterbium ions, located at the corners of an octahedron, and 12 bridging chloride ions. A single chloride ion occupies the center of the shell.^[49]

3.6.4. Rhombicuboctahedron (5)

Müller et al. have shown that 24 oxygen atoms of the polyoxometalate $[\text{As}_4\text{Mo}_6\text{V}_7\text{O}_{39}]^{2-}$ may be attributed to the structure of a rhombicuboctahedron.^[57] Notably, a strong “tetrahedral distortion” of the ion is required for the host to correspond to the polyhedron. This shell has been shown to complex a sulfate ion in the solid state.^[57]

3.6.5. Snub Cube (6)

We have recently demonstrated the ability of six calix[4]-resorcinarenes and eight water molecules to assemble in apolar media to form a spherical molecular assembly which conforms to a snub cube (Figure 24).^[8] The shell consists of 24 asymmetric units, (each calix[4]arene lies on a fourfold axis and each H_2O molecule on a threefold axis), in which the vertices of the square faces of the polyhedron correspond to

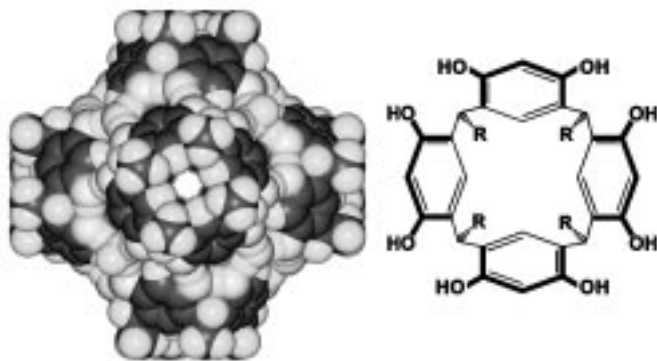


Figure 24. X-ray crystal structure of the calix[4]resorcinarene spheroid, a shell based upon the snub cube (inset: iconic representation of calix[4]resorcinarene, R = Me).

the corners of the calixarenes and the centroids of the eight triangles that adjoin three squares correspond to the water molecules. The assembly, which exhibits an external diameter of 24 Å, possesses an internal volume of about 1375 Å³ and is held together by 60 O–H...O hydrogen bonds.

3.6.6. Truncated Icosahedron (10)

Buckminsterfullerene, an allotrope of carbon, is topologically equivalent to a truncated icosahedron, an Archimedean solid that possesses 12 pentagons and 20 hexagons (Figure 25).^[4] Each carbon atom of this fullerene corresponds to a vertex of the polyhedron. As a result, C_{60} possesses 120 asymmetric units and is held together by 90 covalent bonds, the number of edges of the solid.^[9]

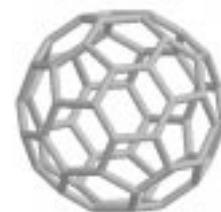


Figure 25. X-ray crystal structure of buckminsterfullerene, C_{60} , a shell based upon the truncated icosahedron.

3.7. Archimedean Duals

The polyhedra described thus far comprise two finite and two infinite families in which each solid consists of identical vertices, edges, and either a single or two or more regular polygons. It is of interest to note, however, that there exists a family of solids that are made up of irregular polygons and which may also be used as models for spheroid design. Known as Archimedean duals,^[9] and sometimes referred to as the Catalan solids, these polyhedra are constructed by simply connecting the midpoints of the faces of an Archimedean solid together. Such a treatment gives rise to 13 polyhedra that possess two or more different vertices and identical irregular polygon faces (Figure 26). As a result, chemical subunits used to construct hosts that conform to these polyhedra cannot be based upon regular polygons.

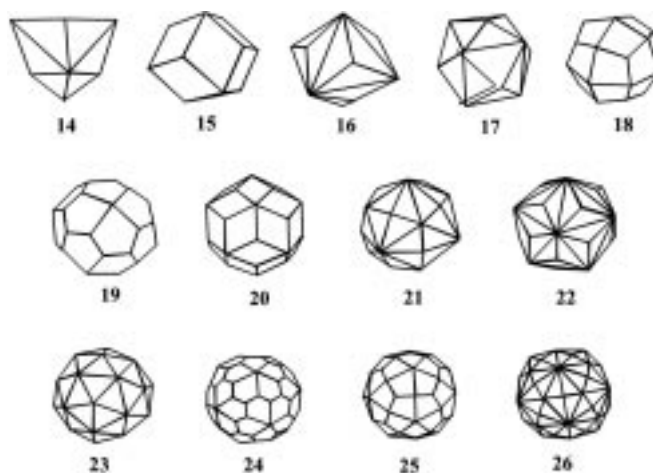


Figure 26. The 13 Archimedean duals derived from corresponding Archimedean solids (see Figure 6). Triakis tetrahedron (14), rhombic dodecahedron (15), triakis octahedron (16), tetrakis hexahedron (17), deltoidal icositetrahedron (18), pentagonal icositetrahedron (19), rhombic tricontahedron (20), disdyakis dodecahedron (21), triakis icosahedron (22), pentakis dodecahedron (23), deltoidal hexecontahedron (24), pentagonal hexecontahedron (25), disdyakis triacontahedron (26).

3.7.1. Rhombic Dodecahedron (15)

To the best of our knowledge, there is one host that conforms to the structure of an Archimedean dual. Harrison et al. have pointed out that the quaternary structure of ferritin, a major iron storage protein in animals, bacteria, and plants, corresponds to the structure of a rhombic dodecahedron.^[58] This protein, which is approximately 125 in diameter, consists of 24 identical polypeptide subunits (Figure 27 a), and

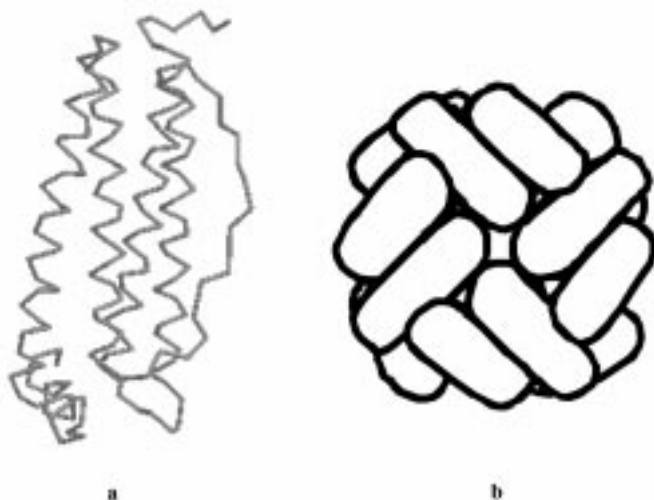


Figure 27. X-ray crystal structure of ferritin, a spherical host based upon the rhombic dodecahedron, a) carbon trace of the polypeptide subunit, b) the assembly displayed by the subunits. Blue ovals represent single subunits.

holds up to 4500 iron atoms in the form of hydrated ferric oxide with varying amounts of phosphate $[\text{Fe}_2\text{O}_3(\text{H}_2\text{O}/\text{H}_3\text{PO}_4)_n]$.^[59] The subunits, which consist of four helix bundles, assemble by way of noncovalent forces and form dimers that correspond to the faces of the solid (Figure 27b).

3.8. Irregular Polygons

It is also important to point out that if “partial” truncation is applied to the Platonic solids such that Archimedean solids are not realized, or if truncation is applied to the Archimedean solids, then the resulting polyhedra will not possess regular faces but, like the Archimedean duals, may be used as models for spheroid design as a consequence of their cubic symmetries. Indeed, it is striking to note that of the spherical molecular hosts designed to date, all have been constructed using chemical subunits that either correspond to regular polygons (for example, calix[4]arenes, VO_5 pyramids, tridentate bridging ligands) or form regular polygons (for example, carbon based hexagons and pentagons). Moreover, the realization that spherical shells may be constructed from polyhedra with irregular faces, as in the case of ferritin, implies that spherical shells based upon irregular polygons may be rationally designed.

3.9. Lower Symmetries

We have demonstrated that there exists three classes of convex uniform polyhedra, the Platonic solids, Archimedean solids, and prisms and antiprisms, along with two shells—the rugby ball and tennis ball shell—and the Archimedean duals, the frameworks of which may be used as models for the design of spherical and pseudo-spherical molecular hosts. Each host described here has been shown to conform to one of these figures, the lowest symmetry system belonging to the point group D_{2d} . It is, however, possible to construct shells that possess symmetry lower than D_{2d} . In some instances, such hosts can exhibit unique forms of stereoisomerism, which we will address.

3.9.1. Shells with D_n , S_{2n} , or C_{nh} Symmetry

Of the remaining eight point groups there are three that possess a principal C_n axis and either a C_2 axis or a mirror plane perpendicular to the C_n axis: D_n , S_{2n} , and C_{nh} .^[60] If a shell belongs to one of these point groups, it can be demonstrated that the shell is based upon either a convex uniform polyhedron, the rugby ball or tennis ball shell, or an Archimedean dual. Such a host would not possess all the symmetry elements of one of these figures but would possess points along its surface that correspond to points along the surface of the solid or shell.

Three examples from the literature demonstrate these principles. The first involves the D_{4d} calix[4]arene dimer of Shimizu and Rebek (Figure 16a).^[34] As pointed out previously, this capsule possesses D_{4d} symmetry only if the hydrogen bonds that hold the shell together are ignored. Close inspection of this host with the hydrogen bonds intact, however, reveals that the shell possesses S_8 symmetry as a result of the unidirectionality of the urea hydrogen bond network.^[34] Since the north and south poles of this assembly are identical, this host is based upon a square antiprism. The second involves Collet's cryptophanes.^[61] It is well known that the synthesis of cryptophanes yields two geometrical isomers, *anti* and *syn*, which describe the relationship of the R substituents with respect to the bridges (Figure 28).^[61] Although the *anti* and *syn* forms belong to different point

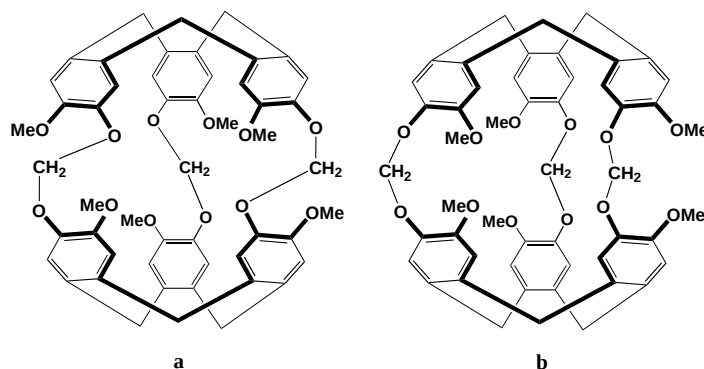


Figure 28. Collet's cryptophanes, shells based upon the trigonal prism, a) *anti* isomer (D_3 symmetry), b) *syn* isomer (C_{3h} symmetry).

groups, D_3 and C_{3h} , respectively, both isomers are based upon a trigonal prism. The third involves the calix[4]resorcinarene spheroid.^[8] Symmetry considerations reveal that the highest symmetry representation of the assembly, with its hydrogen bonds intact, is D_2 .^[8] Nevertheless, the positions of the calixarenes and water molecules of this conformer correspond to points along the surface of a snub cube.

3.9.2. Shells with C_n , C_{nv} , C_s , C_i , or C_1 Symmetry

The five point groups that remain, C_n , C_{nv} , C_s , C_i , and C_1 , cannot provide combinations of symmetry operations to produce a prism or antiprism.^[60] As a result, shell-like hosts that belong to these point groups are neither spherical nor pseudo-spherical but can exhibit unique forms of stereoisomerism as a consequence of their non-uniform interiors and exteriors.

An example involves a carcerand designed by Reinhoudt et al. (Figure 29).^[62] This molecule, which is constructed from two different calix[4]arenes, belongs to the point group C_{4v} and, as a result, forms carceplexes in which the guest can

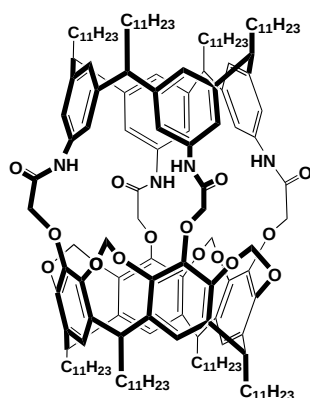


Figure 29. A C_{4v} carcerand (Reinhoudt et al.)

adopt two different orientations within its cavity and hence correspond to two different isomeric states.^[62] Rebek et al. have also described the synthesis of a chiral molecular capsule with C_2 symmetry.^[63] Specifically, the host was designed such that the ends of its subunits are different and, as Rebek et al. have shown,^[63] form hitherto unknown diastomeric host–guest complexes with chiral guests. A number of higher fullerenes also belong to such point groups (for example, C_{2v} – C_{78}).^[4] As expected, in contrast to C_{60} , these cages possess increasing numbers of nonequivalent carbon atoms, as demonstrated by their ^{13}C NMR spectra.^[4]

4. Synthesis

A symmetry based approach for constructing molecular architecture does not concern itself with the nature of the atoms that comprise the framework since it is the symmetry of the structure that ultimately dictates its design (see above).

Selecting appropriate chemical subunits and devising synthetic schemes, however, remains a job of the chemist.

In such a context, further analysis of the data reveals that the syntheses of spherical and pseudo-spherical hosts may be divided into two categories: 1) spontaneous formation and 2) multistep design (Table 5). In the case of the former, hosts are constructed in a “single step” by simply adding the subunits together in an appropriate medium and allowing the

Table 5. Shell-like hosts—synthetic pathways and symmetries.

Spontaneous Formation—host (point group)	Multi-step design
buckminsterfullerene (I_h), spherical viruses (I)	DNA-based polyhedra (O)
ferritin (O), calix[4]resorcinarene spheroid (O)	spherands (T_d)
polyoxometallates (O_h , T_d , D)	cryptands, carcerands, cryptophanes (D_{nh})
metal-organic cages (T_d , T , D)	
molecular capsules (D)	

assembly process to occur. Examples include polyoxometallates, molecular capsules, metal–organic cages, fullerenes, the calix[4]resorcinarene spheroid, ferritin, and spherical viruses. In each case, identical amounts of information are present in identical components and the final structure possesses properties not found in the individual subunits.^[34] In the case of the second approach, a multistep procedure is employed in which many synthetic steps involve dissimilar components. Examples include cryptands, cryptophanes, carcerands, spherands, and DNA-based polyhedra. The subunits of these systems are typically held together by covalent bonds.

5. Self-Assembly

From the onset of our discussion we presented a notion that self-assembly is the building of molecular architecture with identical subunits. If this is the case, then each host described here has been designed by way of self-assembly as a consequence of symmetry requirements imposed by space. Cryptands and carcerands would be examples of self-assembly since identical subunits are used to design their frameworks. It seems to us, however, that the term self-assembly requires the resulting molecular framework to form in a single step.^[11] Indeed, such a view would only encompass those shell-like hosts that form spontaneously rather than by way of multistep design. As a result, we believe that self-assembly, in relation to shell-like hosts, may be described as the single-step construction of molecular architecture from identical chemical subunits such that the final structure is ordered, and conforms to principles of geometry. Notably, such a description, in principle, also includes infinite frameworks, such as the assembly of atoms and molecules into crystals, and, as a result of structural organization, requires directional forces (for example, covalent, noncovalent, coordinate covalent bonds) to effect the assembly process.

6. Why the Platonic and Archimedean Solids?

With current interests in chemical synthesis moving towards the fabrication of nanometre scale molecular frameworks^[64–67]

and the miniaturization of functional microstructures,^[68] it becomes apparent why the Platonic and Archimedean solids are appropriate models for shell design.

First, for a given chemical subunit, these solids inherently give rise to larger structures.^[69] This may be illustrated by comparing four molecules that adopt the structures of three Platonic solids and an Archimedean solid: tetrahedrane,^[70] cubane,^[71] dodecahedrane,^[72] and C_{60} .^[4] In each molecule a carbon atom is located at the vertex of a polyhedron. Upon traversing the series, however, one observes a gradual increase in the size of these molecules. In fact, the increase is such that C_{60} is capable of entrapping a guest.^[4]

Second, we note that these solids incorporate economy of design. This may be illustrated upon considering a sphere (guest) inscribed within either a tetrahedron (T_d), cube (O_h), or icosahedron (I_h) (host). One realizes upon comparing these hosts that the icosahedron facilitates encapsulation of the guest with the largest number of subunits (Table 4). Moreover, from a chemical perspective, the icosahedron allows the host to complex the guest with the smallest chemical subunits. Indeed, this feature could eliminate errors in subunit design and shell formation and, in principle, reduce certain "costs" of a given framework.

Thus, the Platonic and Archimedean solids not only provide a means for host design, but a way in which to maximize chemical information, and allows the chemist to, in effect, engineer host–guest systems.

7. Summary and Outlook

We have presented general principles for the design of spherical molecular hosts. The principles rely on the use of convex polyhedra as models for spheroid design. To demonstrate the utility of this approach, we have structurally classified a variety of organic, inorganic, and biological hosts as frameworks that can be rationally compared on the basis of symmetry, which reveals an interplay between symmetry, structure, and function.^[73] Indeed, we anticipate these models to be amenable to the construction of hosts that conform to those solids not yet realized and to additional members of each family, where self-assembly will play a major role in their design, and usher in an era of spherical host–guest chemistry.^[8, 74]

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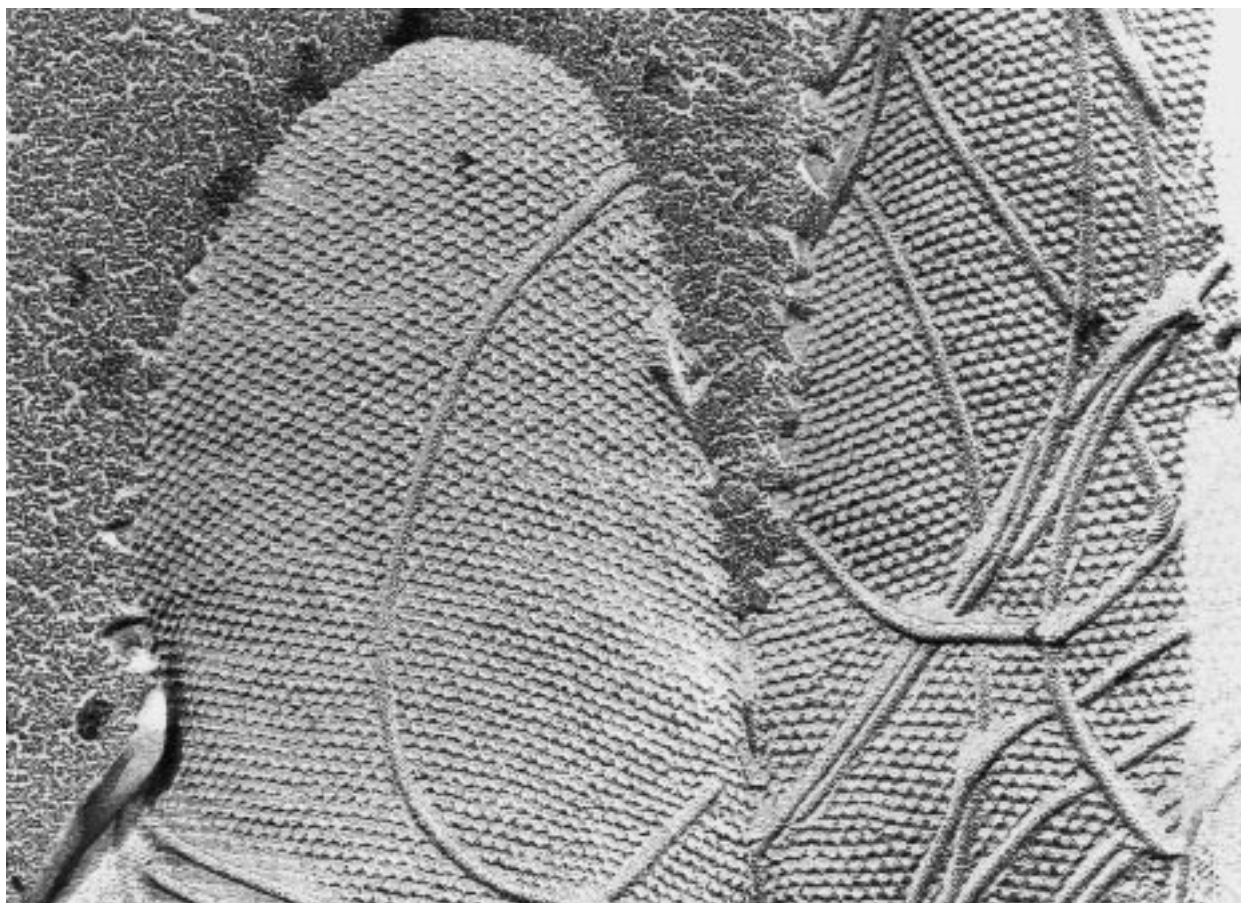
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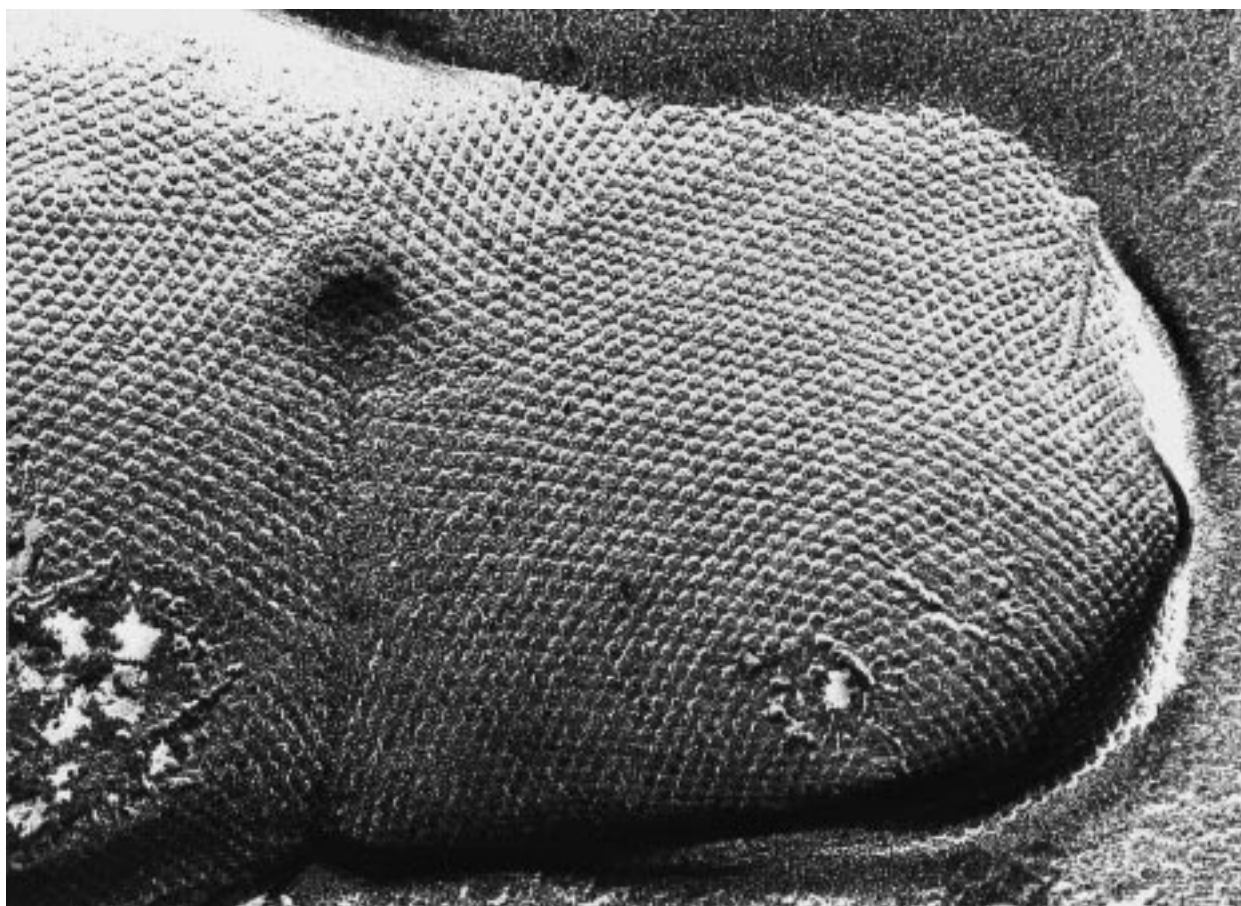
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Electron micrographs of two S layer lattices on the surface of bacteria cells.
 Top: *Thermoanaerobacter thermohydrosulfuricus* L111-69 (magnification 241 000 times);
 bottom: *Methanocorpusculum sinense* DSM 4274 (magnification 343 000 times)



Crystalline Bacterial Cell Surface Layers (S Layers): From Supramolecular Cell Structure to Biomimetics and Nanotechnology

Uwe B. Sleytr,* Paul Messner, Dietmar Pum, and Margit Sára

One of the most remarkable features of many prokaryotic organisms (archaea and bacteria) is the presence of a regularly ordered surface layer (S layer) as the outermost component of the cell envelope. Surface layers are composed of a single protein or glycoprotein species and represent the simplest biological membrane developed during evolution. Owing to the fact that S layers possess a high degree of structural regularity they represent unique

systems for studying the structure, synthesis, genetics, the dynamic process of assembly, and the function of a supramolecular structure. Isolated S layer subunits of numerous organisms are able to assemble into monomolecular arrays either in suspension, at liquid–surface interfaces, on lipid films, on liposomes, and on solid supports (for example, silicon wafers). Pores in S layers are of regular size and morphology, and functional groups on the

protein lattice are aligned in well-defined positions and orientations. These unique features of S layers have led to a broad spectrum of applications in biotechnology, diagnostics, vaccine development, biomimetics, molecular nanotechnology, and controlled biomineralization.

Keywords: biomimetics • immobilization • membranes • nanotechnology • S layers

1. Introduction

It is now generally accepted that in the course of evolution life on this planet has divided into three domains, bacteria, archaea, and eucarya.^[1, 2] Both bacteria (formerly eubacteria) and archaea (formerly archaebacteria) are prokaryotes, resembling extremely small unicellular life forms. With the exception of those prokaryotes which dwell as naked protoplasts in very specialized ecological niches, most bacteria and archaea possess a supramolecular layered cell-wall structure outside the cytoplasmic membrane. The cell wall and the cytoplasmic membrane constitute the prokaryotic cell envelope complex which regulates the molecular exchange between the cell and its environment. In most organisms the cell wall has to be stable enough to withstand the turgor pressure of the protoplast and is also involved in the determination of cell shape. Since most prokaryotic cells have to survive in highly competitive habitats the diversity observed in the molecular architecture of cell walls, particularly the structure

of the outermost boundary layer, reflects specific adaptations to environmental and ecological conditions.^[3]

One of the most commonly observed surface structures on archaea and bacteria are crystalline arrays of proteinaceous subunits, termed surface layers (S layers).^[4, 5] Structural, chemical, genetic, and morphogenetic studies revealed that S layers represent the simplest type of biological membrane developed during evolution.^[6–8] They are generally composed of a single protein or glycoprotein species endowed with the ability to assemble into closed, highly porous lattices on the cell surface during all stages of cell growth and division.

The wealth of information accumulated on the general principles of S layers led to a broad spectrum of applications.^[9–11] The most relevant features exploited in applied S layer research are:

- 1) Isolated S layer subunits from many organisms are capable of recrystallizing as closed monolayers onto solid supports, at the air/water interface, on Langmuir lipid films, and on liposomes.
- 2) Pores passing through S layers show identical size and morphology and are in the range of pores in ultrafiltration membranes.
- 3) Functional groups on the surface and in the pores are aligned in well-defined positions and orientations and are accessible for binding functional molecules in a very precise fashion.

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These repetitive physicochemical properties down to the subnanometer scale make S layer lattices unique structures for functionalizing surfaces and interfaces close to the ultimate resolution limit. The remarkable supramolecular principles of S layers are presently exploited in the fields of biotechnology, biomimetics, biomedicine, and molecular nanotechnology.

2. Occurrence and Ultrastructure

Despite considerable variations in the supramolecular structure and complexity of prokaryotic cell envelopes, most envelope profiles can be classified into three main groups, which also reflect the phylogenetic position of the organism (Figure 1). Surface layers have now been identified on organisms of nearly every taxonomic group of walled bacteria

and appear to be an almost universal feature of archaea. Unlike most other prokaryotic cell surface structures, S layers can be unequivocally identified only by electron microscopy, in particular by freeze-etching (Figure 2). In both bacteria and archaea the regular arrays completely cover the cells at all stages of cell growth and division.^[4, 12–15]

The three-dimensional spatial organization of S layers has been obtained primarily by electron crystallography and more recently through scanning force microscopy. Mass distribution analysis by computer image enhancement procedures revealed structural information down to a range of 0.5 to 1.5 nm.^[14, 16–19] Surface layers are planar assemblies of identical protein or glycoprotein subunits which can be aligned in lattices with oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetry with center-to-center spacings of the morphological units of approximately 2.5–35 nm (Figure 3). Hexagonal lattice symmetry is predominant among archaea.^[20] Surface layers of bacteria are generally 5–20 nm thick and

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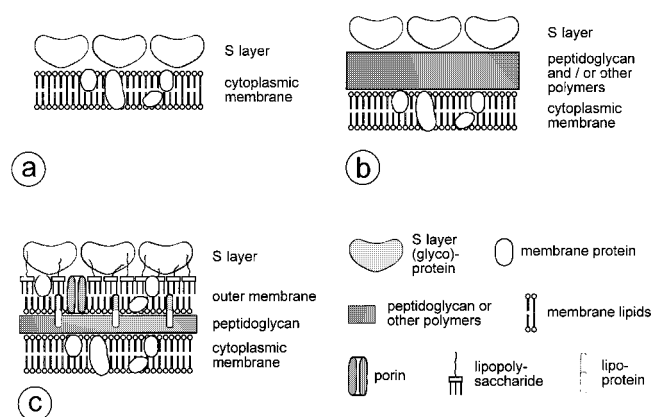


Figure 1. Schematic illustration of the supramolecular architecture of the three major classes of prokaryotic cell envelopes containing crystalline bacterial cell surface layers (S layers). a) Cell envelope structure of Gram-negative archaea with S layers as the only cell-wall component external to the cytoplasmic membrane. b) Cell envelope as observed in Gram-positive archaea and bacteria. In bacteria the rigid wall component is primarily composed of peptidoglycan. In archaea other wall polymers (e.g. pseudomurein or methanochondroitin) are found. c) Cell envelope profile of Gram-negative bacteria composed of a thin peptidoglycan layer and an outer membrane. If present the S layer is closely associated with the lipopolysaccharide of the outer membrane (modified based on reference [13]).

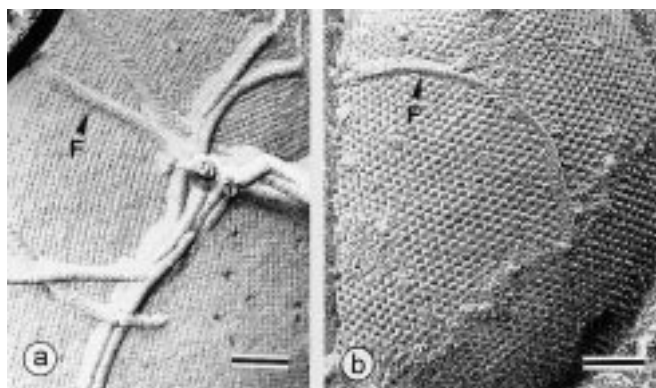


Figure 2. Electron micrograph of freeze-etched preparations of intact cells from a) *Aneurinibacillus thermoaerophilus* DSM 10155 showing a square (p4) S layer lattice and b) archaeon *Methanomicrobium mobile* DSM 1539 covered with a hexagonal (p6) array. F: flagella. The bar corresponds to 100 nm.

reveal a rather smooth outer and a more corrugated inner surface. Amongst archaeal S layers pillarlike domains associated with the plasma membrane have been described.^[17, 21–23] These characteristic structural features may lead to S layer lattices with their heavy domains located at a distance of up to 70 nm from the surface of the cytoplasmic membrane.

High-resolution electron microscopical and scanning force microscopy studies have shown that S layers are very porous membranes with pores occupying up to 70 % of their surface area (Figure 4). Since S layers are assemblies of identical subunits they exhibit pores of identical size and morphology. In many protein lattices two or more distinct classes of pores in the range of approximately 2–8 nm have been identified.^[13–15, 18, 22]

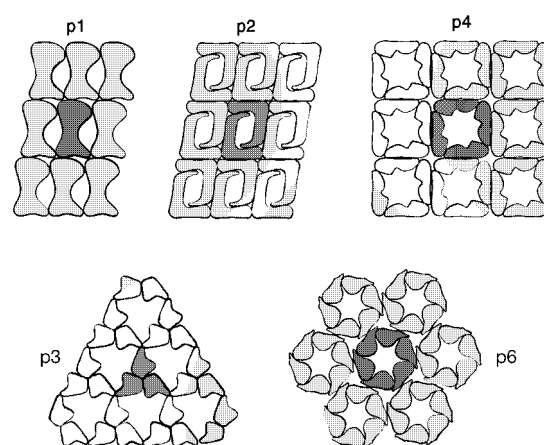


Figure 3. Schematic drawing of different S layer lattice types. The regular arrays exhibit either oblique (p1, p2), square (p4), or hexagonal lattice symmetry (p3, p6). The morphological units are composed of one, two, three, four, or six identical subunits.

3. Assembly and Morphogenesis

Surface layers represent a fascinating model system for studying the dynamic process of assembly of a supramolecular structure during cell growth. An intact closed S layer on an average-sized, rod-shaped prokaryotic cell consists of approximately 5×10^5 monomers. Thus, for maintaining a complete S layer on the surface of a cell growing with a generation time of 20 to 30 min at least 500 copies of a single polypeptide species have to be synthesized, translocated to the cell surface, and incorporated into the existing lattice per second. Detailed electron microscopical examinations of S layers on growing cells and in vitro self-assembly studies of isolated S layer subunits have been performed to elucidate the dynamic process of assembly in the course of S layer morphogenesis.

3.1. Self-Assembly In Vitro

A great variety of methods has been developed for the detachment of S layers and their disintegration into protomeric units. Generally a complete disintegration of S layer lattices can be achieved using high concentrations of chaotropic agents (for example, guanidine hydrochloride, urea), or by lowering or raising the pH value. Particularly S layers from Gram-negative bacteria may also be disintegrated by applying metal-chelating agents (for example, ethylenediaminetetraacetic acid (EDTA), ethylenbis(oxyethylenitrilo)tetraacetic acid (EGTA)) or cation substitution.^[4, 12–14, 24–28] These data have confirmed that S layers are held together and onto the supporting envelope component by noncovalent forces, including hydrophobic interactions and hydrogen bonds as well as ionic bonds involving divalent cations or direct interactions of polar groups. The extraction experiments have also shown that the bonds holding the subunits together are stronger than those involved in the association of the S layers to the underlying layer.

Surface layers from some archaea are very resistant to extraction and disintegration, suggesting even covalent

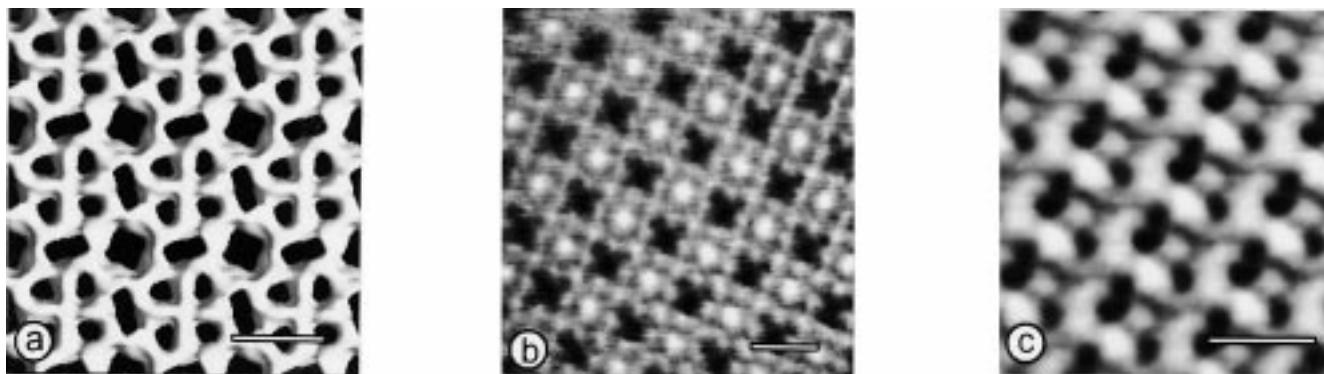


Figure 4. a) Three-dimensional model of the protein mass distribution of the S layer of *Bacillus stearothermophilus* NRS 2004/3a/V2 (outer face). The S layer is about 8 nm thick and exhibits a center-to-center spacing of the morphological units of 13.5 nm. The protein meshwork shows one square-shaped, two elongated, and four small pores per morphological unit. The model was obtained after recording several tilt series of a negatively stained preparation in a transmission electron microscope, performing Fourier-domain computer image reconstructions over each individual tilted view, and combining all processed views to a three-dimensional data volume. b), c) Computer image reconstruction of the scanning force microscopic images of the topography of the inner face of the S layer lattice from *B. sphaericus* CCM 2177 (b) and *Bacillus coagulans* E38–66/V1 (c). The images were taken under water. The surface corrugation corresponding to a gray scale from black to white is 1.8 nm. The center-to-center spacing of the morphological unit of the square lattice (b) is 14.5 nm. The S layer lattice in (c) shows an oblique unit cell ($a = 9.4$ nm, $b = 7.5$ nm, base angle = 80°). The bars correspond to 10 nm.

intersubunit bonds.^[14, 29a] Isolated S layer subunits from many prokaryotic organisms have shown the ability to assemble into regular lattices identical to those observed on intact cells upon removal of the disrupting agent used for their isolation (for example, upon dialysis).^[29b] These S layer self-assembly processes may lead to the formation of flat sheets, open-ended cylinders, or spheres (Figure 5).^[12, 15, 27, 30] Ionic strength,

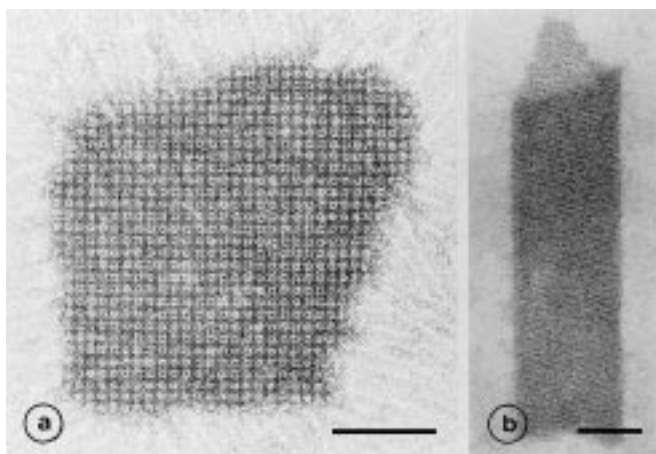


Figure 5. Electron micrographs of negatively stained preparations of a) sheet-like S layer self-assembly products of *Desulfotomaculum nigrificans* NCIB 8706 showing a p4 lattice and b) cylindrical S layer self-assembly products of *Bacillus stearothermophilus* NRS 2004/3a showing a p2 lattice. The bars correspond to 100 nm.

temperature, protein concentration, and polymers associated with S layers can determine both the rate and extent of association. With S layers from Bacillaceae it was shown that the assembly kinetics are multiphasic, with a rapid initial phase leading to oligomeric precursors and slow consecutive rearrangement steps, which finally lead to extended lattices.^[31] The capability of some S layer proteins to reassemble into large coherent monomolecular arrays on solid supports, at the air/water interface, and on Langmuir lipid films (Figure 6) has led to numerous applications (see Section 7).

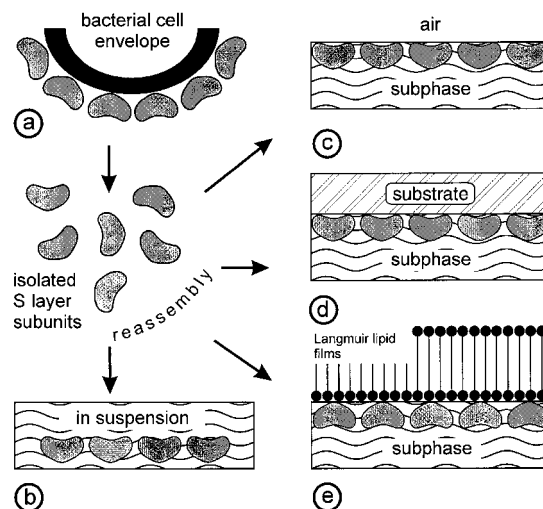


Figure 6. Schematic illustration of the recrystallization of isolated S layer subunits into crystalline arrays (a). The self-assembly process can occur in suspension (b), at the air/water interface (c), on solid supports (d), and on Langmuir lipid films (e).

3.2. Self-Assembly In Vivo

With S layers from various bacteria it was demonstrated that distinct surface properties of the subunits (charge distribution, hydrophobicity, specific interactions with components of the supporting envelope layer) are essential for the proper orientation of the S layer subunits during local insertion in the course of lattice growth. Labeling experiments (for example, with colloidal gold or fluorescent markers) provided information about the extension of the S layer lattices and incorporation sites of subunits during cell growth. In Gram-positive and Gram-negative bacteria S layer lattice growth is dependent on the extension of the supporting rigid envelope component (Figure 1) and may occur in defined domains^[32, 33] or at random sites.^[34, 35]

In high-resolution freeze-etching preparations dislocations and disclinations have been identified (Figure 7). Topologi-

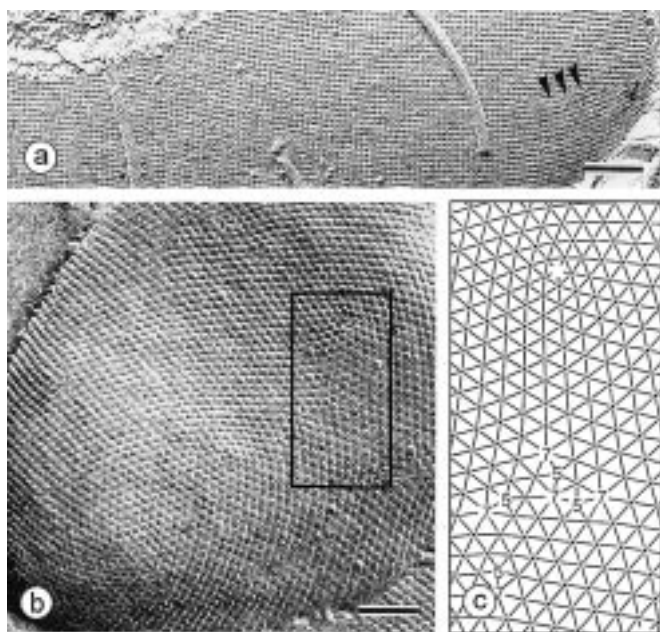


Figure 7. Freeze-etched preparations of intact bacteria showing common lattice faults of S layers. a) The arrows indicate dislocations in the square lattice on the rounded surface of the cell pole of *Aneurinibacillus thermoaerophilus* DSM 10155. b) Wedge disclinations and edge dislocations are seen as point imperfections in the crystalline array of *Methanococcus sinense* DSM 4274. c) Representation of the neighboring S layer units from the boxed region in (b). The central unit cells of the $+60^\circ$ and -60° wedge disclinations are pentagons (labeled 5) and heptagons (labeled 7), respectively. The representation is modified based on reference [39]. The bars correspond to 100 nm.

cally, such lattice faults are required for covering the curved surface of the hemispherical cell poles of rod-shaped cells or the spherical surface of a coccus. On the other hand, as theoretically predicted by Harris and co-workers,^[36–38] dislocations and disclinations can serve as preferential sites for incorporation of new subunits in crystalline arrays which grow by “intussusception”.^[36] Further, as a geometrical necessity, closed surface crystals must contain local wedge (screw) disclinations which themselves can act as source of edge disclinations.^[38]

Analysis of the distribution of lattice faults in the hexagonal S layer of archaea which possess an S layer as its sole cell-wall constituent (Figure 1a) provided strong evidence that complementary pairs of pentagons and heptagons play an important role as sites for the incorporation of new subunits in the formation and maintenance of the lobed cell structure and in the fission process.^[27, 39] The latter was suggested to be determined by the ratio between the increase in protoplast volume and the increase in actual S layer surface area during cell growth. In rod-shaped archaea with S layers as exclusive cell-wall component, wedge disclinations at both hemispherical poles resembling the theoretical required numbers could be visualized. Elongation of the cylindrical part of the cell most probably involves incorporation of S layer subunits at sites of sliding dislocations at the poles.^[40, 41]

It is now evident that S layers are “dynamic closed surface crystals” with the intrinsic capability to assume continuously a structure of low free energy during cell growth. Further, the morphogenetic potential of S layer lattices is determined by the molecular structure of its constituent subunits.^[4, 12, 14, 27, 42]

4. Chemical Composition and Biosynthesis

Chemical characterization of highly purified S layers from bacteria as well as archaea revealed that most of these cell surface macromolecules possess a rather similar overall composition, regardless of the phylogenetic origin of the respective bacteria. Extensive reviews of the relevant work have been published recently.^[26, 43–53] Additionally, S layers of various nonpathogenic^[45–48] and pathogenic^[49–51] bacteria and archaea^[52, 53] have been characterized biochemically since then. Typically they are composed of 40 to 60% of hydrophobic amino acids and possess a rather low portion, if any, of sulfur-containing amino acids. From circular dichroism measurements and protein sequence data secondary structure predictions have been performed (Table 1). In general, S layers are weakly acidic proteins or glycoproteins with isoelectric points in the range of 4–6.^[5, 7, 26, 43, 44, 54, 55] For *Methanothermobacter fervidus*, however, a pI value for the S layer protein of 8.4^[56] and for lactobacilli pI values in the range of 9–10 have been determined.^[57]

Table 1. Properties of S layers.^[5, 7, 14, 26, 43, 44, 54, 55]

The S layer lattices can have oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetry.
The center-to-center spacing of the morphological unit can be between 3 and 35 nm.
The lattices are generally 5 to 20 nm thick (in archaea up to approximately 70 nm).
The outer surface is generally less corrugated than the inner surface.
The S layer lattices exhibit pores of identical size and morphology.
In many S layers two or even more distinct classes of pores are present.
The pore sizes range from approximately 2 to 8 nm.
The pores can occupy 30 to 70% of the surface area.
The relative molecular mass of constituent subunits is in the range of 40000 to 200000.
These are weakly acidic proteins (pI $\approx$ 4–6), except for <i>Methanothermobacter fervidus</i> (pI = 8.4) and lactobacilli (pI > 9.5).
Large amounts of glutamic and aspartic acid (about 15 mol%) are present.
There is a high lysine content (about 10 mol%).
There are large amounts of hydrophobic amino acids (about 40–60 mol%).
Hydrophilic and hydrophobic amino acids do not form extended clusters.
In most S layer proteins about 20% of the amino acids are organized as α helices and about 40% occur as β sheets.
Aperiodic foldings and β -turn content may vary between 5 and 45%.
Posttranslational modifications of S layer proteins include cleavage of N- or C-terminal fragments, glycosylation, and phosphorylation of amino acid residues.

General protein secretion pathways are known in a number of bacteria. For secretion of S layers a specific pathway has been described for the first time in *Aeromonas salmonicida*.^[58] A few posttranslational modifications are known to occur in S layer proteins, including protein phosphorylation and protein glycosylation (Table 1). The modification of the S layer of *Aeromonas hydrophila* (termed A layer) has been detected by a comparison of the molecular masses obtained from SDS PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) and the DNA sequence. The molecular mass of the A layer (45 000 Da) was increased by 7000 Da. Western blot analysis using a monoclonal antibody showed the presence of phosphotyrosine residues.^[59]

A more frequently observed modification of S layer proteins is their glycosylation. Many archaea—including halophilic, methanogenic, thermophilic, acidophilic, and alkalophilic strains—possess S layer proteins with covalently linked glycan chains.^[43, 60, 61] The hyperthermostable S layer glycoprotein complex of *Staphylothermus marinus*^[62] and the S layer glycoprotein of *Haloarcula japonica*^[63] were only recently characterized. In the domain bacteria, however, glycosylated S layers have been demonstrated unambiguously only in the Bacillaceae family.^[43, 64, 65]

The purification regimes are identical for nonglycosylated and glycosylated S layers and have been summarized in recent reviews.^[26, 43, 66, 67] In the case of glycosylated S layers, usually complete degradation to S layer glycopeptides by proteolytic enzymes is required for further analysis. Determination of the S layer glycan structure includes chemical degradation experiments of the glycan chains, ¹H and ¹³C NMR spectroscopy, and mass spectrometry.^[43, 68] Glycan structures of selected strains of bacteria and archaea are summarized in Table 2. From the data available the following conclusions can be drawn: Most bacterial S layer glycan chains are composed of 20 to 50 identical repeating units made of neutral hexoses and rarely pentoses, 6-deoxy hexoses, and amino sugars. In terms of structure they are comparable to lipopolysaccharide (LPS) O-antigens of Gram-negative bacteria (Figure 8).^[86]

Between the O-antigen-like glycan chain and the S layer polypeptide there is usually a core oligosaccharide of two to four sugar residues which connects the proximal portions of this macromolecule. Per S layer protein monomer two to six glycosylation sites have been determined,^[68, 73a, 77] where S layer glycans are attached predominantly by O-glycosidic linkages such as galactose → tyrosine,^[68] glucose → tyrosine,^[77] or N-acetylgalactosamine → threonine/serine.^[73a] The reexamination^[87] of an S layer glycoprotein preparation from *Bacillus stearothermophilus* of NRS 2004/3a^[88a] has demonstrated that in this S layer there is only one type of glycan with a certain variation in chain length. A concomitantly isolated oligosaccharide^[88a] is not covalently linked to the S layer polypeptide,^[87] and therefore has to be considered as an associated secondary cell-wall polymer like in other bacilli.^[88b]

Those archaeal S layer glycoproteins whose glycan structures and linkage regions have been investigated (Table 2) consist almost exclusively of short heterosaccharides with up to ten sugar residues and no repeating units.^[43, 61, 85] In halobacteria up to three different glycan species with up to

25 glycosylation sites per S layer monomer can be present.^[61, 84] The dominating linkage types are N-glycosidic linkages such as glucose → asparagine^[61] or N-acetylgalactosamine → asparagine.^[61, 85] However, short O-glycans, which occur in clusters, have also been found.

The remarkable diversity in glycan structures raised interesting questions about the biosynthesis of prokaryotic glycoproteins. Recently Sumper and Wieland have proposed a model for how the different N-linked glycans are synthesized in *Halobacterium halobium*.^[61] It includes transfer of dolichol-linked saccharides to consensus sequences for N-glycosylation on the S layer polypeptide. Similar lipid-activated oligosaccharides with short-chain (C₅₅–C₆₀) dolichol species rather than undecaprenol for S layer glycosylation were also observed in *Haloferax volcanii*^[89] and *M. fervidus*.^[90] In comparison to archaeal S layer glycoproteins, less detailed information is available about the biosynthesis of glycosylated S layers of bacteria. In *Paenibacillus* (formerly *Bacillus*) *alvei* similar dolichol (C₅₅) carrier lipids are involved in glycan biosynthesis,^[91] whereas for *Thermoanaerobacterium* (formerly *Clostridium*) *thermosaccharolyticum* only nucleotide-activated sugars have been characterized so far.^[92] Of particular interest is the observation that in all investigated systems beside nucleotide-activated monosaccharides also nucleotide-activated oligosaccharides play an important role in synthesis.^[90–92] Such activated metabolic intermediates are not known for eukaryotic glycoproteins. The biological relevance of this characteristic difference is currently being investigated.

Elucidation of biosynthetic pathways for prokaryotic glycoprotein glycans should enable the production of tailored S layer glycoproteins as required for different biotechnological applications (for example, S layer vaccines; see Section 7.1.3).

5. Molecular Biology and Genetics

Although it is quite evident that common structural principles must exist in S layer proteins (for example, the ability to form intersubunit bonds and to self-assemble, the formation of hydrophilic pores with low unspecific adsorption, the interaction with the underlying cell envelope layer), sequencing of S layer genes from organisms of all phylogenetic branches led to the conclusion that sequence identities are rare.^[93] Sequencing of S layer genes from closely related organisms (Table 3)^[94–127] revealed that sequence identities depend on evolutionary relationship. For example, the nucleotide sequence of the S layer gene *slpA* from *Lactobacillus acidophilus* ATCC 4356^[115] showed 80% similarity to that of the S layer gene from the closely related *L. helveticus*,^[118] but almost no similarity to that from *L. brevis*.^[116]

For some species the N-terminal part of the S layer proteins was found to represent the conserved structural element which is responsible for anchoring the S layer subunits to the underlying cell envelope layer. The S layer proteins SbsA^[103] and SbsC^[105] from two *B. stearothermophilus* wild-type strains (PV72/p6 and ATCC 12980) showed high identity for the 270 N-terminal amino acids, but only low identity (< 25%) for the

Table 2. Glycan structures of selected bacterial and archaeal S layer glycoproteins.^[a][43, 61]

Bacteria	
<i>Bacillus stearothermophilus</i> NRS 2004/3a ^[69, 70] [→2)-α-L-Rhap-(1→2)-α-L-Rhap-(1→3)-β-L-Rhap-(1→) _{n≈50}	-Rhap-(1→N)-Asn
<i>Aneurinibacillus thermoaerophilus</i> L420–91 (formerly <i>Bacillus thermoaerophilus</i>) ^[71] [→3)-α-D-Rhap-(1→3)-α-D-Rhap-(1→2)-α-D-Rhap-(1→2)-α-D-Rhap-(1→) _{n≈15}	-(D-Rha) ₀₋₂ -β-D-GalNAc-(1→O)-Thr
$ \begin{array}{c} 2 \qquad \qquad 2 \\ \uparrow \qquad \qquad \uparrow \\ 1 \qquad \qquad 1 \\ \alpha\text{-D-Fucp3NAc} \quad \alpha\text{-D-Fucp3NAc} \end{array} $	
<i>Aneurinibacillus thermoaerophilus</i> (DSM 10155) (formerly <i>Bacillus thermoaerophilus</i>) ^[72, 73] [→4)-α-L-Rhap-(1→3)-β-D-glycero-D-manno-Hepp-(1→) _{n≈18}	-(L-Rha) ₀₋₂ -β-D-GalNAc-(1→O)-Thr/Ser
<i>Paenibacillus alvei</i> (CCM 2051) ^[74, 75]	
$ \begin{array}{c} \alpha\text{-D-Glcp} \qquad \qquad \alpha\text{-D-Glcp} \\ 1 \qquad \qquad \qquad 1 \\ \downarrow \qquad \qquad \downarrow \\ 6 \qquad \qquad \qquad 6 \end{array} $	
β-D-Galp-(1→4)-β-D-ManpNAc-(1→[3)-β-D-Galp-(1→4)-β-D-ManpNAc-(1→) _{n≈20} 3)- α-L-Rhap-(1→3)-α-L-Rhap-(1→3)-α-L-Rhap-(1→3)-β-D-Galp-(1→O)-Tyr	
$ \begin{array}{c} 4 \\ \uparrow \\ 1 \\ \text{GroA-(2→O)-PO}_2\text{-(O→4)-β-D-ManpNAc} \end{array} $	
<i>Thermoanaerobacter thermohydrosulfuricus</i> L111-69 and L110-69 (DSM 568) (formerly <i>Clostridium thermohydrosulfuricus</i>) ^[68, 76] 3-OMe-α-L-Rhap-(1→4)-α-D-Manp-(1→[3)-α-L-Rhap-(1→4)-α-D-Manp-(1→) _{n≈27} 3)- α-L-Rhap-(1→3)-α-L-Rhap-(1→3)-α-L-Rhap-(1→3)-β-D-Galp-(1→O)-Tyr	
<i>Thermoanaerobacter thermohydrosulfuricus</i> S102-70 (formerly <i>Clostridium thermohydrosulfuricus</i>) ^[77, 78]	
β-D-Galf-(1→3)-α-D-Galp-(1→2)-α-L-Rhap-(1→3)-α-D-Manp-(1→3)-α-L-Rhap-(1→3)-β-D-Glcp-(1→O)-Tyr	
<i>Thermoanaerobacter thermohydrosulfuricus</i> L77-66 (DSM 569) and L92-71 (formerly <i>Clostridium thermohydrosulfuricus</i>) ^[79] [→3)-α-D-GalpNAc-(1→3)-α-D-GalpNAc-(1→) _{n≈25}	O-glycosidic bond through Tyr?
$ \begin{array}{c} 4 \\ \uparrow \\ 1 \\ \alpha\text{-D-GlcpNAc-(1→2)-β-D-Manp} \end{array} $	
<i>Thermoanaerobacterium thermosaccharolyticum</i> D120-70 (formerly <i>Clostridium thermosaccharolyticum</i>) ^[80] [→3)-β-D-Manp-(1→4)-α-L-Rhap-(1→3)-α-D-Glcp-(1→4)-α-L-Rhap-(1→) _n	O-glycosidic bond through Tyr
$ \begin{array}{c} 6 \qquad \qquad \qquad 2 \\ \uparrow \qquad \qquad \qquad \uparrow \\ 1 \qquad \qquad \qquad 1 \\ \beta\text{-D-Glcp} \qquad \qquad \alpha\text{-D-Galp} \end{array} $	
<i>Thermoanaerobacterium thermosaccharolyticum</i> E207-71 (formerly <i>Clostridium thermosaccharolyticum</i>) ^[81] [→4)-β-D-Galp-(1→4)-β-D-Glcp-(1→4)-α-D-Manp1-(1→) _{n≈17}	O-glycosidic bond through Tyr
$ \begin{array}{c} 3 \\ \uparrow \\ 1 \\ \beta\text{-D-Quip3NAc-(1→6)-β-D-Galf-(1→4)-α-L-Rhap} \end{array} $	
<i>Clostridium symbiosum</i> HB25 ^[82] [→6)-α-D-ManpNAc-(1→4)-β-D-GalpNAc-(1→3)-α-D-BacpNAc-(1→4)-α-D-GalpNAc-(1→O)-PO ₂ -(O→) _{n≈15}	
<i>Lactobacillus buchneri</i> 41021/251 ^[83] α-D-Glcp-(1→[6)-α-D-Glcp-(1→) _{n=4-6} 6)-α-D-Glcp-(1→O)-Ser	

larger part of the sequences. Actually, the N-terminal part of SbsA and SbsC was found to anchor the S layer subunits through a secondary cell-wall polymer of identical chemical composition and structure to the rigid cell-wall layer,^[128] while the C-terminal part most probably leads to quite diverse cell surface properties even amongst closely related strains within the same species.

Contrary to the hypothesis that evolutionary relationship correlates with sequence identities for structurally homologous domains was the observation that the S layer protein

SbsB showed only low overall identity (< 25 %) to SbsA and that both S layer proteins do not even share an identical N-terminal region.^[104] The S layer protein SbsB is synthesized by *B. stearothermophilus* PV72/p2, an oxygen-induced strain variant from *B. stearothermophilus* PV72/p6. Interestingly, the oxygen-induced strain variant produced a different secondary cell-wall polymer which was recognized by the SbsB as binding site, indicating that a highly coordinated switch in synthesis of cell-envelope components had occurred during oxygen-induced variant formation. Sequencing of 450 bases in

Table 2. (Continued)

Archaea	
<i>Halobacterium halobium</i> R ₁ M ₁ ^[61]	
$\begin{array}{c} \text{OSO}_3^- \\ \\ \text{[}\rightarrow 4\text{]-GlcNAc-(1}\rightarrow 4\text{)-GalA-(1}\rightarrow 3\text{)-GalNAc-(1)}_{n=10-15}\text{-}\rightarrow N\text{)-Asn} \\ \qquad \qquad \\ 6 \qquad \qquad 3 \\ \qquad \qquad \\ 1 \qquad \qquad 1 \\ \text{3-O-Me-GalA} \qquad \text{Gal}^f \end{array}$	$\begin{array}{c} \text{Ala-NH}_2 \\ \\ \text{Ala} \\ \\ \text{Ser} \\ \\ \text{Asn} \\ \\ \text{X} \\ \\ \text{Thr/Ser} \end{array}$
$\begin{array}{c} \text{GlcA-(1}\rightarrow 4\text{)-GlcA-(1}\rightarrow 4\text{)-GlcA-(1}\rightarrow 4\text{)-}\beta\text{-D-Glc-(1}\rightarrow N\text{)-Asn} \\ \qquad \qquad \qquad \qquad \\ 3 \qquad \qquad 3 \qquad \qquad 3 \\ \text{OSO}_3^- \qquad \text{OSO}_3^- \qquad \text{OSO}_3^- \end{array}$	1/3 of GlcA can be replaced by IdA
$\alpha\text{-D-Glc-(1}\rightarrow 2\text{)-Gal-(1}\rightarrow O\text{)-Thr}$	
<i>Haloferax volcanii</i> DS2 ^[61, 84]	
$\begin{array}{c} \beta\text{-D-Glc-(1}\rightarrow [4]\text{-}\beta\text{-D-Glc-(1}\rightarrow]_{n=8}\text{ 4)-}\beta\text{-D-Glc-(1}\rightarrow N\text{)-Asn} \\ \\ \text{Glc-(1}\rightarrow 2\text{)-Gal-(1}\rightarrow O\text{)-Thr} \end{array}$	
<i>Methanothermobacter fervidus</i> V24S ^[85]	
$\begin{array}{c} \text{3-O-Me-}\alpha\text{-D-Manp-(1}\rightarrow 6\text{)-3-O-Me-}\alpha\text{-D-Manp-(1}\rightarrow [2]\text{-}\alpha\text{-D-Manp-(1}\rightarrow]_{n=3}\text{ 4)-D-GalNAc-(1}\rightarrow N\text{)-Asn} \end{array}$	

[a] The formulas represent either the repeating unit structure or the saccharide structure of S layer glycoprotein glycans. Additionally, as far as already determined, the structure of the carbohydrate-protein linkage region is presented. Abbreviations: Glcp: glucose (pyranose form), Gal^f: galactose (furanose form), Man: mannose, Rha: rhamnose, GlcNAc: N-acetylglucosamine, GalNAc: N-acetylgalactosamine, ManNAc: N-acetylmannosamine, GlcA: glucuronic acid, GalA: galacturonic acid, ManA: mannuronic acids, IdA: iduronic acid, 3-O-Me-GalA: 3-O-methylgalacturonic acid, BacNAc: N-acetylbacillosamine (2-N-acetyl-4-amino-2,4,6-trideoxyglucose), Fuc3NAc: 3-N-acetylfucosamine, Qui3NAc: 3-N-acetylquinovosamine (3-acetamido-3,6-dideoxyglucose), BacNAc: N-acetylbacillosamine (2-acetamido-4-amino-2,4,6-trideoxyglucose), GroA: phosphoglyceric acid, Asn: asparagine, Tyr: tyrosine, Thr: threonine, Ser: serine, Ala: alanine, X: interchangeable amino acid.

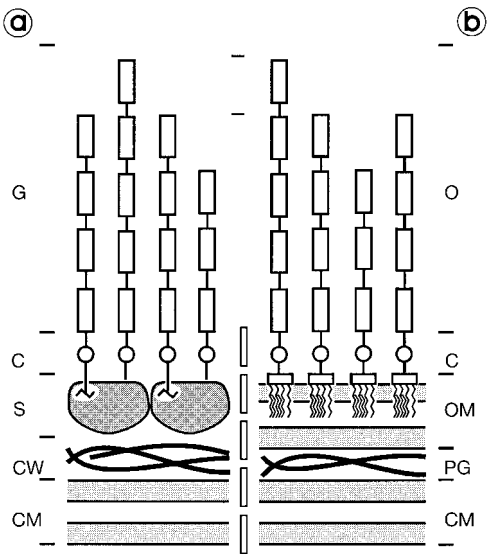


Figure 8. Schematic drawing of the cell envelope profile of Gram-positive (a) and Gram-negative bacteria (b) underlining the structural similarities between S layer glycoproteins and lipopolysaccharides. Abbreviations: C: core region; CM: cytoplasmic membrane; CW: cell wall; G: glycan chain; O: O-antigen; OM: outer membrane; PG: peptidoglycan (taken from reference [43] with permission of Landes/Academic Press).

the variable region of the 16S rDNA confirmed that the similarity values for the wild-type strain and the variant are 100%. These findings indicated that despite evolutionary

relationship, other factors such as growth conditions or environmental stress are determinative for sequence identities of structurally homologous domains of S layer proteins.

Since at a generation time of 20 minutes about 500 S layer subunits have to be produced per second to keep the bacterial cell surface completely covered with S layer protein,^[26] promoters preceeding S layer genes must be very strong.^[93] Indeed, the promoter of the S layer gene from *L. acidophilus* is two times more efficient than that of the gene encoding lactate dehydrogenase, which is considered to be one of the strongest promoters in bacteria.^[129] In *L. brevis* two promoters (P1 and P2) have been identified for S layer gene expression. The P2 promoter is located closer to the start codon and is efficiently transcribed during the exponential and early stationary growth phase.^[130] The use of three promoters has been reported for the expression of the *cwp* operon of *B. brevis*.^[99, 131] With exception of those from *Campylobacter* and *Caulobacter*, all S layer proteins are produced with a signal peptide, suggesting the classical route of secretion.^[57]

Important for understanding S layer gene regulation has been the observation that single bacterial strains can express different (silent) genes. The most detailed studies regarding S layer variation were carried out with *Campylobacter fetus* subsp. *fetus*, a pathogen that interferes with reproductive function in ungulates.^[132] In case of pathogens S layer variation can be considered as antigenic variation which is

Table 3. Survey of S layer proteins with known amino acid sequence.

Species	Strain	Gene	Number of amino acids including the N-terminal leader peptide/N-terminal leader peptide	Lattice type ^[a]	Gene bank accession number	Ref.
<i>Aeromonas hydrophila</i>	TF7	<i>ahs</i>	467/19	S	L37348	[59]
<i>Aeromonas salmonicida</i>	A 450	<i>vapA</i>	502/21	S	M64655	[94]
<i>Bacillus anthracis</i>	Sterne derivative sub-strain 9131	<i>sap</i>	814/29	O	Z36946	[95]
<i>Bacillus anthracis</i>	Sterne substrain 9131	<i>eag</i>	862/29	O	X99724	[96]
<i>Bacillus brevis</i>	47	<i>owp</i>	1004/24	–	M14238	[97]
<i>Bacillus brevis</i>	47	<i>mwp</i>	1053/23	H	M19115	[98]
<i>Bacillus brevis</i>	HPD 31	<i>HWP</i>	1087/23 or 53	H	D90050	[99]
<i>Bacillus licheniformis</i>	HM 105	–	874/29	–	U38842	[100]
<i>Bacillus sphaericus</i>	P1	sequence 8	1252/30	S	A45814	[101]
<i>Bacillus sphaericus</i>	2362	Gen 125	1176/30	S	M28361	[102]
<i>Bacillus sphaericus</i>	2362	Gen 80	745 (silent)	–	–	[102]
<i>Bacillus stearothermophilus</i>	PV72/p6	<i>sbsA</i>	1228/30	H	X71092	[103]
<i>Bacillus stearothermophilus</i>	PV72/p2	<i>sbsB</i>	920/31	O	X98095	[104]
<i>Bacillus stearothermophilus</i>	ATCC 12980	<i>sbsC</i>	1099/30	O	AF055578	[105]
<i>Bacillus thuringiensis</i>	ssp. <i>israelensis</i> 4Q2	<i>slp</i>	393/putative	–	X62090	–
<i>Campylobacter fetus</i> , ssp. <i>fetus</i>	–	<i>sapA</i>	933/No	H, S ^[b]	J05577	[106]
<i>Campylobacter fetus</i> , ssp. <i>fetus</i>	23B	<i>sapA1</i>	920/No	H, S ^[b]	L15800	[107]
<i>Campylobacter fetus</i> , ssp. <i>fetus</i>	82-40LP3	<i>sapA2</i>	1109/No	H, S ^[b]	S76860	[108]
<i>Campylobacter fetus</i> , ssp. <i>fetus</i>	84-91	<i>sapB</i>	936/No	–	U25133	[108]
<i>Campylobacter fetus</i> , ssp. <i>fetus</i>	CIP 5396T	<i>sapB2</i>	1112/No	–	AF048699	–
<i>Campylobacter rectus</i>	314	<i>crs</i>	1361/No	–	AF010143	[109]
<i>Caulobacter crescentus</i>	CB 15	<i>rsaA</i>	1026/No	H	M84760	[110]
<i>Corynebacterium glutamicum</i>	ATCC 17965	<i>csp2</i>	510/30	H	X69103	[111]
<i>Deinococcus radiodurans</i>	–	<i>HPI</i>	1036/31	H	M17895	[112]
<i>Halobacterium halobium</i>	–	<i>csg</i>	852/34	H	J02767	[113]
<i>Haloferax volcanii</i>	–	–	828/34	H	M62816	[114]
<i>Lactobacillus acidophilus</i>	ATCC 4356	<i>slpA</i>	444/24	O	X89375	[115]
<i>Lactobacillus acidophilus</i>	ATCC 4356	<i>slpB</i>	456 (silent)	–	X89376	[115]
<i>Lactobacillus brevis</i>	ATCC 8287	–	465/30	O	Z14250	[116]
<i>Lactobacillus crispatus</i>	JCM 5810	<i>cbsA</i>	440/30	O	AF001313	–
<i>Lactobacillus fermentum</i>	BR 11	<i>bspA</i>	265/putative	O	U97348	[117]
<i>Lactobacillus helveticus</i>	CNRZ 892	<i>slpH1</i>	440/30	O	X91199	[118]
<i>Lactobacillus helveticus</i>	CNRZ 1269	<i>slpH2</i>	440/30	O	X92752	[119]
<i>Methanococcus voltae</i>	–	<i>sla</i>	565/12	H	M59200	[120]
<i>Methanosarcina mazei</i>	S-6	<i>slgB</i>	652/31	H	X77929	[121]
<i>Methanothermobacter fervidus</i>	DSM 2088	<i>slgA</i>	593/22	H	X58297	[56]
<i>Methanothermobacter sociabilis</i>	DSM 3496	<i>slgA</i>	593/22	H	X58296	[56]
<i>Rickettsia prowazekii</i>	Brein 1	<i>spaP</i>	1612/32	–	M37647	[122]
<i>Rickettsia rickettsii</i>	R	<i>p120</i>	1645/32	–	X16353	[123]
<i>Rickettsia typhi</i>	Wilmington	<i>slpT</i>	1645/32	–	L04661	[124]
<i>Serratia marcescens</i>	isolate 8000	<i>slaA</i>	1004	–	AB007125	–
<i>Staphylothermus marinus</i>	F1	–	1524/putative	–	U57967	[125]
<i>Thermoanaerobacter kivui</i>	DSM 2030	<i>slp</i>	762/26	H	M31069	[126]
<i>Thermus thermophilus</i>	HB-8	<i>slpA</i>	917/27	H, S	X57333	[127]

[a] H: hexagonal, S: square, O: oblique. [b] In *Campylobacter fetus*, ssp. *fetus*, the lattice type depends on the molecular weight of the S layer subunits (H: 97000; S: 127000 and 149000).

induced in response to the lytic activity of the immune system and leads to modified cell-surface properties. Two serotypes (type A and type B) are known which depend on the type of lipopolysaccharides in the outer membrane.^[133] In both serotypes the N-terminal part of the S layer proteins recognizes a distinct type of lipopolysaccharide. Surface layer proteins from type A cells are encoded by genes designated *sapA* and *sapA*_{1–n}, while S layer proteins from type B cells represent a parallel family. Comparison of *sapA* and *sapA*₁ showed that two regions of identity exist: The first (5'-conserved region) begins 74 bp “upstream” of the open reading frame (ORF), proceeding 552 bp into the ORF to encode the 184 N-terminal amino acids, whereas

the second (3'-conserved region) begins “downstream” of the ORF.^[107, 134]

Eight to nine S layer gene cassettes are present in *C. fetus* wild-type strains,^[135, 136] which are tightly clustered in a region less than 93 kb, representing less than 8% of the genome.^[134] Several studies confirmed that only a single promoter exists^[137] and that antigenic variation is due to recombination events. In addition to promoter inversion between two oppositely oriented gene cassettes, one of the S layer gene cassettes bracketing the invertible element with a nonflanking, previously silent gene cassette is exchanged.^[132, 138] Thus, *C. fetus* reassembles a single promoter strictly by a single DNA inversion event, and at frequencies independent of the

size of the DNA fragment (6–16 kb), permitting expression of different S layer gene cassettes.

L. acidophilus ATCC 4356 was isolated from human pharynx and possesses two S layer genes, termed *slpA* and *slpB*.^[115] The *slpA* gene is actively transcribed, whereas the *slpB* represents the silent gene. Interestingly, the 5' and 3' ends of both S layer genes are very similar, while considerable differences could be observed for the middle region.^[115] The two S layer genes are located at a distance of 3 kb on the chromosome in reverse orientation relative to each other.^[57]

Environmental factors inducing change in S layer gene expression in nonpathogens have been studied for *B. stearothermophilus* strains. The S layer gene *sbsA* was stably expressed by *B. stearothermophilus* PV72/p6 in continuous culture under oxygen limitation.^[139–141] After relieving oxygen limitation, variant formation and expression of the second S layer gene *sbsB* was induced. The decrease in *SbsA* revealed that change in S layer gene expression is a synchronous process in the whole culture. Although the mechanism leading to change in S layer gene expression is still unclear, PCR and hybridization studies indicated that *sbsB* is generated from partial coding sequences and that *sbsA* is disrupted during the switch.^[142]

6. Functional Aspects

The biosynthetic effort to keep the cell surface completely covered with S layer protein raises the question of what advantage of selection S layer carrying organisms have in their natural habitats in comparison to their unlayered counterparts. Although many of the functions assigned to S layers are still hypothetical, it is now recognized that they can act as 1) frameworks to determine cell shape and to aid in the cell division process in those archaea that possess S layers as an exclusive wall component (see Section 3.2), 2) structures involved in cell adhesion and surface recognition, and 3) protective coats, molecular sieves, and molecule and ion traps.^[7, 11, 14, 20]

Because S layers possess pores identical in size and morphology in the 2 to 8 nm range, they work as precise molecular sieves with sharp cut-off levels for the cells. Interestingly, the smallest pores were reported for the S-layer-like sheaths of *Methanospirillum hungatei*,^[143, 144] which uses only small molecules such as gases and salts. In contrast, S layers from Bacillaceae, which are Gram-positive bacteria producing large amounts of exoenzymes, possess pores with a size of 4 to 5 nm.^[145] Since S layers from Bacillaceae reject macromolecules with a molecular weight of 30000 to 40000, which is just within the dimension of the pores and of the exoenzymes produced, S layers from these organisms were suggested to delineate a kind of periplasmic space and control the speed of exoenzyme release into the environment.^[146–148] The largest pores in the 5 to 8 nm range were reported for S layers of archaea lacking a rigid cell-wall layer, in which the S layer fulfills shape-determin-

ing or shape-maintaining function.^[16–18, 21–23, 39–41] In these organisms, the S layer subunits generally possess long hydrophobic protrusions which integrate into the plasma membrane. Thereby, a kind of periplasmic space is formed between the S layer and the plasma membrane in which secreted macromolecules involved in nutrient degradation and transport as well as folding and export of proteins could be stored.^[113, 149–151]

Regarding protective functions, S layers from Gram-negative bacteria such as *A. salmonicida*, *C. fetus*, *A. serpens*, and *C. crescentus* were found to prevent the cells from attack by bacteria parasites such as *Bdellovibrio bacteriovorus*, but they could not shield the cells from other predators like protozoa.^[152, 153] As described for *L. crispatus* and *L. acidophilus*, S layers mediate the adhesion of these bacteria to intestinal epithelial cells.^[154, 155] In comparison to variants lacking S layers, strains carrying S layers have a more hydrophobic surface.^[156, 157]

Surface layers from Bacillaceae were found to function as adhesion sites for cell-associated exoenzymes. The high molecular weight exoamylases from two *B. stearothermophilus* wild-type strains were bound to the S layer surface in a density that did not disturb diffusion of nutrients or metabolites through the S layer lattice.^[158, 159] Exoenzymes associated with S layers were also described for *Thermoanaerobacterium thermohydrosulfurigenes*^[160, 161] and *Clostridium thermocellum*.^[162] A quite different function was reported for the S layer lattice from *Synechococcus* GL-24,^[29a, 163] a cyanobacterium capable of growing in lakes with exceptionally high calcium and sulfate ion concentrations. The hexagonally ordered S layer lattice functioned as template for fine grain mineralization and was continuously shed from the cell surface.

Surface layers can contribute to virulence when they are present as structural components of the cell envelope of pathogens. Detailed studies have been performed with the fish pathogen *Aeromonas salmonicida*. The S layer (termed A layer) endows this organism with high or intermediate resistance towards the bactericidal activity of complement systems, in both immune and nonimmune serum. Moreover, the A layer plays an important role in uptake of porphyrins and shows unique binding capabilities for immunoglobulins and extracellular matrix proteins.^[164–166] Similar observation was reported for the S layer from *B. cereus* which was recently isolated from periodontal infections.^[156] The cells carrying S layers adhered to various matrix proteins and were resistant against polymorphonuclear leucocytes in absence of opsonins. Serum resistance of *C. fetus* was found to be due to the inability of complement component C3b to bind to the S layer surface, and effective opsonization occurred only after addition of specific antibodies.^[132] In *Rickettsia prowazekii* and *R. typhi*, which cause either epidemic or endemic typhus, the S layer protein is responsible for humoral and cell-mediated immunity.^[167] *B. anthracis*, the causative agent of anthrax, possesses two S layer proteins: Sap and EA1.^[95, 96] Synthesis of EA1 in *sap*-disrupted mutants revealed that EA1 is the major cell surface antigen which is exclusively cell-associated, while Sap is also shed into the culture fluid.

7. Applications of Surface Layers

7.1. Biotechnological and Biomimetic Applications

The specific structural and self-assembly properties of S layers have led to a broad spectrum of applications in biotechnology and biomimetics.^[10, 11, 67]

For obtaining S layer proteins with defined properties for bio- and nanotechnological applications, organisms carrying S layers are grown in continuous culture at a constant specific growth rate.^[140, 168] However, future development is directed to the production of recombinant S layer proteins^[11, 169] with integrated functional domains. Moreover, the use of truncated forms or recombinant S layer proteins with exchanged amino acids will enable the definition of those domains that are responsible for intersubunit bonding and for interactions between the S layer subunits and natural or artificial supports. These data are also required for the construction of recombinant S layer fusion proteins capable of self-assembly.

7.1.1. Surface Layers as Isoporous Ultrafiltration Membranes

Owing to the presence of pores identical in size and morphology in the 2 to 8 nm range, S layers can be considered as isoporous ultrafiltration membranes^[10, 11] with a porosity in the range of 30 to 70%. Permeability studies using proteins with defined molecular size according to the space technique were performed with S layers from *B. stearothermophilus* strains.^[145] The nominal molecular weight cut-off determined by this method was in good agreement with the pore size estimated from negatively stained preparations of isolated S layers or S layer self-assembly products.^[148, 170] Because of the sharp molecular weight cut-off, S layers from various Bacillaceae could be exploited for the production of ultrafiltration membranes (SUMs).^[171, 172] SUMs are produced by depositing S layer self-assembly products or cell-wall fragments carrying S layers on microfiltration membranes, cross-linking the S layer protein with glutaraldehyde, and reducing Schiff bases with sodium borohydride (Figure 9a).

Chemical modification of the carboxy groups from the acidic amino acids exposed on the SUM surface and in the pore areas allowed the preparation of neutral, or positively or negatively charged SUMs of different surface hydrophobicity.^[173, 174] The isoporous structure and the defined surface properties of (chemically modified) SUMs enabled detailed

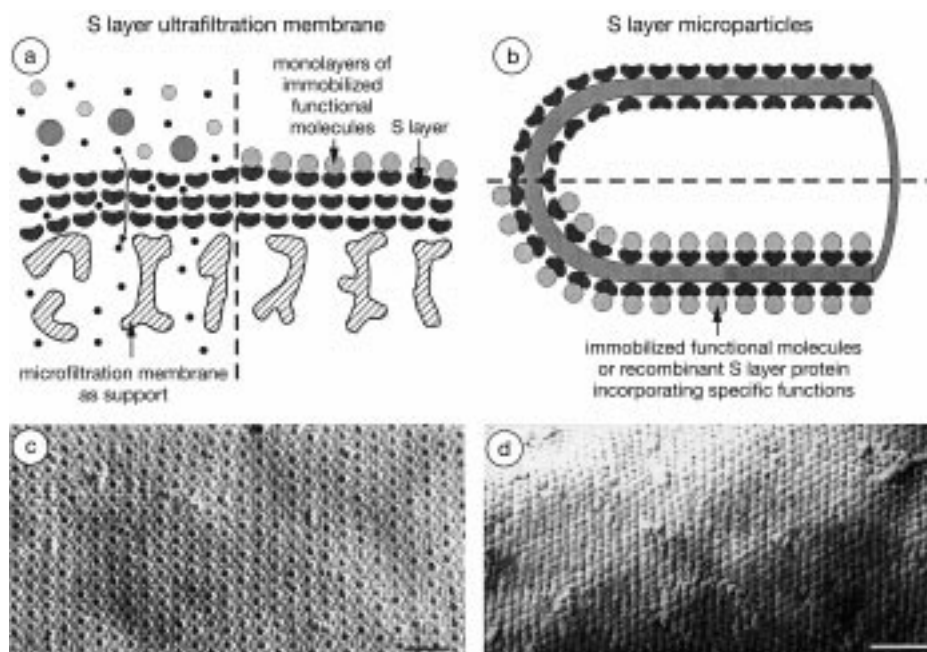


Figure 9. Schematic drawing showing a) S layer ultrafiltration membranes (SUMs) and b) S layer microparticles (SMPs) as matrix for the immobilization of functional macromolecules. c, d) Electron micrographs of freeze-etched preparations of S layers with hexagonal lattice symmetry. In (c) polycationic ferritin (PCF) was used for labeling negatively charged domains on the surface of the archaeon *Thermoproteus tenax*. In (d) ferritin was covalently linked to the carbodiimide-activated carboxy groups of the S layer lattice of *Thermoanaerobacter thermohydrosulfuricus*. The bars correspond to 100 nm.

studies regarding the flux losses of ultrafiltration membranes caused by adsorbed monolayers of protein molecules.^[173, 174]

7.1.2. Surface Layers as Matrix for the Immobilization of Functional Molecules

The high density and defined position of carboxy groups located on the surface of S layer lattices was exploited for the immobilization (covalent attachment) of different (macro-)molecules (Figure 9). Amongst different immobilization procedures, carbodiimide activation of carboxy groups was found to be optimal with respect to the binding density and the reproducibility.^[175–178] For quantification of the amount of foreign (macro)molecules which could covalently be bound to the S layer lattice, S layer microparticles (SMP; cell-wall fragments carrying S layers that show a complete outer and inner S layer and are cross-linked with glutaraldehyde) were used (Figure 9b).^[175, 177] Most enzymes—for example, β -glucosidase, glucose oxidase, invertase, or naringinase—could be immobilized on S layer lattices as densely packed monolayers.^[176, 178] Immobilization through spacers frequently improved the retained enzymic activity, but had no influence on the binding capacity.^[176] After covalent attachment of protein A, which is a ligand that specifically recognizes the Fc part of most mammalian antibodies, SMPs were applicable as affinity particles for the isolation and purification of (monoclonal) antibodies from serum or hybridoma culture supernatants.^[179, 180] Because of the high stability towards pressure and shear forces, SMPs with immobilized protein A could also be used as “escort particles” in affinity cross-flow filtration processes.^[180]

Ultrafiltration membranes with immobilized IgG were used as novel reaction zone for dipstick-style solid-phase immunoassays or SUM-based dipsticks.^[175, 181] Dipsticks based on SUMs are easy to handle and allow rapid determination of various analytes. For preparing the reaction zone, monoclonal antibodies were linked to the carbodiimide-activated carboxy groups of the S layer protein. The binding capacity of the square S layer lattice from *B. sphaericus* for IgG was 375 ng cm⁻². As derived from the saturation capacity of a planar surface and the molecular dimension of IgG, this corresponds to a monolayer of randomly oriented antibody molecules.^[175] So far, different types of SUM-based dipsticks have been developed for the rapid determination of the concentration of tissue-type plasminogen activator (t-PA) in whole blood or plasma, IgE as a marker for type I allergies in serum, and interleukins to differentiate between septic and traumatic shock. The advantage of SUMs as reaction zone for dipsticks in comparison to amorphous polymers can be summed up as follows:

- 1) Surface layers have well-defined surface properties.
- 2) Immobilization of the catching antibody can only occur on the outermost surface of the crystal lattice, preventing diffusion-controlled reactions.
- 3) Since the catching antibody is covalently linked to the S layer protein, no leakage occurs during the test procedure.
- 4) SUMs do not unspecifically adsorb erythrocytes or other serum or blood components.
- 5) Stable, concentration-dependent precipitates are formed by peroxidase or alkaline phosphatase on the SUM surface, from which the intensities can be measured with a reflectometer.^[181]

7.1.3. Surface Layers for Vaccine Development

In the past, crystalline bacterial cell S layers have been utilized in different ways for vaccine development. *Aeromonas salmonicida* and *A. hydrophila* can cause disease in salmonids in fresh-water and marine environments. Since the S layers of those organisms are essential for virulence, whole cell preparations, cell sonicates, or partially purified cell products have been applied as attenuated vaccines.^[44, 182]

On the other hand, native and cross-linked S layers were used as combined carrier/adjuvants system either against infection with pathogenic bacteria, in the immunotherapy of cancers, and in the antiallergic immunotherapy.^[183, 184] Owing to their crystalline nature the functional groups available for antigen/hapten binding occur on each protomer in identical position and orientation.^[185, 186] Thus, ligands can be immobilized onto these protein lattices of S layer self-assembly products in a precisely defined manner (Figures 5 and 9).

The suitability of S layers as combined carrier/adjuvants for therapeutic cancer vaccines was first suggested by Smith et al.^[187] Tumor-associated glycans such as T or Lewis Y antigen, chemically coupled to glutaraldehyde-cross-linked S layer preparations of different Gram-positive bacteria elicited without the use of extraneous adjuvant strong, caused hapten-specific delayed-type hypersensitivity (DTH) responses. In contrast, only low titers of antibody responses were obtained

with those S layer–hapten conjugates. Adoptive transfer experiments indicated that the observed DTH responses are mediated by T-helper cells. Administration of the S layer–hapten conjugates by the oral/nasal route resulted for tumor-associated glycans in similar specific DTH responses.^[187]

Surface layer–antigen conjugates were also prepared by coupling oligosaccharides derived from capsules of different *Streptococcus pneumoniae* serotypes to native, non-cross-linked S layers. Sera from mice immunized with these conjugates were found to be immunoprotective, and they reduced the colony counts of *S. pneumoniae* by 99 % on blood agar plates. Surface layer–polysaccharide conjugates of the same serotype revealed no protective antibodies. The oligosaccharide conjugates could also elicit a cellular (DTH) response on challenge with heat-killed *S. pneumoniae* bacteria. Therefore with these S layer conjugates the thymus-independent response against a bacterial polysaccharide was changed into an immunoprotective thymus-dependent response.^[188]

In type I allergies the production of IgE antibodies is mediated by the Th2 helper lymphocyte subset. Nonallergic individuals, however, produce low levels of specific IgG to allergens, a response mediated by Th0/Th1 cells.^[189] The modulation of regulatory T cell subsets by adjuvant effects of S layers is a promising concept for immunotherapy and perhaps also for prophylaxis of type I allergies. In the experimental S layer vaccine recombinant birch pollen allergen Bet v 1 (rBet v 1) was conjugated to cross-linked S layers. The T cell lines (TCL) and T cell clones (TCC) were established from peripheral blood from patients allergic to birch pollen using either rBet v 1 alone or rBet v 1–S layer conjugates as initial antigen stimulus. For assessment of the respective immune reaction the corresponding cytokine production was investigated (Figure 10).^[190] Surface layers and rBet v 1–S layer conjugates stimulated the production of high levels of interleukin 12 (IL-12). Stimulation of rBet v 1-induced TCC with Bet v 1–S layer conjugates led to an increased production of interferon γ , which could be

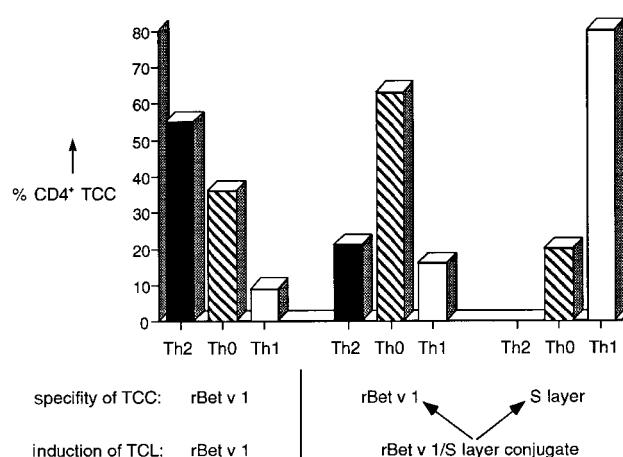


Figure 10. Distribution of T helper cell (Th) subsets within CD4⁺ T cell clones (TCC) induced by recombinant major birch pollen allergen rBet v 1 or rBet v 1–S layer conjugates and stimulated by rBet v 1. Th subsets were classified according to the ratio of interferon γ to interleukine 4 (taken from reference [190] with permission of Oxford University Press).

reversed by neutralizing anti-IL-12 monoclonal antibodies. Together these results indicate an adjuvant effect of S layers mediated by IL-12.^[190]

The experiments conducted so far have indicated that the S layer vaccine technology is suitable for a broad spectrum of applications. A number of immunologically non-cross-reactive S layers, which do not show any measurable toxicity, can already be used as carrier/adjuvants systems. A further strategy for the development of new model vaccines is based on liposomes stabilized by S layers, and a wide range of combinations is possible with suitable protein-, lipid-, or glycolipid-immune modulators. In all applications, the S layer vaccine technology appears to offer the versatility needed to direct vaccination responses toward predominant control by Th1 or Th2 lymphocytes to meet the different therapeutic or prophylactic requirements.

7.1.4. Surface Layers as Supporting Structure for Functional Lipid Membranes

Since a great variety of biological processes are membrane-mediated, there has always been great interest in the meso- and macroscopic reconstitution of biological membranes. Particularly functional transmembrane proteins have a broad potential for bioanalytical, biotechnological, and biomimetic applications. On the other hand, investigations are primarily impeded by a low stability of artificial planar lipid bilayer systems and liposomes.^[191, 192] Consequently, there is a strong demand to develop systems that reinforce such fragile structures without interfering with their function.

The stability of lipid membranes can be increased significantly by recrystallization of isolated S layer (glyco)proteins as coherent monomolecular lattices.^[193, 194] Composite S layer lipid films (Figure 11) are biomimetic structures resembling those archaeal envelope structures which are exclusively composed of monomolecular arrays of (glyco)proteins and a

closely associated plasma membrane (Figure 1a). Phospholipid or tetraetherlipid films stabilized by S layers may span apertures in a solid support that are some 10 μm in diameter.^[194] The possibility for handling such layers by standard Langmuir–Blodgett (LB) techniques opens a broad spectrum of applications in basic and applied membrane research, including physiology, diagnostics, and biosensor development. Composite S layer lipid films are particularly interesting supramolecular structures, since the crystallization does not involve specific key–lock mechanisms between proteins and (modified) lipids, as is the case for antigen–antibody, enzyme–substrate, or biotin–avidin systems.^[195–198]

The recrystallization of S layer proteins into coherent monolayer or phospholipid films depends on 1) the nature of the lipid headgroup, 2) the phase state of the lipid film, and 3) the ionic content and pH value of the subphase.^[198–201]

To obtain more information about the interaction between the S layer protein lattice and lipid membranes and to study how the interaction points influence fluidity in a lipid layer, different biophysical methods including X-ray reflectivity and grazing incidence diffraction,^[202] fluorescence recovery after photobleaching,^[203] atomic force microscopy,^[199] sound velocity and density measurements,^[204] and microcalorimetric studies^[205] have been performed. As the interaction of the lipid head groups in such composite membranes with the repetitive domains of the associated S layer lattice (Figure 11) can significantly modulate the characteristics of the lipid film (particularly its fluidity and local order on the nanometer scale), the terminology “semifluid membranes” has been used to describe membranes supported by S layers.^[194, 200]

Voltage clamp studies at Langmuir films^[206] and black lipid membranes^[207] confirmed that the associated S layer did not impede the function of the lipid membrane or incorporated molecules. Particularly stable composite structures could be obtained after intra- and intermolecular cross-linking of the S layer proteins alone or with molecules from the lipid layer.

The “semifluid membrane” concept was also exploited for the design of different types of solid-supported lipid bilayers and tetraether monolayers (Figure 12),^[199, 203] as required for (highly selective) receptor surfaces of biosensors on electro-optical devices or the biofunctionalization of inorganic surfaces.^[208, 209]

Surface layer subunits can also recrystallize on liposomes^[205, 210, 211] composed of phospholipids and tetraetherlipids (Figure 13). Such liposomes coated with S layers are closed biomimetic structures that resemble archaeal cells (Figure 1a) or virus envelopes. The S layer proteins, once recrystallized on liposomes, can be cross-linked and exploited as a matrix for the covalent attachment of molecules. The high mechanical and thermal stability of liposomes coated with S layers and the possibility for immobilizing or entrapping biologically active molecules reveal a broad application potential, particularly as carrier and/or drug-delivery and drug-targeting systems or in gene therapy (for example, artificial viruses).

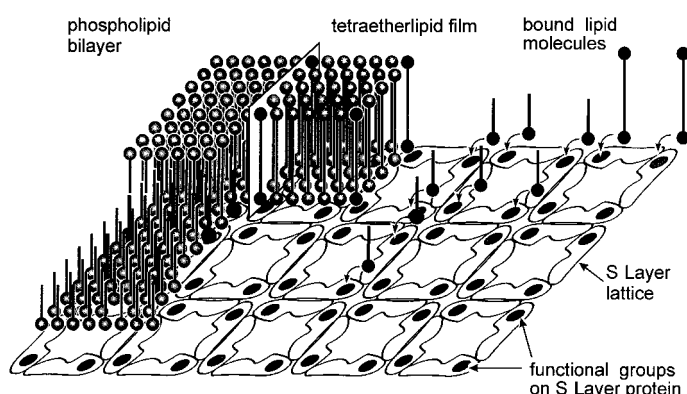


Figure 11. Schematic illustration of the “semifluid” membrane model composed of a phospholipid bilayer or tetraetherlipid monolayer Langmuir film supported by an S layer. The proportion of lipid molecules which can covalently be linked to the porous S layer lattice or which interact by noncovalent forces with domains of the S layer protein subunits significantly modulates the lateral diffusion of the free lipid molecules and consequently the fluidity of the membrane (modified based on reference [200]).

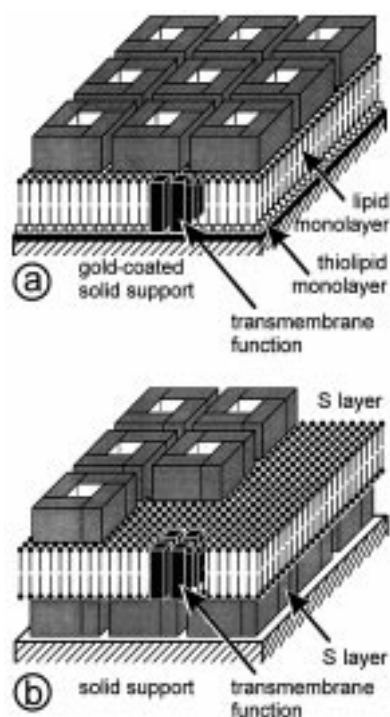


Figure 12. Schematic drawing illustrating the concept of solid-supported lipid membranes stabilized by S layers. In (a) the generation of the lipid bilayer makes use of the strong chemisorption of thiolipids to gold. The second leaflet and the S layer which had recrystallized at the lipid film before in a Langmuir trough were transferred onto the thiolipid-coated solid support by the Langmuir–Schaefer technique. Integrated functional molecules allow the investigation of transmembrane functions. b) As an alternative to soft polymer cushions an S layer is located between the solid support and the lipid layer. Optionally, the external leaflet of the lipid bilayer can be stabilized by the attachment of an S layer (modified based on reference [212]).

7.2. Nanotechnological Applications

7.2.1. Surface Layers Recrystallized on Solid Supports

The intrinsic property of S layer proteins to form extended crystalline arrays on solid supports is one of the most important features for functionalizing surfaces.^[200, 212] For many bio- and nanotechnological applications a defined orientation of the lattice with respect to the substrate is required.^[213] A common feature of most technologically relevant S layers is their asymmetrical topography, surface charge, and hydrophobicity between the outer and inner faces. High-resolution electron microscopy and scanning force microscopy have demonstrated a moderate surface roughness for the outer face and a more pronounced one for the inner face. Chemical modifications of the surfaces as well as adsorption and labeling experiments with differently charged marker molecules demonstrated a charge neutral, more hydrophobic outer face and a net negatively charged, more hydrophilic inner face for S layers of many Bacillaceae.

High-resolution electron microscopical and scanning force microscopical studies have shown that the protein monolayers are not monocrystalline over the entire surface, but consist of a mosaic of randomly aligned crystalline domains.^[29b, 193, 213]

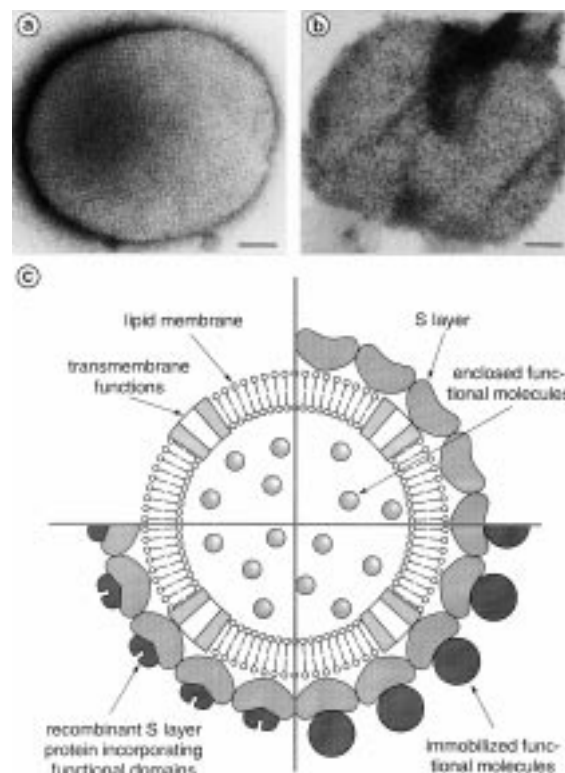


Figure 13. Electron micrographs of negatively stained liposomes coated with an oblique S layer lattice a) before and b) after covalent binding of electron-dense ferritin molecules. c) Schematic drawing of a liposome coated with an S layer lattice which is used as matrix for the immobilization of functional molecules. Alternatively, recombinant S layer proteins incorporating functional domains may be used. The bars correspond to 80 nm.

Analysis of the crystallization process revealed that crystal growth of S layers at interfaces is initiated at randomly distributed nucleation sites composed of proteins or small protein assemblies from the bulk solution. Subsequently, the crystalline domains grow laterally in all directions until neighboring areas meet and a closed coherent monolayer is formed.

Although the S layer lattice exhibits a polycrystalline character, the individual crystalline domains have the same orientation with respect to the interface. This orientation is unambiguously determined by the reciprocal influence of the surface properties of the S layer and support (for example, hydrophobicity, surface charge). The patch size of the crystalline areas depends on the density and lateral mobility of those protein subunits or protein assemblies which have adsorbed in a very early stage of crystal growth at the surface. Beside the surface properties of the substrate, the formation of coherent crystalline arrays depends strongly on the particular S layer used and on the environmental conditions of the bulk phase (for example, temperature, pH value, ion composition, and ionic strength). The average diameter of the crystalline patches is in the range of 1 to 20 μm , while the total covered area is only limited by the size of the substrate. Table 4 summarizes all those technologically important substrates for which the formation of S layer lattices has already been optimized.

Table 4. Technologically important substrates on which S layers recrystallize.^[200]

Silicon and gallium arsenide wafers for novel applications in microelectronics and for miniaturized integrated biosensors

Glass for all developments of optical and electrooptical sensor systems

Noble metals (e.g. gold or platinum) for the development of ultrathin working electrodes for amperometric biosensors

7.2.2. Patterning of Recrystallized Surface Layers

Almost all technological applications for surfaces functionalized with S layers require patterning of the protein lattice in the (sub)micrometer range. For this purpose, S-protein monolayers recrystallized on silicon wafers are irradiated by deep ultraviolet (DUV) laser radiation through a microlithographic mask possessing feature in the range of 1 μm to 200 nm (square and line patterns, Figure 14).^[214] Drying of the S layer was necessary to avoid formation of interference fringes in the patterned S layer by a thin water layer between mask and substrate. The S layer could be completely removed by two pulses of ArF radiation ($\lambda = 193$ nm; dose: approximately 100 mJ cm^{-2} per pulse) in the exposed areas, while in the unexposed regions the structural and functional integrity was completely retained. The height difference between exposed and unexposed areas was determined by scanning force microscopy, and found to be in perfect agreement with the thickness of the S layer as determined at fringes of individual monolayers.

Surface protein monolayers recrystallized on silicon wafers are currently being tested for their potential application as novel ultrathin high-resolution resists. In this application the patterned S layers are reinforced by a thin refractory layer (for example, Zr, deposited by reaction with ZrOCl_2), silylation,^[215] or electroless metallization^[216] in order to yield different etching rates between exposed and unexposed regions for subsequent dry (reactive-ion) etching. Advantages against conventional polymeric resists are expected due to the extreme thinness of S layers (approximately 10 nm).

In comparison to ArF radiation, the S layer could not be ablated when exposed to KrF radiation ($\lambda = 248$ nm).^[214] Investigation of the irradiated S layer lattices showed that even a dosage of about 3500 mJ cm^{-2} (supplied in 10 shots at 350 mJ cm^{-2} each) led only to a carbonization and not to an ablation of the protein.^[214] As determined by scanning force microscopy the step height between exposed and unexposed areas did not reach the thickness of the intact S layers. Since the S layer lattice could not be identified in the unexposed regions after exposure, it was concluded that during exposure heat dissipated into the unexposed areas and destroyed the molecular structure of the S layer lattice there. Nevertheless, this remarkably different sensitivity of the S layer against ArF and KrF radiation could already be utilized for transferring a submicron pattern into a conventional polymeric resist on a silicon wafer.^[217] In this approach an S layers recrystallized on top of a spin-coated polymeric resist (thickness approximately 500 nm), sensitive towards KrF but not towards ArF radiation, was first patterned by ArF radiation as described above. Subsequently, this patterned S layer structure served as mask for a blank exposure of the resist by irradiation with KrF. In

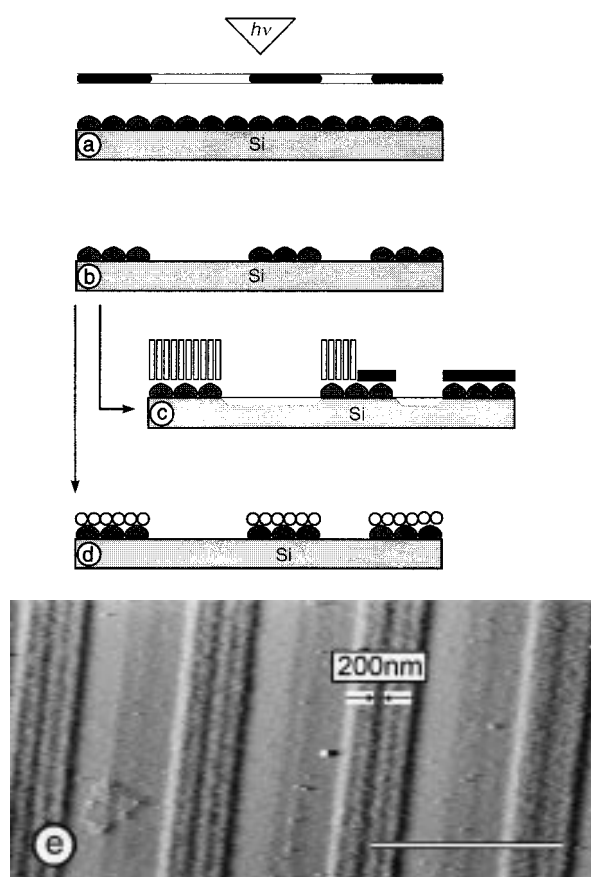


Figure 14. Schematic drawing of the patterning of S layers by exposure to deep ultraviolet radiation. a) A pattern is transferred onto the S layer by exposure to ArF excimer laser radiation ($\lambda = 193$ nm) through a microlithographic mask. b) The S layer is specifically removed from the silicon surface in the exposed regions, but retains its crystalline and functional integrity in the unexposed areas. c) Unexposed S layer areas can be used either to bind enhancing ligands or to enable electroless metallization. In both cases a layer is formed which allows a patterning process by reactive ion etching. d) Alternatively unexposed S layers may also be used for selectively binding biologically active molecules which would be necessary for the fabrication of miniaturized biosensors or biocompatible surfaces. e) Scanning force microscopical image of a patterned S layer on a silicon wafer. The bar corresponds to 3 μm . The ultimate resolution is determined by the wavelength of the excimer laser radiation (modified based on reference [212]).

this approach very steep side walls in the developed polymeric resist could be obtained.

Current research is concentrated on an improvement of the resolution limit of 100 nm, which is caused by the wavelength of the ultraviolet radiation. Preliminary experiments with electron and ion beams have already demonstrated that feature sizes well below the 100-nm limit can be generated in S layer lattices (unpublished results of our research group).

7.2.3. Surface Layers as Templates in the Formation of Regularly Arranged Nanoparticles

The formation of arrays of metal clusters with novel physical properties by colloidal crystallization or monolayer deposition is currently under extensive investigation in the

field of molecular electronics and nonlinear optics.^[218–221] Although, in recent years, these methods have advanced tremendously it is still not possible to fabricate extended arrays on the micrometer scale. As an alternative approach to such methods S layers have already demonstrated their application potential as templates in the formation of regularly arranged nanometric metallic or semiconducting point patterns. Surface protein monolayers on substrates and sheet-like self-assembly products in suspension promote the precipitation of monodisperse cadmium sulfide^[222] and gold nanoparticles (Figure 15).^[223] This observation is not surprising since recent studies on a cyanobacterial S layer clearly demonstrated that a crystalline surface layer can function as template for fine grain mineralization.^[163]

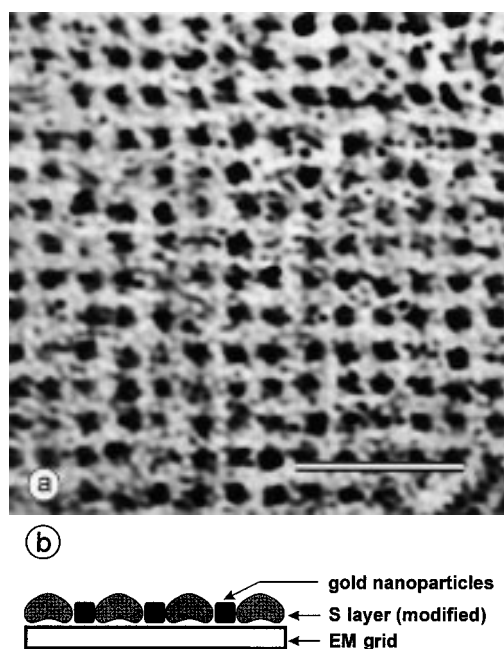


Figure 15. a) Electron micrograph of a gold “superlattice” consisting of monodisperse gold nanoparticles with mean diameters of 4 to 5 nm. b) Schematic drawing of a cross-section through a support coated with S layers, where the gold nanoparticles had formed in the pore region of the protein layer. The bar corresponds to 50 nm.

For the formation of gold nanoparticle arrays thiol groups had to be introduced in the S layer before incubation with a tetrachloroauric(III) acid solution. The gold particles were formed upon electron irradiation of an S layer covered with a thin layer of gold (gold staining of the S layer). High-resolution electron microscopical studies revealed that the particles were formed in the pore region of the S layer lattice and that their shape resembled the morphology of the pore (Figure 15). The interparticle spacing of the gold dots resembled the S layer lattice. Electron diffraction patterns and energy-dispersive X-ray analysis confirmed that the gold particles were crystalline (pure) gold, but not crystallographically aligned in terms of long-range order. Digital image processing also revealed that the mean deviation of the individual particles from their ideal position in the two-dimensional lattice was less than ± 0.5 nm.

Most recently the spectrum of available materials was extended towards the precipitation of platinum and palladium, but also magnetic materials such as iron or nickel (unpublished observations of our research group). Currently the electrical, magnetic, and optical properties of these nanoparticle arrays are being investigated.

Surface layers have also been used as nanometric etching masks in the fabrication of ordered arrays of titanium nanoparticles.^[224–226] In this approach an hexagonal S layer structure was transferred into a silicon surface by (low energy enhanced) electron etching. Subsequently, the S layer mask was removed, and the patterned surface oxidized in an oxygen plasma. After deposition of a thin layer (about 1.2 nm) of titanium on the oxidized surface, an ordered array of nanometric titanium clusters was formed in the etched holes.

In contrast to other current approaches, only S layers appear to allow the synthesis of extended inorganic nanocrystal superlattices with a broad range of particle sizes (3 to 15 nm in diameter), interparticle spacings (up to 30 nm), and lattice symmetries (oblique, square, or hexagonal).

8. Conclusions and Perspectives

Surface layers are the most common envelope structures in prokaryotic organisms. As highly porous protein lattices that completely cover the cell surface, they reflect specific adaptations to ecological conditions and selection criteria. Being composed of a single protein species, S layers represent the most simple self-assembling membranes that developed during the evolution of life. The spontaneous associations of identical subunits under equilibrium conditions result in stable, structurally well defined monomolecular aggregates joined by noncovalent bonds. On growing bacterial cells S layers represent “dynamic closed surface crystals” with the intrinsic capability to assume a structure of low free energy continuously during cell growth.

The study of self-assembly is a new and rapidly growing scientific and engineering field that crosses the boundaries of several existing disciplines.^[29b, 212, 227, 228] Molecular self-assembly processes are ubiquitous in biological systems and are essential for the morphogenesis of complex biological structures. The attractiveness of self-assembly for practitioners of material science lies in its capabilities to form various ultrasmall, uniform, or highly complex meso- or macroscopic area structures spontaneously. Such “bottom up” processes are based on intermolecular forces which determine, in a highly predictable way, the form or pattern of the final structure.

Learning from nature how to create supramolecular units and the elucidation of rules mediating their organization into functional materials is leading to fascinating new technologies. Our goal in applied research on S layers and prokaryotic cell envelopes has been to harness for technical applications the unique self-assembly characteristics and the repetitive structural properties of these supramolecular systems. Especially the possibility to immobilize or grow other materials on top of S layers with an accurately spatially controlled archi-

texture opens up many new possibilities in supramolecular engineering and nanofabrication.

Similarly, the biomimetic approaches copying the supramolecular principle of plasma membranes associated with S layers (developed by archaea in the most extreme and hostile ecosystems, for example, at pH values below 1, at temperatures up to 110 °C, or in concentrated salt solutions) is expected to lead to new technologies for stabilizing functional lipid membranes and their use at the macroscopic scale.

Along the same line, liposomes stabilized by S layers resemble archaeal and viral envelopes and represent unique supramolecular systems that are suitable as carriers for drug delivery and drug targeting or as vehicles for gene therapy.

An important line of development is directed towards the genetic manipulation of S layer proteins. The possibility to change the natural properties of S layer proteins by site-directed mutagenesis opens a new horizon for the tuning of their structural and functional features by genetic engineering techniques. Incorporation of peptide segments corresponding to specific functional domains of other proteins while maintaining the self-assembly capability should lead to completely new affinity structures and enzyme membranes, ion-selective binding matrices, microcarriers, biosensors, diagnostics, vaccines, biocompatible surfaces, or bioabsorbable systems for tissue regeneration.^[11] Finally, S layer technologies provide new approaches for nanopatterning of surfaces, biological templating, and formation of ordered arrays of metal clusters as required for nonlinear optics and nanoelectronics.^[212]

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Miniature Continuous-Flow Polymerase Chain Reaction: A Breakthrough?

Haralabos Zorbas*

On August 27th, 1998, the Associated Press released the following notice: "TI announces tech breakthrough: Dallas—Texas Instruments Inc. says it has developed semiconductor technology based on minuscule transistors that could someday allow hearing aids to be built small enough to be directly implanted into the inner ear. The Texas Instruments transistor is so small, more than 400 million of them will fit onto a chip the size of a fingernail, researchers said Wednesday. Texas Instruments said products based on these chips will be smaller, weigh less, consume less power and execute software much faster than existing technology. (...) While Texas Instruments claims the transistor is the 'smallest announced' researchers at Bell Laboratories, the research and development arm of the New Jersey-based Lucent Technologies, announced in November that they had created the 'world's smallest complete metal oxide semiconductor transistors.' (...) "[1] This news is symptomatic of the notion that the human zeal for the creation of giant items, such as buildings, machines, and carriages,—frantically driven by the superpowers dozens of years ago—can seemingly nowadays only be beaten by the fervor to miniaturize devices down to micrometer and nanometer range. But not only so in electronic application sciences; the triumphant advance of this enterprise has conquered chemical and biochemical science as well.

We all want to save time, space, and costs. During the last decade a plethora of miniature systems have been studied and some prototypes developed. In particular, microfabricated devices for microfluidic systems (pumps, valves) and microfiltration systems, micro heat exchangers and microseparators, micromixers, microreaction chambers (for reviews see references [2, 3]), and electric field directed nucleic acid hybridization on microchips[4] have been presented. High-speed capillary electrophoresis (CE)[5] has been further developed successfully into capillary array electrophoresis[6, 7] and also two-dimensional capillary array electrophoresis[8] for DNA analyses on chips. Chip technology and performance also proved suitable for the powerful DNA analysis techniques for ligase chain reaction (LCR)[9] and polymerase chain reaction (PCR).[10] Moreover, microsystems with several functionally integrated features have been elaborated. These include CE

with an integrated volume-defined sample injector for an unbiased injection procedure,[11] CE and PCR-coupled-CE with integrated real time monitoring,[12, 13] integrated cell isolation with PCR analysis using silicon microfilter chambers,[14] and even more complex systems, for example combining a microdialysis sampling interface with a microflow manifold and integrated biosensor array for glucose and lactate detection with associated computer software.[15] A new paradigm for genetic analysis are DNA chips with high density arrays of polynucleotide probes for gene discovery, observation of the expression of thousands of genes in concert under defined conditions, as well as for genotyping and DNA sequencing by array hybridization, epifluorescence confocal scanning, and data analysis.[16] These last developments gave rise to the so-called miniaturized total analysis systems (μ TAS) that bring to reality the lab-on-a-chip concept (for critical recent overviews and perspectives see references [17–21]). According to Affymetrix (Santa Clara, California), a DNA array pioneer and the best known DNA-chip maker, it is in fact possible today that a plastic cartridge smaller than a credit card carries out seven different experimental procedures after an extracted DNA sample has been injected into a storage chamber. By a computer-controlled system, the sample is bound to a tiny glass wall, rinsed, dissolved again, amplified, converted into RNA, labeled, chopped up, and delivered to one of the company's microarray test.[22]

Compared with traditional "normal size" machines, miniature systems come off quite impressively. Processes are ultrafast (ranging from some seconds to some minutes,[12, 23] the sensitivity of detection of the analyzed substances touches the order of attomoles (10^{-18} moles)[12] and even zeptomoles (10^{-21} moles)[24], and the resolving power of the microdevices is similar to those of conventional systems.[5, 25] In summary, fabrication of micromachines, although miles apart from the minute dimensions of their electronic trailblazers, realize reproducible, low-cost manufacturing,[26] a very small sample volume (usually $<10\ \mu\text{L}$), reduction of reagents, excellent thermal conductivity, a fast response, automated assays, and are suitable for high-throughput analysis through parallel sample processing. Evidently, these new analytical tools are useful in chemical and medical analysis, for example, disease diagnosis and research, and also meet the needs of the pharmaceutical industry in drug development.

The promising virtues of the biochemical micromachines led to a summit of enthusiasm as expressed by the bold and rousing announcement last May by J. Craig Venter, president

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of The Institute for Genomic Research (Rockville, Maryland), to sequence the human genome at a fraction of the time and the costs anticipated for the US governmental endeavor.^[27] He focuses on miniaturized, nonstop working “pocket DNA sequencers” that will be delivered by Perkin–Elmer, the world’s largest manufacturer of automated sequencing machines.^[22] Although Perkin–Elmer’s CE-microfluidics are still in the testing stage and doubts about their suitability have been expressed,^[28] David Burke and co-workers of the University of Michigan in Ann Arbor, provided a convincing foretaste towards the realization of this direction. These researchers described recently a DNA analysis chip that integrates and performs at the nanoliter scale most of the steps needed in DNA amplification, restriction endonuclease digestion, or DNA sequencing, and detection of discrete DNA products with virtually no human intervention.^[26]

However, specific problems show up with microfluidics that are not encountered with normal size machines. For example, handling and processing of micro-sized samples are still challenging, and have a series of attendant problems, such as loss of sample on the walls of transfer devices, loss by evaporation, loss of components from the sample during manipulation and processing, and obtaining a representative sample from a nonhomogeneous specimen. A further difficulty is that the expected concentration of the analyte restricts the scale of miniaturization of the sample.^[2] Miniaturization inevitably leads to a reduction of the surfaces needed for efficient chemical or enzymatic reactions, which, however, can be successfully controlled by the use of porous silicon.^[29] In addition, care has to be taken with respect to the compatibility of the new materials, other than polyethylene tubes, with the desired reaction, such as PCR, to take place in fabricated micromachines on chips.^[30] Obviously, there’s still a lot to do.

Additional, nontechnical problems may also jeopardize or slow down a rapid advent and a large-scale application of microfluidics. In particular, DNA arrays are still very expensive, and for medical use, for example, in pinpointing the onset of diseases or tailoring medication to patients with specific genetic makeups, they have in addition “to wind their way through clinical trials and regulatory approval”.^[31] Moreover, patent battles and questions of proprietary rights over key aspects of technology and gene sequences in arrays could bring this new gold rush to a halt. However, as a matter of fact these legal fights of companies and the fear of being defeated also nurture the burgeoning of novel ideas and the invention of new principles.

In this context, a significant new development in the world of miniature systems was reported by Kopp et al. (Zeneca/SmithKline Beecham, UK) in the May 15, 1998 issue of *Science*.^[32a] These authors described a PCR chip for continuous-flow DNA fragment amplifications. The glass microchip was fabricated at the Alberta Microelectronic Centre, Canada, and is about 1 mm thick and 4 cm wide and long. The PCR mixture together with the DNA sample to be amplified is introduced by a precision syringe pump under simultaneous and constant buffer flow through a tiny hole drilled into the cover plate into a single channel (40 μm deep, 90 μm wide, 2.2 m long) etched into the glass chip (Figure 1A). The

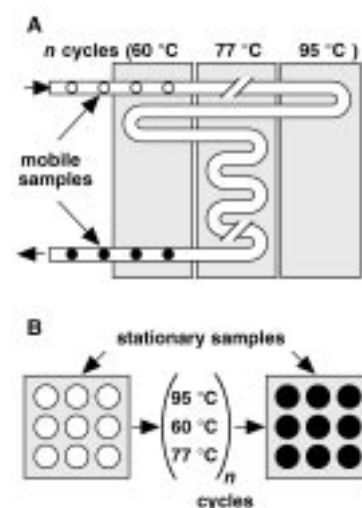


Figure 1. Schematic presentation of the continuous-flow PCR (A) and the classical batch process (B). ○ = input DNA (template); ● = product DNA (amplicates).

channel passes twenty times through three temperature zones that are kept at 95 °C, 60 °C, and 77 °C by controlled thermostated copper blocks, thus defining a thermal cycling process of 20 identical cycles in total. The sample (10 μL) is hydrostatically pumped through the channel and the PCR product (also 10 μL) is collected at an outlet capillary. Analysis is performed by standard slab-gel electrophoresis. By varying the flow rates of this set up, the authors demonstrated that they could achieve satisfactory amplifications, that is several nanograms final product of a 176 bp fragment, after only 3–4 min of total cycling time, and starting with approximately 10^8 copies of the template. The range of the flow rates was between 5.8 and 72.9 nL s^{-1} , which corresponds to between 18.8 and 1.5 min total amplification time for the 20 cycles.

For a critical evaluation of this report the presented results must be compared not only to the declared goals of the work, but also to existing alternatives and must be judged with regard to opening the gate for further developments as well. Kopp et al. claim to have presented an example of a “chemical amplifier”. The latter is defined as a system that should increase a weak signal in a *continuous manner* (continuous input and output of material) and by a large *constant* factor, which means independently of the signal’s initial size or quantity. As opposed to electronic amplifiers, which allow weak signals to be increased with virtually no time delay, chemical amplification does require time because of both diffusion-limited mass transport and dependence on the specific reactivities of the substances. Following the authors’ requirements for a “true” chemical amplifier, however, amplification should also occur with the *same* time dependence, again regardless of the signal’s initial magnitude.

To my knowledge, a micromachined, continuous-flow PCR has been presented here for the first time. This device in fact amplifies the input DNA in a continuous manner. This has been accomplished by a time–space conversion, that is by keeping the temperatures constant at different locations and moving the sample to the different temperature zones. In

general, conventional thermocyclers alternately heat and cool the stationary samples^[33] (Figure 1B). In addition, they perform the amplification of the target DNA in a batch process, precluding them from being “true” chemical amplifiers and, more importantly, from the potential to be coupled with other, continuous-flow systems (see below). As a consequence of the fact that cross-contamination is not a major problem, the continuous-flow PCR chip may be used for serial injection of different samples to be analyzed. By only one-step intervening buffer washings, the samples can be introduced one after the other and give rise to a very high throughput, which in other microdevices is accomplished by an array set-up of the elements (see above).

However, with regard to the required constancy of the factor and invariable time dependence of the amplification, the achievements of the manufactured microdevice are not so clear. Neither feature has been shown in this report experimentally. Moreover, the amplification factor of an input DNA target is by no means determined solely by the (constant) number of PCR cycles. The theoretical value of a PCR amplification is shown in Equation (1), with N being the

$$N = N_0 2^n \quad (1)$$

number of amplified molecules (product), N_0 the initial number of molecules before amplification (input), and n the number of the cycles. In this ideal formula the number of molecules in fact is doubled in each cycle. However, in practice the outcome is better described by Equation (2),

$$N = N_0 (1 + E)^n \quad (2)$$

where E is the efficiency of the reaction and can be any value between 0 and 1. Experimental values of $E = 0.8$ and $E = 0.9$ have been found for an optimized PCR.^[34] Moreover, at the final stages of a PCR, E is subject to a vast decline because of exhaustion of ingredients, polymerase inactivation, etc. Therefore, an amplification above about 10^{13} molecules in the final product of one PCR is usually not possible. As a result of the nonlinear response of amplification, methods for determining and correcting these deviations have been sought in the last few years (“quantitative”, “competitive” PCR).^[35]

The continuous-flow PCR on a chip shares several features with existing microdevices, such as a small sample volume, rapid thermal conductivity (heating and cooling times less than 100 ms), and a very fast performance. In some aspects, however, thermocyclers of conventional size and batch processing are in fact superior to the presented continuous-flow PCR microdevice. For example, up to 12 primer-annealing temperatures can be checked in a single experiment with a commercial machine (RoboCycler from Stratagene) thereby increasing the rate of successful amplification. Another apparatus on the market (LightCycler from Boehringer Mannheim) combines not only high speed analysis (15–30 min for 30 cycles) and a reasonably small volume (5–20 μL) of up to 32 samples, but also on-line and real-time detection of the amplicate by a kinetic approach.

What is the significance of this work then? First of all, for Zeneca/SmithKline Beecham this new invention may help to

avoid legal scuffles by eluding restrictions of existing patents. Besides, in my opinion two—more scientific—points must also be emphasized. First, the demonstration of the feasibility of continuous-flow PCR. PCR is known to be a highly sensitive method that will amplify traces of template and is thus prone to false amplifications because of contaminations. To me, it is very surprising—pleasing though!—that the described PCR chip obviously operates without this shortcoming. Probably, the process of mutual mixing and contaminating of the consecutive analyte peaks is efficiently restricted by the low-dispersion characteristics of the capillary-shaped chemical reactor in combination with the silanized surface of the channel walls and the use of a non-ionic surfactant in the buffer. However, it would have been interesting to examine, whether or not a *different* template injected after the first, a much longer template and, finally, less than 10^8 copies of a template would have led to similar satisfactory results. Second, the potential of further development of total analysis and synthesis systems by means of incorporation of the continuous-flow microamplifier is very high. For example, one can imagine linking the microamplifier to microfabricated devices for cell isolation,^[14] volume-defined sample injectors,^[11] and on-line sensors^[6, 12] to elaborate a μTAS for a fast, integrated, and continuous DNA analysis of cell populations. For this purpose, all modular parts should operate in a continuous manner similar to the microamplifier in the report of Kopp et al. Indeed, as pointed out by the authors, continuous-flow microsystems are already available.^[32b] These combinations would allow the continuous-flow PCR microamplifier to be useful not only in fast and reliable routine clinical diagnosis, but also versatile enough to challenge research laboratories as well. In conclusion, does the report of Kopp et al. mean a breakthrough in PCR technology? Perhaps no, but it is a (good) step in the right direction.

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Keywords: analytical methods • capillary electrophoresis • microreactors • nucleic acids • polymerase chain reaction

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Heterogenization of Metallocene Catalysts for Alkene Polymerization**

Hendrikus C. L. Abbenhuis*

Metallocenes, when used in combination with suitable activating agents, are extremely active catalysts for alkene polymerization.^[1] At present, research in this area has reached such a level of sophistication that new metallocenes and activating agents can be more or less designed in a rational way. Current work focuses on controlling the stereoselectivity of alkene polymerization and, increasingly, minimizing competing insertion and chain-transfer reactions as well as determining the mechanisms that account for catalyst deactivation.

In developing large-scale industrial processes for gas-phase and slurry polymerization of alkenes it is extremely important to heterogenize the essentially homogeneous metallocene/activator catalyst systems. Without heterogenization of metallocenes many new polyolefins could not be produced at all on an industrial scale! The reason is that although homogeneous polymerization catalysts form at best finely divided polymer

powders, a heterogeneous catalyst can control the morphology of the polymer formed. As a result the formed polymer beads are enlarged duplicates of the catalyst particles (Figure 1).^[2]

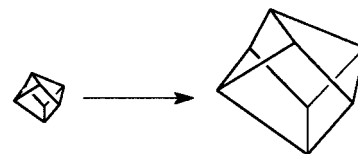


Figure 1.

Due to the complexity of the systems, catalyst immobilization is difficult to approach at the molecular level. Common supports, such as high-surface-area silica and alumina have received the most attention and have proved to be very successful for the commercial-scale production of polyolefins.^[3] Yet despite their apparent simplicity, these supports have undesirable or ill-exploited properties that can lead to multiple active sites, catalyst deactivation, and leaching. Clearly, support characteristics such as microstructure, surface functionalities, and polarity must be further investigated, and catalyst-anchoring techniques including, for instance, grafting, tethering, physisorption, and electrostatic immobilization must be devised. This highlight describes some approaches to developing new catalyst supports and the corresponding methods to best exploit valuable metallocene catalysts.

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Table 1. Selected data on alkene polymerization by (supported) metallocenes.

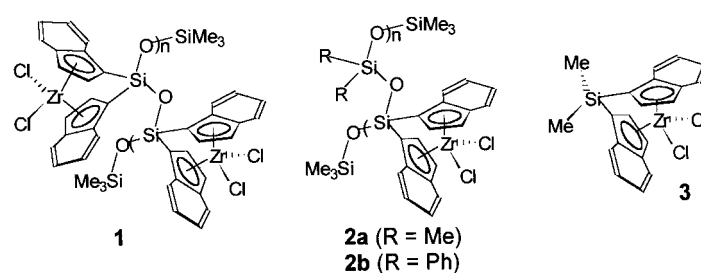
Entry	Support	Metallocene ^[a]	Al:M	Alkene	Activity ^[b]	$M_n \times 10^{-3}$ ^[c]	M_w/M_n ^[d]	Ref.
1	MCM-41	$[\text{C}_2\text{H}_4(1\text{-ind})_2\text{Zr}(\text{CH}_3)_2]$	40:1	ethene/propene	3.88	1.28	1.94	[7]
2	$\text{SiO}_2\text{-SiMe}_3$	$[\text{Cp}_2\text{ZrMe}_2]$	0	ethene	0.046	—	—	[8]
3	poly(siloxane)	1	5000:1	ethene	7.80	290	1.9	[9]
4	poly(siloxane)	1	5000:1	propene	3.40	20 ^[e]	3.3	[9]
5	poly(siloxane)	2a	5000:1	ethene	2.20	120	3.2	[9]
6	poly(siloxane)	2b	5000:1	ethene	2.90	120	1.7	[9]
7	—	3	250:1	ethene	12.0	900	—	[10a]
8	—	3	10000:1	propene	22.0	78	1.7	[10b]
9	polystyrene derivative	4	—	ethene/1-hexene	0.422	99.6	2.9	[11]
10	polystyrene derivative	5	1000:1	ethene	0.041	—	—	[12]

[a] Abbreviations: ind = indenyl; Cp = cyclopentadienyl. [b] In units of 10^6 g of polymer per mole of Zr per atmosphere per hour. [c] Weight-average molecular weight. [d] Ratio of weight- to number-average molecular weight. [e] Ratio of the fraction soluble to that insoluble in boiling pentane is 33.5:66.5.

When the common activator methyl aluminoxane (MAO) is used in homogeneous metallocene catalysis, the required ratio of activator to metallocene can be on the order of 1000:1 or even higher!^[4] With the new heterogenization methods the amount of activator can be reduced considerably, and MAO:metallocene ratios as low as 40:1 have been used successfully in specific cases.^[5] In one interesting approach to in situ heterogenization aluminosilane-coated silica is prepared: the adsorbed water on silica gel reacts directly with a trimethylaluminum solution to form an aluminosilane-coated silica.^[6] Treatment of the resulting support with a metallocene precursor results in the desired immobilized catalyst with a high catalytic activity. This methodology was recently applied to prepare mesoporous MCM-41 type silicates used as supports for $[\text{C}_2\text{H}_4(1\text{-indenyl})_2\text{Zr}(\text{CH}_3)_2]$. This immobilized catalyst was found to be active for the copolymerization of ethene and propene, and a low Al:Zr ratio (40:1 to 360:1) was also possible.^[7] Representative polymerization data for the catalysts described here are given in Table 1. A noteworthy finding was that the molecular weight of the copolymer formed increases with the decreasing pore radius of the MCM-41 support. Since the copolymers were either oils (m.p. -20°C) or waxes (m.p. -8°C), the relation between catalyst and polymer morphologies could not be investigated. This should be a topic for further research because the uniform hexagonal structure of MCM-41 is clearly different from that of the commonly employed amorphous silica. Finally, silylated silicas were recently reported to be suitable supports for $[\text{Cp}_2\text{Zr}(\text{CH}_3)_2]$. This system enables morphology control in ethene polymerization even without activation by aluminosilane.^[8] The catalytic activity, however, was low $[46 \text{ kg PE}(\text{mol Zr h bar})^{-1}]$.

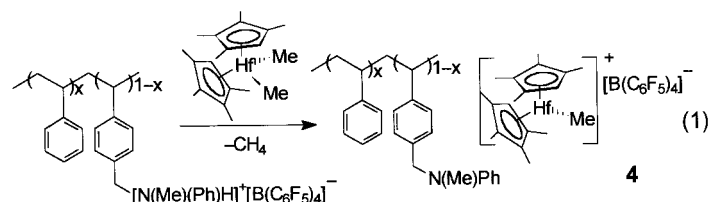
Other current research is aimed at linking metallocenes to organic supports having a more defined structure. This work may eventually result in heterogeneous catalysts in which the spacing of the active sites over the support can be controlled. For instance, the new poly(siloxane)-supported zirconocene precatalysts **1** and **2** can be activated with methylaluminoxane in the conventional way, in other words, with high Al:Zr ratios (5000:1 to 10000:1).^[9]

The zirconocene catalysts supported on poly(bisindenyl-siloxane) and poly(bisfluorenylsiloxane) gave high activities for ethene polymerization $[\text{up to } 10300 \text{ kg PE}(\text{mol Zr h bar})^{-1}]$



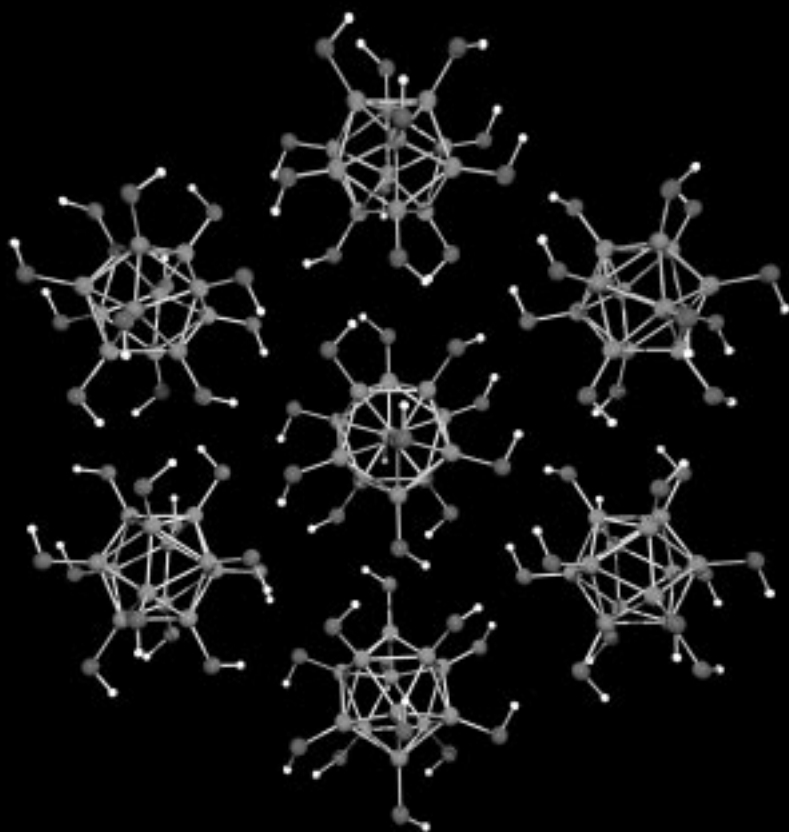
and moderate activities for propene polymerization $[\text{up to } 5900 \text{ kg PP}(\text{mol Zr h bar})^{-1}]$. For comparison, the activity of the closely related complex $[\text{Me}_2\text{Si}(1\text{-indenyl})_2\text{ZrCl}_2]$ (**3**) in homogeneous catalysis is also given in Table 1.^[10] The molecular mass distributions of the polymers were rather broad (ca. 1.7 to 9.1), and during propene polymerization large amounts of atactic polymer formed. Both observations might be related to the way the supported metallocenes were generated, which likely provided mixtures of *rac* and *meso* isomers.

Modified polystyrene beads have been examined as interesting catalyst supports and have led to several new immobilization techniques. One of these is the phase immobilization of active cationic metallocene sites on noninteracting polystyrene.^[11] Supported catalysts were prepared by treating lightly cross-linked, chloromethylated polystyrene beads successively with a secondary amine, an ammonium salt of a weakly coordinating anion (the actual activating agent), and finally a neutral dialkylmetallocene. The last step of this sequence generating the active heterogeneous catalyst **4** is

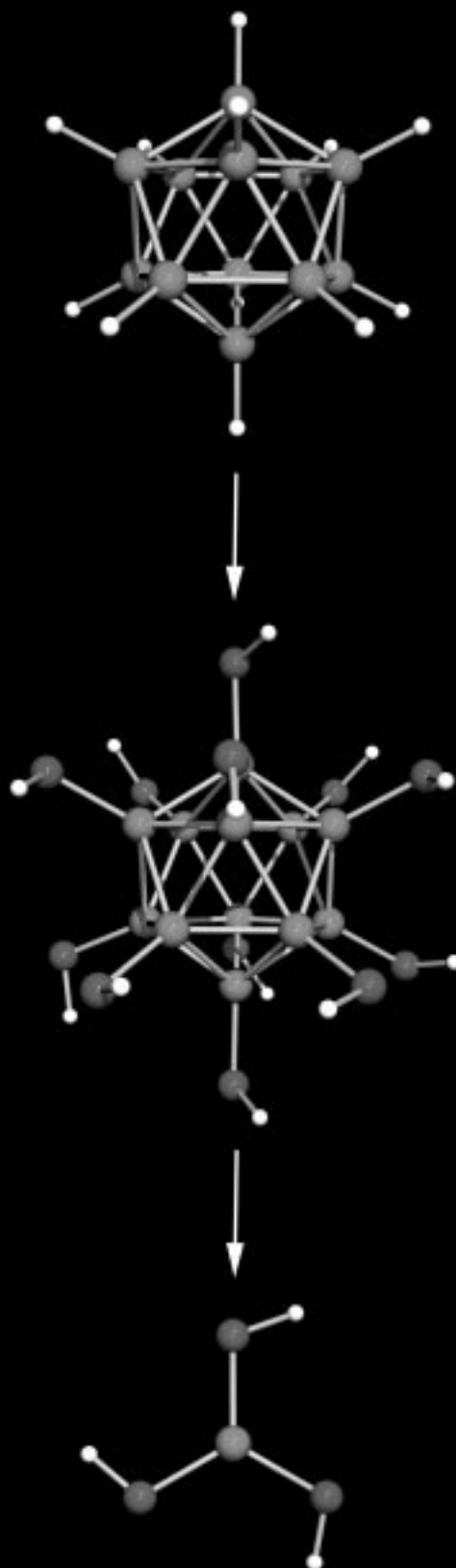


shown in Equation (1). Catalytic sites were found to be distributed homogeneously throughout the support particle. In contrast to reactions with surface-supported metallocene catalysts, the polymerization occurs within the catalyst

Oxidation of icosahedral boranes such as $[\text{B}_{12}\text{H}_{12}]^{2-}$ by hydrogen peroxide leads to a fascinating group of per-B-hydroxylated borane clusters.



These “inorganic sugars” are crossbreeds of borate minerals and boron hydrides. More information is given on the following pages.



Aromatic Polyhedral Hydroxyborates: Bridging Boron Oxides and Boron Hydrides**

Toralf Peymann, Axel Herzog, Carolyn B. Knobler,
and M. Frederick Hawthorne*

Polyhydroxylated molecules have fascinated scientists pursuing various disciplines. Carbohydrates are ubiquitous in nature and serve as a source for chemical energy (glucose), the backbone for genetic information (ribose), and the organic constituents of plants (cellulose) or insects (chitin).^[1] The three-dimensional network of silica and its derived minerals^[2] results from the condensation of polyhydroxylated silicates. The most prominent polyhydroxylated boron compound is boric acid $B(OH)_3$. Its alkaline solutions deposit $Na_2[B_4O_5(OH)_4] \cdot nH_2O$, found in two abundant boron minerals, kernite ($n = 2$) and borax ($n = 8$).^[3] Except for the trigonal and tetrahedral boron–oxygen units common to borate minerals,^[4] the recurring structural motif in boron chemistry is the icosahedron. The allotropes of elemental boron,^[5] boron-rich solids,^[6] and the parent anion of the polyhedral boranes, $[closo-B_{12}H_{12}]^{2-}$ (**1**),^[7] all contain B_{12} icosahedra.

The charge-delocalized icosahedral ion **1** may be considered as the parent aromatic species that serves borane chemistry as benzene serves organic chemistry.^[8] Isoelectronic substitution of one or two $:B-H$ vertices in **1** by $:C-H^+$ provides the aromatic derivatives $[closo-1-CB_{11}H_{12}]^-$ (**2**) and three isomeric dicarbaboranes (1,2- or *ortho*; 1,7- or *meta*; and 1,12- or *para*) $closo-C_2B_{10}H_{12}$.^[9] Each of these isoelectronic derivatives of **1** undergoes characteristic hydrogen substitution reactions at the $B-H$ vertices resulting in a huge number of known icosahedral species. Of special interest are derivatives in which every available $B-H$ vertex has been substituted. Thus, hydrophobic derivatives of **1**, **2**, and the three isomeric dicarbaboranes, such as $[closo-B_{12}Cl_{12}]^{2-}$,^[10] $[closo-CB_{11}(CH_3)_{12}]^-$,^[11] and $closo-1,12-C_2B_{10}(CH_3)_{12}$,^[12] have been synthesized. Similar highly substituted polyhedral borane derivatives with hydrophilic substituents such as hydroxyl are, until now, unknown. We here report the synthesis of per-*B*-hydroxylated icosahedral borane derivatives, which may be considered to be derivatives of a new type of polyhedral subboric acid. Described are the per-*B*-hydroxylated icosahedra $Cs_2[closo-B_{12}(OH)_{12}]$, $Cs_2\text{-3}$; $Cs[closo-1-H-1-CB_{11}(OH)_{11}]$, $Cs\text{-4}$; and $closo-1,12-H_2-1,12-C_2B_{10}(OH)_{10}$ (**5**); prepared by the oxidation of the icosahedral boranes $Cs_2\text{-1}$, $Cs\text{-2}$, and $closo-1,12-(CH_2OH)_2-1,12-C_2B_{10}H_{10}$ (**6**), respectively, with 30% hydrogen peroxide at the reflux temperature. The new compounds **3–5** were characterized by ^{11}B NMR spectro-

scopy, electrospray mass spectrometry, and, for $Cs_2\text{-3}$ and **5**, single-crystal X-ray diffraction.

The ^{11}B NMR spectra of species **3–5** are consistent with their structures in that **3** (point group I_h) gave a singlet at $\delta = -17.1$, **4** (C_{5v}) exhibited three singlets at $\delta = -7.3$, -15.3 , and -17.1 , and **5** (D_{5d}) displayed a singlet at $\delta = -17.0$. Electrospray mass spectrometry confirmed the m/z values expected for the assigned polyhydroxylated structures **3** and **4**. The water-soluble salts $Cs_2\text{-1}$ and $Cs\text{-2}$ served as precursors to ions **3** and **4**, whereas the synthesis of **5** employed the slightly water-soluble precursor **6**, because $closo-1,12-C_2B_{10}H_{12}$ is water-insoluble and hence not available to the hydrogen peroxide reagent. During this reaction sequence, the diol **6** is most likely oxidized to the corresponding dicarboxylic acid, which subsequently decarboxylates during *B*-hydroxylation to afford species **5**.

Because the per-*B*-hydroxylated species are thermodynamically unstable with respect to boric acid, continued degradation to this product under experimental conditions is expected and observed. The yields of the syntheses reported here are: **5** (80%), **3** (65%), and **4** (31%). This distribution reflects the higher kinetic stabilities of the two centrosymmetric cage structures **1** and $closo-1,12-C_2B_{10}H_{10}$ relative to that of the polarized cluster **2**.

One unexpected feature of the anion **3** is the low solubility of its alkali metal salts in water even though its surface is covered with hydroxyl groups. Thus, $Cs_2\text{-3}$ can be recrystallized from water, whereas $Li_2\text{-3}$, $Na_2\text{-3}$, and $K_2\text{-3}$ precipitate quantitatively upon addition of the corresponding alkali metal chloride to warm aqueous solutions of $Cs_2\text{-3}$. The anion **3** probably functions as a strong multihapto ligand for the smaller alkali metal ions and displaces their water of hydration, cross-linking and precipitating the aggregated alkali metal salts. The per-*B*-hydroxylated $closo-1,12$ -carborane **5** is insoluble in water as well as in organic solvents. This property of **5** can be attributed to the absence of a dipole moment and the strong network of the hydrogen bonding involving every pendant hydroxy group and $C-H$ as well as water in the crystal lattice. The close packing of this network is reflected by the density of **5** ($\rho = 1.73 \text{ g cm}^{-3}$), which is unusually high for a covalent organic compound (for comparison: the density of $closo-1,12-H_2-1,12-C_2B_{10}(CH_3)_{10}$ is 0.97 g cm^{-3}).^[12] The overall geometry of the icosahedral framework in both **3** and **5** is not affected by per-*B*-hydroxylation.

In the solid-state structure of **5** (Figure 1),^[13] the $B-B$ (177.6(5)–183.7(5) pm) and $B-C$ (171.6(5)–173.4(5) pm) bond lengths are similar to those of unsubstituted $closo-1,12-C_2B_{10}H_{12}$ determined by electron diffraction ($B-B$ 177.2(13)–179.2(7) pm, $B-C$ 171.0(11) pm).^[14] Similarly, the $B-B$ distances^[15] of anion **3** (Figure 2) (177.5(9)–181.4(9) pm) do not deviate significantly from those present in **1** (175.5(7)–178.0(7) pm).^[7] The $B-O$ bond lengths of the anion **3** are elongated by about 5 pm compared with those of **5** (**3** 143.3(7)–145.9(9) pm; **5**: 138.6(4)–140.3(4) pm), which may be due to the interaction of the delocalized negative charge in **3** and the $B-O$ dipoles.

The dianion **3** is only the second derivative of **1** to be described that is saturated with twelve chalcogen substituents. The first to be synthesized was the selenoborate $Cs_8[closo-$

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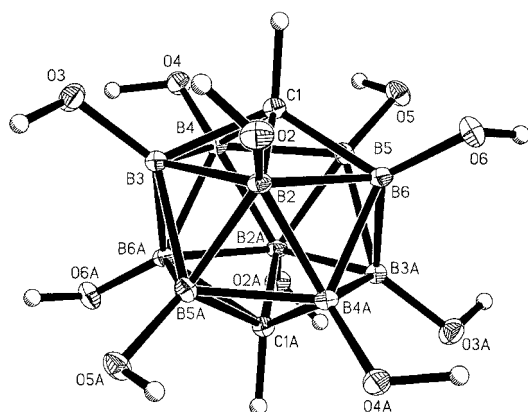


Figure 1. ORTEP diagram of **5**. The ellipsoids represent a 30 % probability level. Selected bond lengths [pm]: B–B 177.6(5)–183.7(5), B–O 138.6(4)–140.3(4), B–C 171.6(5)–173.4(5).

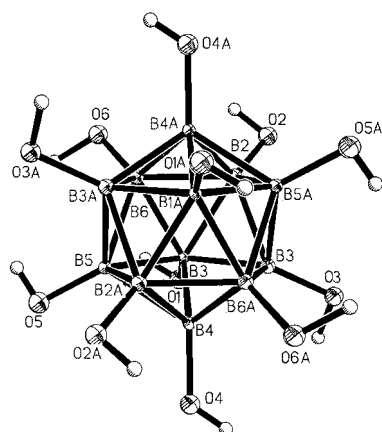


Figure 2. ORTEP diagram of **3**. The ellipsoids represent a 30 % probability level. Selected bond lengths [pm]: B–B 177.5(9)–181.4(9), B–O 143.3(7)–145.9(9).

$B_{12}(Se_2BSe)_6$ obtained directly from elemental boron, Cs_2Se , and selenium at 700 °C in a sealed glass ampoule.^[16] The structure of this species displays Se–B(Se)–Se bridges between neighboring boron vertices of the icosahedron. Related species, in which two or three neighboring B–O vertices of **3–5** are bridged by a trigonally or tetrahedrally surrounded boron atom, appear to be likely targets for synthesis ($Cs_2[closo-B_{12}(O_2BOH)_6]$ as an example).

Species **3–5** suggest the potential existence of a new field of boron cluster chemistry, wherein the aromatic icosahedral cluster functions only as the scaffolding for reactions that would be performed on its oxygen sheathing, such as the use of **3** as the central core for dendrimeric chemistry.^[17] Furthermore, it is conceivable that the high-temperature pyrolysis of a simple salt of **3** might form a very stable polymeric network of icosahedra connected by covalent B–O–B bonds. Species of this sort should be very robust due to strong B–O bonds, but their dianionic cages should serve as a source of electrons for chemical processes such as $B_{12}(OH)_{12}^{2-} + 24 OH^- \rightarrow 12 B(OH)_3 + 26 e^-$ and perhaps provide some water solubility. In addition, the possibilities for the covalent incorporation of species such as **3** in metal oxide lattices are boundless. The organic chemistry of **4** and **5** and

their derivatives similarly opens new areas of novel chemical cross-fertilization.

Experimental Section

Caution! On this scale and under these conditions no explosions occurred. Nevertheless, this does not preclude such an event when dealing with these species. Extreme precautions should be taken!

The ^{11}B NMR spectra were obtained with a Bruker AM-500 spectrometer at 160 MHz. ^{11}B NMR chemical shifts were externally referenced to $BF_3 \cdot Et_2O$; peaks upfield of the reference are designated as negative. ESI mass spectra were recorded by injecting the sample dissolved in water into an ionspray source. The mass spectrometer was operated in the negative-ion mode.

Cs₂-3: A suspension of **Cs₂-1** (1.00 g, 2.50 mmol) in 30 % hydrogen peroxide (40 mL) was refluxed for three days. The obtained solution was cooled overnight in a refrigerator to precipitate crude **Cs₂-3**, which was recrystallized from water. The product was collected by filtration and dried to yield pure **Cs₂-3** (0.97 g, 65 %) as a white powder. ^{11}B NMR (H_2O): $\delta = -17.1$ (s); ESI-MS: m/z : 335.1 $[H][B_{12}(OH)_{12}]^-$.

Cs-4: A suspension of **Cs-2** (0.10 g, 0.36 mmol) in 30 % hydrogen peroxide (10 mL) was refluxed for five hours. The obtained solution was cooled overnight in a refrigerator to precipitate crude **Cs-4**, which was recrystallized from water. The product was collected by filtration and dried to yield pure **Cs-4** (0.05 g, 31 %) as a white powder. ^{11}B NMR (H_2O): $\delta = -7.3$ (s, B12), -15.3 (s, B2-6), -17.1 (s, B7-11); ESI-MS: m/z : 319.0 $[HCB_{11}(OH)_{11}]^-$.

5: A suspension of **6** (1.00 g, 4.90 mmol) in 30 % hydrogen peroxide (75 mL) was refluxed for 12 hours. A colorless precipitate formed, which was filtered off and washed with water. After drying at 150 °C/ 5×10^{-5} Torr pure **5** (1.19 g, 80 %) was obtained as a white powder. ^{11}B NMR (H_2O): $\delta = -17.0$.

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- [13] a) $5 \cdot 2 H_2O$, monoclinic, space group $C2/c$, $a = 15.960(13)$, $b = 7.812(6)$, $c = 11.867(10)$ Å, $\beta = 118.28(2)^\circ$, $V = 1303(2)$ Å³, $\rho = 1.73$ g cm⁻³, $2\theta_{max} = 60^\circ$, $\lambda(MoK\alpha) = 0.71069$ Å, $\theta - 2\theta$ scan mode, 298 K. Of the 1902 unique reflections measured, 1013 were considered observed [$I > 2\sigma(I)$]. Data were corrected for Lorentz and polarization effects but not for absorption, $\mu = 1.5$ cm⁻¹. Atoms were located by use of

statistical methods (SHELX86), and 115 parameters were refined. Water hydrogen atoms were included as located. All other hydrogen atoms were included in calculated positions after they had been located. $R = 0.067$, $wR = 0.193$, refined against $|F^2|$. b) Further details of the crystal structure investigations can be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49)7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository numbers CSD-410414 (**5**) and CSD-410413 (**3**).

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The Equilibrium between Localized and Delocalized States of Thermochromic Semibullvalenes and Barbaralanes—Direct Observation of Transition States of Degenerate Cope Rearrangements

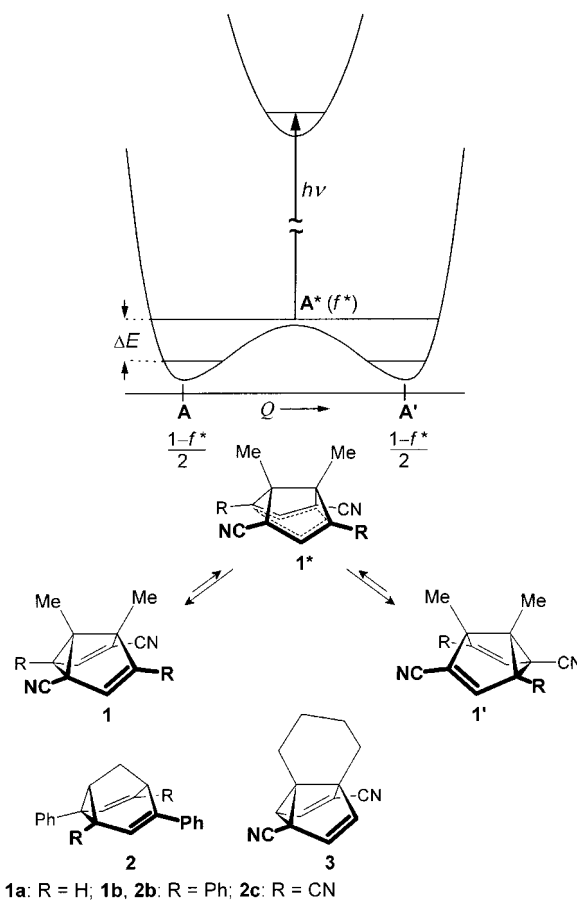
Helmut Quast* and Maximilian Seefelder

Dedicated to Professor William von Eggers Doering on the occasion of his 80th birthday

The Möbius–Hückel concept provides a *theoretical* link between pericyclic transition states and normal molecules.^[1] As yet an *experimental* link has not existed. Von E. Doering's seminal design and first examples of molecules that are fluxional because of a very fast degenerate Cope rearrangement^[2] were followed by Zimmerman's discovery of semibullvalene^[3] and the demonstration that the ground state and the transition state for its Cope rearrangement differ in energy by just a few kcal mol⁻¹.^[4] Hoffmann and Stohrer^[5] and Dewar and Lo^[6] predicted substitution patterns for semibullvalenes that would lower and eventually abandon this energy gap, and thus result in stable delocalized, bishomoaromatic semibullvalenes.^[7] A similar formal mutation of shallow degenerate double-well potentials into single-minimum systems is known from the pyramidal inversion of ammonia and its derivatives.^[8]

The discovery that the semibullvalenedicarbonitrile **1a** is thermochromic suggested an equilibrium between the classical, localized structure **1a** and its delocalized, bishomoaro-

matic isomer **1a***, which was estimated to be higher in energy by about 11 kJ mol⁻¹ (Scheme 1).^[9] Subsequently, the semibullvalenes **1b**^[10] and **3**^[11] and the barbaralanes **2b**^[12] and **2c**^[13]



Scheme 1.

showed that thermochromism is common to degenerate systems with extremely low Cope barriers.^[14] We report here the quantitative analysis of this phenomenon. Enthalpy differences of as little as 1 kJ mol⁻¹ are found to govern the equilibrium between the two degenerate ground states **A**, **A'** and the species **A*** which absorb at long wavelengths. The latter are interpreted as higher, delocalized states, located just above the flat potential barrier between the two ground states, namely, where vibronic interaction is strong.

UV/Vis spectra were recorded at various temperatures for degassed solutions of **1–3** in butyronitrile. This solvent was chosen because it is sufficiently transparent in the near-UV region and has a broad temperature range in the liquid state. Careful attention has been given to the reversibility of the temperature-driven changes in the spectra. The absorbance was corrected for the thermal expansion of the solvent with the help of density data^[15] and refers to a temperature of 298 K.

For the determination of the enthalpy difference ΔH^0 between the differently absorbing species by means of the temperature-dependent long-wavelength bands, we regard their integrated absorbance A_{int} as a measure of the fraction f^* which exists in the higher state **A***. The integrated absorbance A_{int} is obtained by deconvolution of the UV/Vis spectra with Gauss curves^[16] (Figures 1–3). Toward this end, Gauss curves

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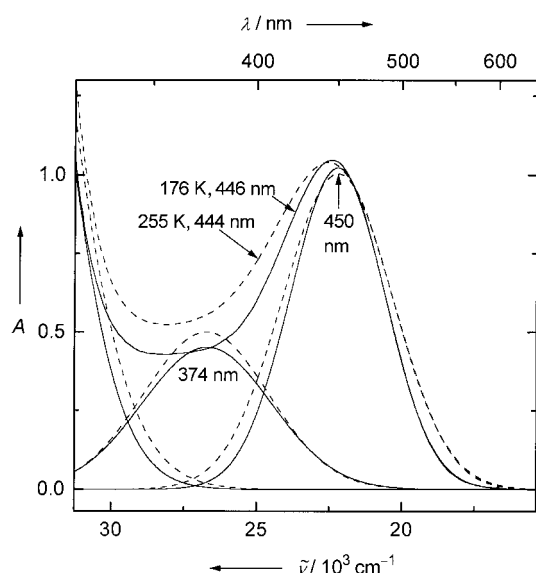


Figure 1. UV/Vis spectra of **1b** ⇌ **1b*** ⇌ **1b'** recorded in degassed butyronitrile ($c = 7.69 \times 10^{-4} \text{ mol L}^{-1}$), and the calculated Gauss curves at the highest (dashed curves) and lowest temperature (solid curves).

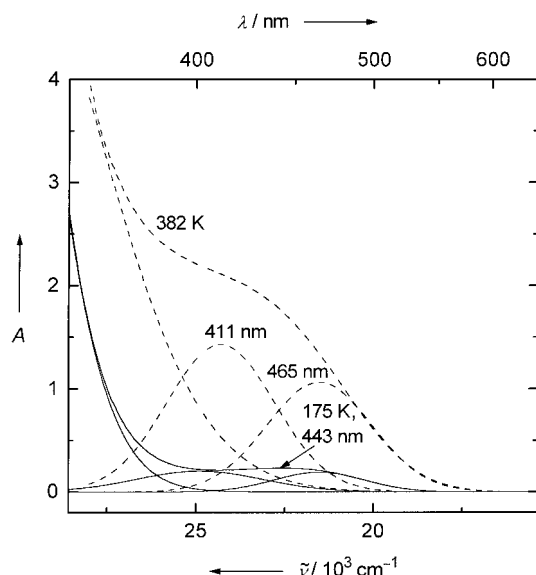


Figure 2. UV/Vis spectra of **2b** ⇌ **2b*** ⇌ **2b'** recorded in degassed butyronitrile ($c = 2.84 \times 10^{-3} \text{ mol L}^{-1}$), and the calculated Gauss curves at the highest (dashed curves) and lowest temperature (solid curves).

with fixed maxima were fitted to the long-wavelength tails of the spectra. The wavelength of the maxima were determined from the spectra recorded at the lowest temperatures, where the interfering Doppler broadening of the intense short-

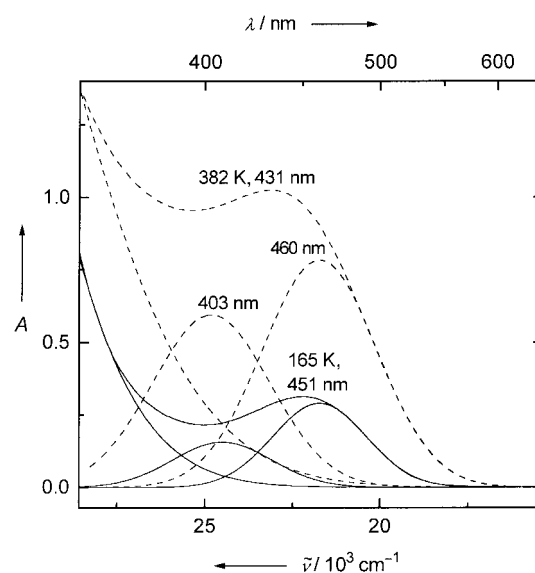


Figure 3. UV/Vis spectra of **2c** ⇌ **2c*** ⇌ **2c'** recorded in degassed butyronitrile ($c = 1.25 \times 10^{-3} \text{ mol L}^{-1}$), and the calculated Gauss curves at the highest (dashed curves) and lowest temperature (solid curves).

wavelength absorptions is smallest. The best fit obtained by variation of the wavelength of the maxima afforded the λ_{max}^* values that were also employed at higher temperatures. We note that the ΔH^0 values depend only slightly on the precise position of these maxima. In fact, deliberate variation of λ_{max}^* by $\pm 10 \text{ nm}$ resulted in only the small errors given in Table 1. Likewise, insignificant errors of less than 0.05 kJ mol^{-1} were introduced by the choice of smaller or larger ($\pm 10 \text{ nm}$) long-wavelength sections of the UV/Vis spectra for the fitting of the Gauss curves.

While single Gauss curves sufficed for the near-UV spectra of the semibullvalenedicarbonitriles **1a**^[9] and **3**,^[11] the near-UV/Vis spectra of the higher substituted systems **1b**, **2b**, and **c** could not be reproduced in that way. Surprisingly, subtraction of the calculated long-wavelength Gauss curve from the total spectrum uncovered a pronounced shoulder at the steep ascent toward shorter wavelengths, which indicated the presence of a second temperature-dependent band of similar intensity (Figures 1–3). The strong overlap with the intense absorptions at shorter wavelengths precluded the calculation of the integrated absorbance of this band with a precision sufficient for a quantitative analysis.^[17]

The expression of the equilibrium constant K for the two degenerate states **A**, **A'** and the higher state **A*** [Eq. (1)] is solved for f^* . Replacement of f^* in the Lambert–Beer law [Eq. (2); where ϵ_{int}^* = integrated extinction coefficient of the

Table 1. Enthalpy differences (ΔH^0) between the ground states **A**, **A'** and the higher states **A***, as well as the integrated extinction coefficients (ϵ_{int}^*) as computed by a least-squares fit of Equation (4) to the integrated absorbance versus temperature data. The error in ΔH^0 results from a deliberate variation of λ_{max}^* by $\pm 10 \text{ nm}$; the statistical error is about $0.005 \text{ kJ mol}^{-1}$. The squares of the correlation coefficients (r^2), and estimated maxima (λ_{max}^*) and calculated extinction coefficients (ϵ_{max}^*) for the long-wavelength band of **A*** are also listed.

Compound	ΔH^0 [kJ mol ⁻¹]	$\epsilon_{\text{int}}^* 10^{-3}$ [L mol ⁻¹ cm ⁻²]	r^2	λ_{max}^* [nm]	$\epsilon_{\text{max}}^* 10^{-3} (T [\text{K}])$ [L mol ⁻¹ cm ⁻¹]
1a ⇌ 1a* ⇌ 1a'	11.06 ± 0.44	50.6	1.0000	360	9.2 (255)
1b ⇌ 1b* ⇌ 1b'	0.82 ± 0.14	24.5	0.9997	450	5.1 (255)
2b ⇌ 2b* ⇌ 2b'	5.51 ± 0.18	17.3	0.9989	465	5.0 (259)
2c ⇌ 2c* ⇌ 2c'	3.30 ± 0.23	17.0	0.9991	460	4.7 (253)
3 ⇌ 3* ⇌ 3'	8.58 ± 0.23	38.3	0.9999	360	5.3 (259)

temperature-dependent long-wavelength band [$\text{L mol}^{-1} \text{cm}^{-2}$]; c = total concentration [mol L^{-1}]; d = path length [cm],^[18] which has been shown to be valid for **1a**^[9] and **2c**, yields the relationship between A_{int} and the thermodynamic parameters of the equilibrium [Eq. (3)]. The overall entropy difference ΔS^0 is the sum of the entropy difference that is due solely to the difference in symmetry numbers σ between **A**, **A'** and **A***, $R \ln(\sigma_{\text{A,A'}}/\sigma_{\text{A*}})$, and a second term that contains all other contributions to ΔS^0 .^[19] We obtain the simplified, approximate Equation (4) by assuming that the latter have the same value for **A**, **A'** and **A***, and **A*** has a structure of twofold rotational symmetry, that is, $\sigma_{\text{A*}} = 2$ (see below).^[20] Equation (4) may also be derived on the basis of the symmetry-corrected equilibrium constant $K_{\text{corr}} = [\text{A*}]/[\text{A}] = 2f^*/(1-f^*)$ instead of $K = [\text{A*}]/([\text{A}] + [\text{A'}])$ [Eq. (1)] and the assumption that the entropy difference between **A** and **A*** is zero. The least-squares fit of Equation (4) to the A_{int} versus temperature data yielded the two parameters $\varepsilon_{\text{int}}^*$ and ΔH^0 . The approximation is supported by satisfactory agreement between the experimental data points and the computed curves, and by excellent correlation coefficients. The squares of the latter are listed in Table 1 together with the calculated parameters $\varepsilon_{\text{int}}^*$ and ΔH^0 .

$$K = \frac{f^*}{1-f^*} = \exp\left(-\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}\right) \quad (1)$$

$$A_{\text{int}} = \varepsilon_{\text{int}}^*(f^*c)d \quad (2)$$

$$A_{\text{int}} = \varepsilon_{\text{int}}^*cd \left(1 + \exp\left(-\frac{\Delta S^0}{R} \exp\left(\frac{\Delta H^0}{RT}\right)\right)\right)^{-1} \quad (3)$$

$$A_{\text{int}} \approx \varepsilon_{\text{int}}^*cd \left(1 + 2 \exp\left(\frac{\Delta H^0}{RT}\right)\right)^{-1} \quad (4)$$

Credence is lent to the analytical procedure by its application to the thermochromism reported by Okazaki et al. for the fluxional system **4** $\rightleftharpoons$ **5**.^[21] Calculation of A_{int} ($\lambda_{\text{max}}^* = 350 \text{ nm}$) from the five published UV/Vis spectra and the least-squares fit of an equation, which is identical to Equation (4) except for the lack of the pre-exponential factor 2, to the A_{int} versus T data yielded $\Delta H^0 = 3.8 \text{ kJ mol}^{-1}$. This value agrees well with the difference between the free enthalpies $\Delta G^0(303 \text{ K}) = 4.2 \text{ kJ mol}^{-1}$, which was estimated on the basis of chemical shifts in the ^{13}C NMR spectra.^[21]



The temperature dependence of the fractions f^* that exist in the higher state **A*** was calculated from Equations (2) and (4) and is displayed in Figure 4. The f^* versus T curves are determined only by the enthalpy difference ΔH^0 between the two states. A cursory inspection of Figure 4 allows an explanation of previous, puzzling observations: The yellow semibullvalenedicarbonitriles **1a** and **3** completely fade at temperatures below 200 K, while, in the case of **1b** sizeable amounts of the orange-red species survive even on cooling with liquid nitrogen.

The enthalpy differences ΔH^0 , which were derived from variable-temperature UV/Vis spectra recorded for butyroni-

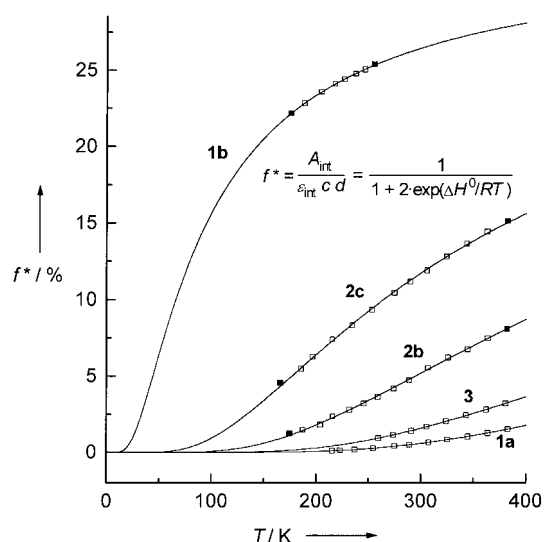


Figure 4. Temperature dependence of the fractions f^* that exist in the higher state **A*** of the thermochromic semibullvalenes **1**, **3**, and the barbaralanes **2**. Filled squares correspond to the UV/Vis spectra displayed in Figures 1–3.

trile solutions (Table 1), are composed of the gas-phase enthalpy differences $\Delta H_{\text{A/A*}}^0$ and the solvation terms $(\Delta H_{\text{A}})_{\text{BN}}$ and $(\Delta H_{\text{A*}})_{\text{BN}}$, which stem from the transfer of **A**, **A'** and **A***, respectively, from the gas phase to the butyronitrile solvent [Eq. (5)].

$$\Delta H^0 = \Delta H_{\text{A/A*}}^0 + (\Delta H_{\text{A*}})_{\text{BN}} - (\Delta H_{\text{A}})_{\text{BN}} \quad (5)$$

We interpret^[12] the gas-phase enthalpy differences $\Delta H_{\text{A/A*}}^0$ on the basis of the ubiquitous system of adiabatic energy hypersurfaces that consists of a symmetrical, shallow double-well ground-state potential and a single-minimum excited-state potential (Scheme 1).^[22] Recently, Zilberg et al. demonstrated that this system can be readily derived for semibullvalene with the valence bond configuration-mixing method. They calculated the energy gap between the two adiabatic surfaces at the geometry of the transition structure on the lower surface to be 5.16 eV, a value which corresponds to a Franck–Condon transition at $\lambda_{\text{max}} = 240 \text{ nm}$.^[23] By taking into account the effects of the substituents, we note that the UV/Vis properties of the higher states **A*** of the present substituted semibullvalenes and barbaralanes (Table 1) are in accord with Zilberg's λ_{max} value for the transition structure of semibullvalene and the UV absorptions predicted for C_{2v} -semibullvalene by Borden, von R. Schleyer et al.^[24]

Within the limits of this simple model, the species **A*** which absorb at long wavelengths are considered as vibronic eigenstates that are located just above the low potential barrier toward the degenerate Cope rearrangement **A** $\rightleftharpoons$ **A'**. Thus $\Delta H_{\text{A/A*}}^0$ corresponds to $N_{\text{L}} \Delta E$ (N_{L} = Loschmidt's number), where ΔE is the energy of the transition with the exceptional fundamental frequency associated with the vibration along the reaction coordinate.

Unfortunately, gas-phase enthalpy differences are not directly available, and possible relations between ΔH^0 values measured for solutions and the structures of the compounds are obscured by solvent effects.^[25] The values of ΔH^0 are very

small indeed and in the range of van der Waals and other weak solute–solvent interactions.^[26] Only in the case of **1a** can the enthalpy difference ΔH^0 between **A**, **A'** and **A*** be compared with the barrier for the degenerate Cope rearrangement $\mathbf{A} \rightleftharpoons \mathbf{A}'$, $\Delta G^\ddagger(200\text{ K}) = 14.4\text{ kJ mol}^{-1}$ (in chlorodifluoromethane/ $[\text{D}_6]$ dimethyl ether).^[27] Those barriers are too low to be determined by ^{13}C NMR spectroscopy for the other compounds, and any estimates based on increments for substituents^[27] must assume their additivity, which can hardly be justified. Nevertheless, qualitative arguments on the basis of cooperative steric and electronic substituent effects, and constant solvation terms support the conclusion that the ΔH^0 values roughly parallel the heights of the Cope barriers.

Taking together all experimental observations and theoretical arguments, one cannot escape the conclusion that the species **A*** are indeed vibronic states above the potential energy maximum between the shallow wells **A** and **A'**, and, consequently, correspond to transition states. In terms of a conventional structural representation, they are delocalized, bishomoaromatic semibullvalenes and barbaralanes. While experimental investigations of transition states have so far been the domain of femtosecond chemistry^[28] or other highly sophisticated techniques,^[29] thermochromic semibullvalenes and barbaralanes allow the observation of transition states even with one's naked eye.

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Solvent Effects on the Equilibrium between Localized and Delocalized States of Thermochromic Semibullvalenes and Barbaralanes

Maximilian Seefelder and Helmut Quast*

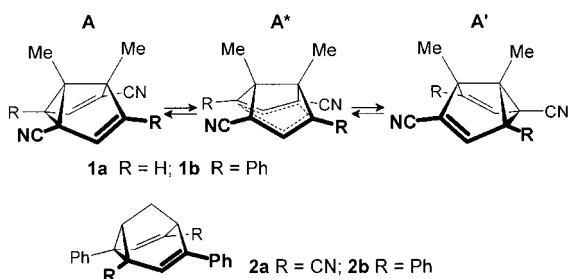
Dedicated to Professor Matthias Seefelder

Thermochromic semibullvalenes **1** and barbaralanes **2** undergo extremely rapid degenerate Cope rearrangements and, therefore, provide an opportunity to study equilibria between the two degenerate, localized ground states **A**, **A'** and the delocalized higher states **A*** that correspond to the transition states. The analysis of variable-temperature UV/Vis spectra with the help of Equation (1) showed that considerable fractions f^* exist in the higher states **A*** and disclosed enthalpy differences ΔH^0 of 1–11 kJ mol⁻¹ for solutions in butyronitrile (A_{int} and $\varepsilon_{\text{int}}^*$ = integrated absorbance and integrated extinction coefficient [L mol⁻¹ cm⁻²] of the temperature-dependent long-wavelength band; c = total concentration [mol L⁻¹]; d = path length [cm]). These enthalpy differences comprise the gas-phase enthalpy differences $\Delta H_{\text{A,A}^*}^0$ between **A**, **A'** and **A*** and the solvation terms $(\Delta H_{\text{A}})_L$ and $(\Delta H_{\text{A}^*})_L$ for the transfer from the gas phase to solution [Eq. (2)].^[1]

$$A_{\text{int}} \approx \varepsilon_{\text{int}}^* c d \left(1 + 2 \exp \frac{\Delta H^0}{RT} \right)^{-1} \quad (1)$$

$$\Delta H^0 = \Delta H_{\text{A,A}^*}^0 + (\Delta H_{\text{A}^*})_L - (\Delta H_{\text{A}})_L \quad (2)$$

Here we report the surprising influence of solvents on the relative stabilities. In fact, we have uncovered solvents that reverse the usual order of **A**, **A'** and **A***: In such solutions, the delocalized states **A*** which absorb at long wavelengths are more stable than the classical localized structures **A**, **A'**!



UV/Vis spectra were recorded at different temperatures for degassed and subsequently with argon-saturated solutions of **1** and **2** in different solvents and corrected for the temperature-driven change of the volume with the help of density data (reference temperature 298 K). The temperature ranges covered were dictated by the limited stability of the semi-

bullvalenes **1**, in particular **1b**, at higher temperatures^[2] and, of course, the freezing point of the solvents.

Selected UV/Vis spectra of solutions of the barbaralanedicarbonitrile **2a** recorded at the lowest and highest temperatures in different solvents are displayed in Figure 1. The

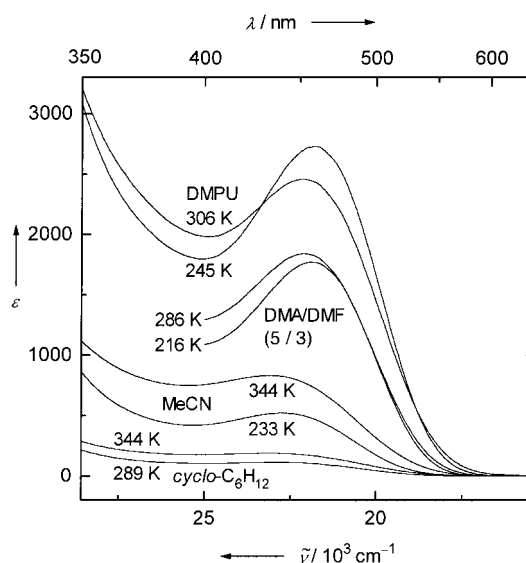


Figure 1. Near-UV/Vis spectra (ε [L mol⁻¹ cm⁻¹]) taken from solutions of the barbaralanedicarbonitrile **2a** $\rightleftharpoons$ **2a*** $\rightleftharpoons$ **2a'** in different solvents at the highest and lowest temperature.

extinction coefficients ε of the long-wavelength band may be considered as an approximate measure of the concentration of the delocalized species **2a***. A cursory inspection of Figure 1 immediately reveals the enormous influence of the solvent. The exchange of cyclohexane for *N,N'*-dimethylpropylene urea (DMPU) not only shifts the maximum of the band in the visible region toward longer wavelengths but increases ε and hence the concentration of **2a*** by one order of magnitude. We note that the deconvolution of the near-UV/Vis spectra of **1b**, **2a** and **2b** (butyronitrile solution) with Gauss curves disclosed the presence of a second temperature-dependent band at 374, 403, and 411 nm, respectively.^[1] This explains why the near-UV part of the spectra is influenced not only by the Doppler broadening of the strong UV bands but also by the variable absorbance of this second band.

The second intriguing result associated with the variation of the solvent is the reversal of the temperature dependence of the extinction coefficients. While they increase with rising temperatures for the solutions of **2a** in cyclohexane, acetonitrile, and most of the other solvents, they are temperature-invariant for a mixture of *N,N*-dimethylacetamide (DMA) and *N,N*-dimethylformamide (DMF) (5:3), and decrease for solutions in *N,N'*-dimethylpropylene urea and similar solvents, such as *N*-methylpyrrolidin-2-one, hexamethylphosphoric triamide (HMPA) and pure *N,N*-dimethylacetamide. This demonstrates the dominance of the solvation terms over the gas-phase enthalpy difference $\Delta H_{\text{2a/2a}^*}^0$ in these cases [Eq. (2)]: The two degenerate, localized states **2a**, **2a'** are more stable than the delocalized state **2a*** in the former solvents, while the opposite is true for solutions in the latter.

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Table 1. Enthalpy differences ΔH^0 [kJ mol⁻¹] calculated from Equation (1), as well as the solvents and temperature ranges T [K], in which the given number n of UV/Vis spectra were recorded.

Compound	No.	Solvent	ΔH^0 ^[a]	T	n	r^2 ^[b]
1a	1	DMF	9.42 ± 0.25	236–322	7	0.9973
	2	butyronitrile ^[1]	11.06 ± 0.04	215–383	11	1.0000
1b	3	DMA/DMF (5/3)	< 0	218–250	8	
	1	DMF	≈ 0	219–251	8	
	2	butyronitrile ^[1]	0.82 ± 0.05	176–236	8	0.9997
2a	4	DMPU	< 0	245–306	7	
	5	1-methylpyrrolidin-2-one	< 0	309–364	4	
	6	DMA	< 0	255–400	9	
	7	HMPA	< 0	308–363	4	
	3	DMA/DMF (5/3)	≈ 0	216–286	6	
	8	dimethyl sulfoxide	0.43 ± 0.09	308–363	4	0.9083
	1	DMF	1.80 ± 0.06	218–364	10	0.9999
	9	diiodomethane	2.15 ± 0.25	308–364	4	0.9659
	10	tetrahydrofuran	2.20 ± 0.14	225–287	4	0.9893
	11	acetone	2.82 ± 0.04	217–291	5	0.9993
	12	1,4-dioxane	3.23 ± 0.28	288–363	5	0.9718
	2	butyronitrile ^[1]	3.30 ± 0.03	165–382	13	0.9991
	13	acetonitrile	3.36 ± 0.02	233–344	10	0.9997
	14	pyridine	3.36 ± 0.04	236–290	4	0.9996
	15	ethyl acetate	3.49 ± 0.07	217–291	5	0.9984
	16	benzonitrile	3.80 ± 0.04	263–420	9	0.9992
	17	benzene	3.90 ± 0.04	307–345	5	0.9996
	18	methanol	3.95 ± 0.05	221–325	8	0.9990
	19	1,2-dichlorobenzene	3.99 ± 0.01	307–344	5	1.0000
	20	nitromethane	4.02 ± 0.06	308–363	4	0.9994
	21	toluene	4.08 ± 0.06	220–381	11	0.9979
	22	ethanol	4.38 ± 0.05	225–288	4	0.9996
	23	[D]trichloromethane	4.41 ± 0.09	308–332	4	0.9988
	24	dibutyl ether	4.72 ± 0.19	307–400	7	0.9901
	25	tetrachloromethane	5.52 ± 0.01	308–332	4	1.0000
	26	cyclohexane	7.93 ± 0.26	289–344	4	0.9978
2b	1	DMF	1.82 ± 0.13	217–323	8	0.9646
	4	DMPU	1.92 ± 0.22	248–320	9	0.8955
	2	butyronitrile ^[1]	5.51 ± 0.06	175–382	14	0.9989
	27	methylcyclohexane	7.49 ± 0.44	221–323	8	0.9821

[a] With the statistical error for the least-squares fit of Equation (1) to the A_{int} versus T data. [b] Square of the regression coefficient.

The quantitative exploitation of the thermochromism through the calculation of the integrated absorbance of the long-wavelength band after deconvolution with Gauss curves, and the determination of ΔH^0 and $\varepsilon_{\text{int}}^*$ by a least-squares fitting of Equation (1) to the A_{int} versus T data has been detailed in the preceding paper.^[1] The results obtained in this way for a variety of different solvents are listed in Table 1 and are displayed in part in Figures 2 and 3, where the fractions of $\mathbf{A}^*$, calculated according to Equation (3), are plotted against the temperature.

$$f^* = \frac{A_{\text{int}}}{\varepsilon_{\text{int}}^* c d} = \left(1 + 2 \exp \frac{\Delta H^0}{RT} \right)^{-1} \quad (3)$$

The spectra obtained for solutions of **2a** in *N,N'*-dimethylpropylene urea (Figure 1) can be used to define the limits for the calculation of A_{int} . Since the strong near-UV absorptions are subject to particularly strong Doppler broadening while the (relatively weak) band in the visible region simultaneously broadens and decreases with rising temperature, overlap becomes deleterious and thus the calculated A_{int} data are no longer sufficiently accurate for a good least-squares fitting of Equation (1). One can only state with confidence that ΔH^0 is negative in these cases. Attempts to fit Equation (1) to A_{int} versus T data are not meaningful, of course, if the UV/Vis

spectra are virtually temperature-invariant and, accordingly, ΔH^0 is very close to zero (Figures 2 and 3, Table 1).

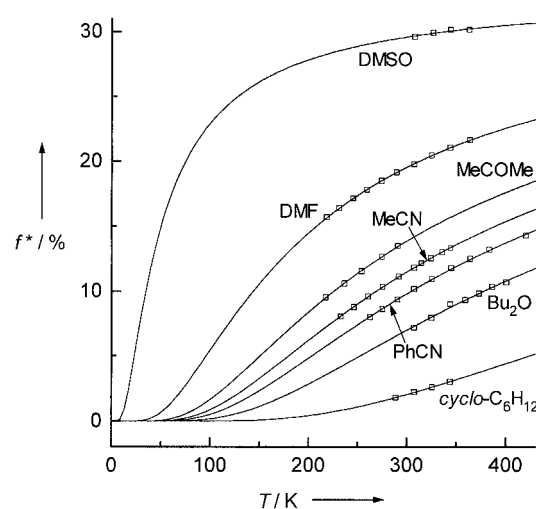


Figure 2. Temperature dependence of the fractions f^* [Eq. (3)] of the barbaralanedicarbonitrile **2a*** in various solvents. Filled squares correspond to the UV/Vis spectra displayed in Figure 1.

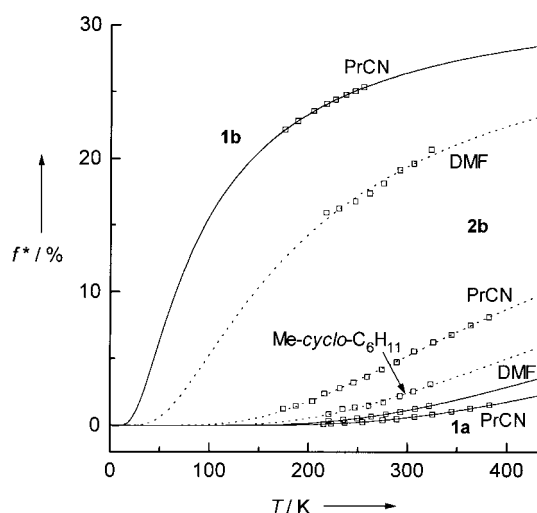


Figure 3. Temperature dependence of the fractions f^* of the semibullvalenedicarbonitriles **1a***, **b*** and tetraphenylbarbaralane **2b*** in various solvents as calculated from Equation (3).

Table 1 shows that the experimentally accessible enthalpy differences ΔH^0 , which are composed of the gas-phase enthalpy differences and the solvation terms [Eq. (2)], are very small indeed and actually in the range of van der Waals interactions, potential barriers toward conformational changes, and enthalpy differences between cyclohexane conformations.^[3] In view of the large data set obtained for **2a**, it appeared promising to correlate ΔH^0 with solvent properties. Thus one could hope to recognize the predominant solvent contributions to the stabilization of **2a*** in solution and extrapolate the gas-phase term $\Delta H_{2a/2a^*}^0$ from the solution ΔH^0 data. Toward this end, a number of multi-parameter equations for the correlation of solute–solvent interactions were tried.^[4] Reasonable results were obtained with the equation from Abraham, Kamlet, and Taft.^[5] The omission of the fourth parameter, namely, the cohesive energy density, in their equation does not change the correlation coefficient and therefore need not be included.^[6] The coefficients of the resulting three-parameter Equation (4); π^* = solvatochromic parameter which describes a combination of dipolarity and polarizability (0.00 for cyclohexane, 1.00 for dimethyl sulfoxide);^[7a] α = hydrogen bond donation ability (0.00 for hexane, 1.96 for hexafluoroisopropanol);^[7b] β = hydrogen bond acceptance or electron pair donation ability to form a coordinative bond (0.00 for hexane, 1.43 for 1,2-diaminoethane))^[7b] are small and interestingly resemble those

$$\Delta H^0 = (6.69 \pm 0.45) - (3.79 \pm 0.67)\pi^* + (1.53 \pm 0.66)\alpha - (2.55 \pm 0.72)\beta \quad (4)$$

that are obtained when this equation is applied to the solvent dependence of the rate constants of the two irreversible Cope rearrangements reported by Mitsuhashi et al.^[8] Figure 4 shows a good correlation between the experimental [Eq. (1)] and calculated enthalpy differences [Eq. (4)] for all 21 solvents that afforded positive ΔH^0 values. The correlation coefficient ($r^2 = 0.782$) might be improved by a more even distribution of the solvents over the whole range.

Equation (4) shows that dipolarity and polarizability (π^*) and the electron pair donation ability (β) of the solvents play the

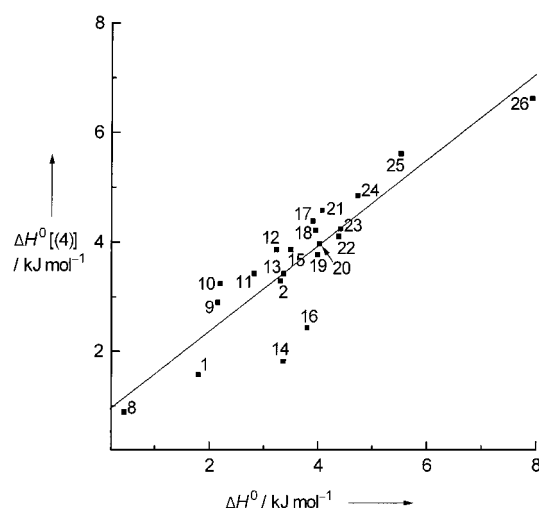


Figure 4. Calculated [Eq. (4)] versus experimental enthalpy differences [Eq. (1)] for all 21 solvents that afforded positive ΔH^0 values. The key to the solvent numbers is given in Table 1. The linear least-squares fit of the data has the same correlation coefficient as Equation (4).

predominant role. The negative sign of both coefficients signifies that an increase of these solvent properties will lower ΔH^0 . This is in good accord with the expectation that the delocalized species **2a*** is more polarizable than the classical, localized structures **2a**, **2a'**. Neither **2a**, **2a'** nor **2a*** possess sizeable dipole moments, but they are certainly expected to have different quadrupole moments. That of **2a*** is most probably larger, because the donor (=phenyl) and the acceptor (=cyano) groups interact more effectively in the delocalized system. Of two equilibrating molecules devoid of a dipole moment, dipolar solvents favor the one with the larger quadrupole moment.^[3]

The first term in Equation (4) is the statistically defined enthalpy difference between **2a**, **2a'** and **2a*** for solutions in saturated hydrocarbons. Its value agrees reasonably well with the experimental one (Table 1) and represents the closest possible approximation to the gas-phase enthalpy difference $\Delta H_{2a/2a^*}^0$ of Equation (2).

Although such an extended series of solvents have not been employed in the study of the other compounds, it is evident from Figure 3 that only the influence of dipolar solvents confers higher stability to the delocalized species **A***, that is, none of these compounds prefers the delocalized structure in the gas phase.

Finally, we emphasize that the conventional cross section along the Cope reaction coordinate through the adiabatic energy hypersurfaces of the ground and excited state, which is useful for a simple pictorial description of the relative enthalpies of **A**, **A'** and **A*** in the gas phase,^[1] cannot be employed for solutions. This is because it does not involve the solute–solvent interactions, which are indeed of the same order of magnitude or even greater than the gas-phase enthalpy differences. Though *solvated*, bishomoaromatic semibullvalenes and barbaralanes may be more stable than their classical, localized isomers, provided that the former enjoy stronger stabilization by the surrounding solvent molecules, single-minimum systems of this sort, which consist

exclusively of a *single*, delocalized, bishomoaromatic species in the gas phase, remain as yet an intriguing myth.

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Efficient Cleavage – Cross-Coupling Strategy for Solid-Phase Synthesis—A Modular Building System for Combinatorial Chemistry**

Stefan Bräse* and Maarten Schroen

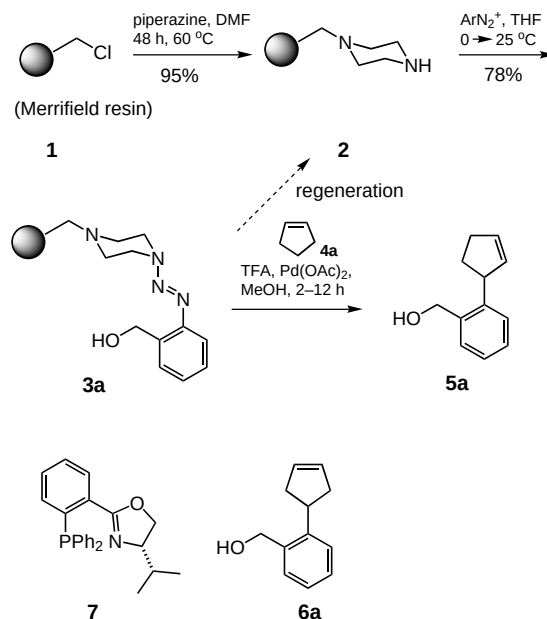
Dedicated to Professor Armin de Meijere on the occasion of his 60th birthday

Solid-phase reactions play an important role in parallel synthesis and combinatorial chemistry, particularly in the area of medicinal chemistry, where its potential has emerged due to the possibility of automation.^[1] Research has focused on the synthesis of small, nonpeptidic molecules, because they are applicable systemically and their properties can be modified so that they can cross the blood–brain barrier more easily.^[2] Cross-coupling reactions serve as efficient methods for their

synthesis because they proceed under mild conditions with the formation of C–C bonds.^[3, 4] Recently, we have shown that triazenes are suitable for the linkage of anilines to solid supports and that they may be cleaved to form hydrocarbons.^[4] An advantage of this linker type is its accessibility from a pool of aminoarenes. The cleavage of this traceless linker proceeds via arene diazonium ions. We report herein on the possibilities of a cleavage – cross-coupling strategy starting from these diazonium ions.

The Heck reaction^[5] of arene diazonium salts bearing little or no functionalization or triazenes, cleaved by acids, with simple olefins proceeds in general under mild thermal conditions to give the expected products in moderate to good yields.^[5, 6] Preliminary experiments with triazenes in methanol as the solvent showed that the reduction products are formed in various amounts together with aryl ether by-products through reaction with the solvent.

The reactions on solid supports were conducted with the known amine resin **2**^[4] (Scheme 1). Benzyl alcohol **3** bound through the triazene system initially served as a test system. The one-pot cleavage – cross-coupling reaction was conducted with cyclopentene (**4a**), a moderately reactive alkene for the Heck reaction. The resin was cleaved with two equivalents of trifluoroacetic acid (TFA) in MeOH at 0 °C to give the diazonium ion. In situ coupling with the alkene in the



Scheme 1. Synthesis and cleavage – cross-coupling reactions on the triazene resin **3a**.

presence of catalytic amounts (5 mol %) of palladium(II) acetate furnished 2-cyclopent-2-enylbenzyl alcohol (**5a**) in over 92 % purity (HPLC, GC, and NMR) and up to 95 % yields after 12 h at 40 °C in MeOH, filtration to remove the resin, and removal of the solvent with a rotary evaporator. The regioisomeric, achiral cyclopentene derivative **6a** was isolated as a by-product in 5 % yield. An examination of the use of various phosphane types as ligands gave the following result: addition of triphenylphosphane lowered the purity

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(80 %, HPLC) and led to a change of the ratio of the isomers (**5a**:**6a** = 3:1). 2,3-*O*-Isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphinyl)butane (DIOP) as ligand reversed this ratio and gave the cyclopentene derivative **6a** in 63 % yield. The oxazoline **7**^[5,7] furnished the chiral product **5a**, but with low asymmetric induction (75 % yield, <10 % *ee*). The HPLC analysis of the crude product showed the presence of the corresponding phosphane oxides: presumably under the reaction conditions the ligand is oxidized, for example through the diazonium salt, resulting in a partial dissociation that suggests that the ligand type is not suitable for asymmetric induction. By using palladium on charcoal, the coupling is achieved in lower purity (87 %); however, the method is advantageous in regard to the ease of removal of the palladium catalyst, that is the filtrate is nearly colorless. A remarkable feature of this new concept is that a multiple phase change (solid–liquid–solid–liquid) is observed.^[8]

Cleavage with functionalization from the resin has been used only seldomly,^[9] since the coupling reagent or partner are in general difficult to separate; however, this is possible in the present case due to the volatility of the alkenes used. This case is the first example of a solid support linker being used to generate a reactive species for Heck reactions.

After these successful reactions a series of activated alkenes (styrene, *tert*-butyl acrylate, 2-vinylpyridine) were employed in coupling reactions and yielded the desired *trans*-configured products **10a–c** in excellent purities and yields (>92 % HPLC, GC) (Scheme 2). Even nonactivated alkenes such as 1-hexene or 1-octene were found to react smoothly at the

terminal position. However, an unexpected mixture of double-bond regio- and diastereomeric products **10d, e** (1*E*:2*E*:2*Z* ≈ 50:45:5) were isolated. Cyclic alkenes (cyclopentene to cyclooctene, **4a–e**) also reacted smoothly (in general >92 % purity for **5a–d**). In addition, the palladium catalyst on charcoal was still active for hydrogenation reactions (1 bar H₂, 25 °C, 2 h) and allows the formal coupling of alkyl units in high yields and purities. Thus, the phenyl-alanine derivative **12** was furnished by Heck coupling of the resin **3** (X = *p*-I) with acetamido acrylate, cleavage Heck reaction with cyclopentene (**4a**), and subsequent hydrogenation of the double bond.

Allyl alcohols such as but-1-en-3-ol were found to react smoothly and gave the ketones **8**. Enol ethers were however not suitable for this reaction and yielded mixtures of products by acid hydrolysis.

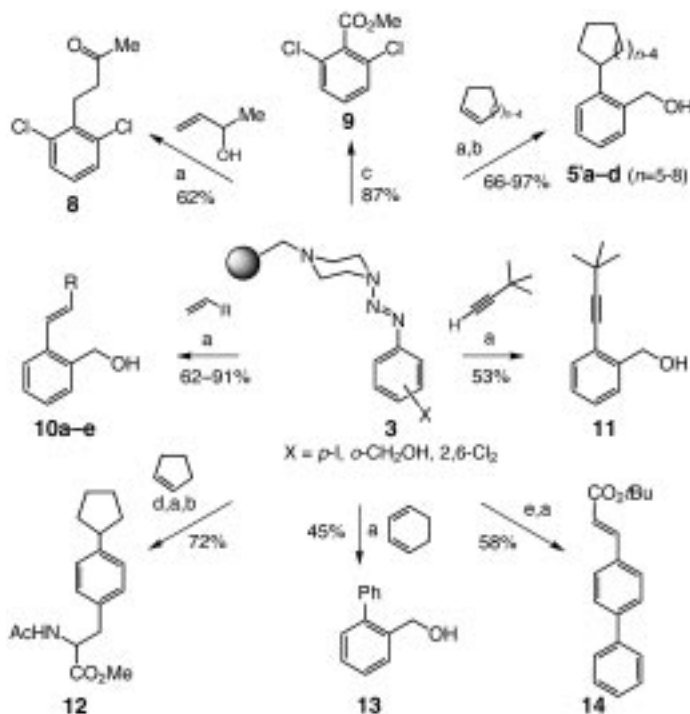
The reaction with 1,3-cyclohexadiene gave neither the expected dienes nor the possible cyclization product;^[5] only the corresponding phenyl derivative **13** was detected (45 % yield, 92 % purity). Seemingly, under the acidic conditions various oxidative insertion/reductive elimination processes or transfer hydrogenation can occur. These novel reaction sequences offer the possibility of a formal biaryl coupling and thus provide a potential alternative to Stille and Suzuki couplings.^[3a] A comparable experiment with phenyl boronic acid for a Suzuki coupling yielded the expected product, however, separation of excess of the coupling component is more laborious.

The reaction under an atmosphere of carbon monoxide (1 bar) in the absence of an alkene furnished the corresponding benzoate **9** in 87 % yield (92 % purity). Basic amine functionalities such as pyridine derivatives did not interfere with the coupling procedure providing that the reaction was conducted with an appropriate excess of trifluoroacetic acid.

Alkynes may also be used as the coupling component: *tert*-butylacetylene furnished the expected product **11** in 85 % purity. The alkyne di- and trimerized under the reaction conditions, thus, separation by chromatography or solid-phase extraction was required to ensure sufficient purity. Alkyne coupling reactions with diazonium salts have not been reported to date.

In this context, a novel unsymmetric biaryl synthesis on solid support has been developed. The observation that Heck reactions with unreactive alkenes led to dimerization of the halogen component has allowed the development of a synthesis of symmetrical biaryl units.^[5,10] The reaction of the polymer-bound iodoarene **3** (X = *p*-I) with iodobenzene in presence of a palladium catalyst under Heck conditions (Pd(OAc)₂, PPh₃, NEt₃, DMF, 24 h, 80 °C) furnished a biaryl resin **3** (X = *p*-Ph), which could be subsequently used in a second, cleavage–cross-coupling reaction with acrylate to give the functionalized arene **14** in good yield and purity.

The examples above have been chosen based on the boiling point of the alkene to be coupled in order to ensure the removal of the excess upon evaporation. With a boiling point lower than or in the range of dimethyl sulfoxide, one of the standard solvents for the high-throughput screening, coupling with components such as 1-decene or crotonic acid can be achieved. Up to this molecule size the cleavage products can



Scheme 2. Cleavage–cross-coupling reactions on the triazene resin **3**: a) **3**, alkene, alkyne, or diene, Pd(OAc)₂ or Pd/C, 2 equiv TFA, MeOH, 2 h, 40 °C; b) H₂ (1 bar), 2 h, 25 °C; c) **3** (X = 2,6-Cl₂), CO (1 bar), Pd(OAc)₂, 2 equiv TFA, MeOH, 2 h, 40 °C; d) (*p*-I), CH₂=C(NHAc)CO₂Me, Pd(OAc)₂, PPh₃, NEt₃, DMF, 24 h, 80 °C; e) (*p*-I), PhI, Pd(OAc)₂, PPh₃, NEt₃, DMF, 24 h, 80 °C; **10a**: R = Ph; **10b**: R = CO₂tBu; **10c**: R = 2-pyridyl; **10d**: R = butyl; **10e**: R = hexyl.

be obtained without purification. For preparative purposes involving chromatographic purification higher boiling components are also accessible.^[11]

This cleavage–cross-coupling reaction is in all cases salt-free, that is the exposed resin **2** functions as a “scavenger-resin” for the trifluoroacetic acid. The filtered, slightly yellowish resin is, after washing, active for coupling steps with diazonium salts and can therefore be recycled.

In conclusion, this salt-free cleavage–cross-coupling strategy allows the clean synthesis of a series of (cyclo)alkenyl-, alkynyl-, (cyclo)alkyl-, and aryl-substituted (hetero)arene derivatives and is especially suitable for automated synthesis. This building system comprising virtually any aminoarene or nitroarene after their reduction as well as alkenes or alkynes allows synthesis of highly lipophilic molecules and tolerates most functional groups.^[4, 12, 13] Multicomponent Heck reactions (domino Heck Diels–Alder reaction, Heck–Stille reaction etc.)^[14] should be possible in this context and might lead to a higher diversification.

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An Oxidation-Labile Traceless Linker for Solid-Phase Synthesis**

Frank Stieber, Uwe Grether, and Herbert Waldmann*

The combinatorial synthesis of small-molecule libraries on polymeric supports is a powerful method for the discovery and development of new molecules with a predetermined profile of properties.^[1] Vital to all solid-phase methodologies is the design and utilization of suitable anchor groups (linkers) that allow facile attachment, functionalization, and release of the molecules of interest. Typically, linkage to the polymeric support is achieved through functionality already present in the target molecule. However, after cleavage from the support

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Nucleotides

Part LX¹⁾

Synthesis and Characterization of New 2'-O-Methylriboside 3'-O-Phosphoramidites Useful for the Solid-Phase Synthesis of 2'-O-Methyloligoribonucleotides

by Hagen Cramer²⁾ and Wolfgang Pfeleiderer*

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A series of new 2'-O-methylribonucleoside 3'-O-[2-(4-nitrophenyl)ethyl dialkylphosphoramidites] **27–31**, **33–38**, **40–44**, and **45–50** were synthesized and their stability and reactivity compared in automated oligonucleotide synthesis with the standard 2'-O-methylribonucleoside 3'-O-(β -cyanoethyl diisopropylphosphoramidites) **32**, **39**, **45**, and **51**, respectively. The 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) groups were used for the protection of the base moieties.

1. Introduction. – The first nucleoside phosphoramidites as building blocks for oligodeoxyribonucleotide synthesis which were introduced by *Caruthers* and coworkers in the beginning of the 1980's [2–5] were very reactive and thus difficult to work with. The stability of the phosphoramidites were subsequently increased by the replacement of the dimethylamino with the diisopropylamino group in the phosphoramidite moiety. The introduction of 2-cyanoethyl as phosphate-protecting group by *Köster* and coworkers [6][7] led to the 2-cyanoethyl diisopropylphosphoramidites, which are so successful that they are still predominant today.

In oligoribonucleotide synthesis, the additional 2'-protecting group makes the phosphoramidites less reactive. The replacement of the diisopropylamino group by smaller dialkylamino groups, such as the diethylamino group, may make the phosphoramidites more reactive [8][9]; however, this leads to a less stable phosphoramidite as well. Therefore, *Stengele* [10], in a search for other β -eliminating phosphate groups which would stabilize the diethyl phosphoramidites, reported equally reactive but more stable phosphoramidites when using 2-(4-nitrophenyl)ethyl instead of 2-cyanoethyl as phosphate-protecting group. The 2-(4-nitrophenyl)ethyl group was introduced by *Pfeleiderer* and coworkers [11–15] as a phosphate-protecting group.

Inspired by the good results of the 2-(4-nitrophenyl)ethyl phosphoramidites in oligoribonucleotide synthesis reported by *Stengele*, we synthesized different 2'-O-methylnucleoside 2-(4-nitrophenyl)ethyl phosphoramidites and compared their stability and reactivity with those of the 2-cyanoethyl diisopropyl phosphoramidites in 2'-O-methyloligoribonucleotide synthesis. Specifically, we employed for our investigations

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diisopropyl-, diethyl-, isopropyl(methyl)-, and ethyl(isopropyl)phosphoramidites protected as their 2-(4-nitrophenyl)ethyl esters.

The 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) groups were also used as base-protecting groups, because they can be removed after oligonucleotide synthesis selectively by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in aprotic solvents, while the oligonucleotide is still attached to the solid support. This offers the advantage of synthesizing very pure oligonucleotides in a direct manner without further additional purification steps.

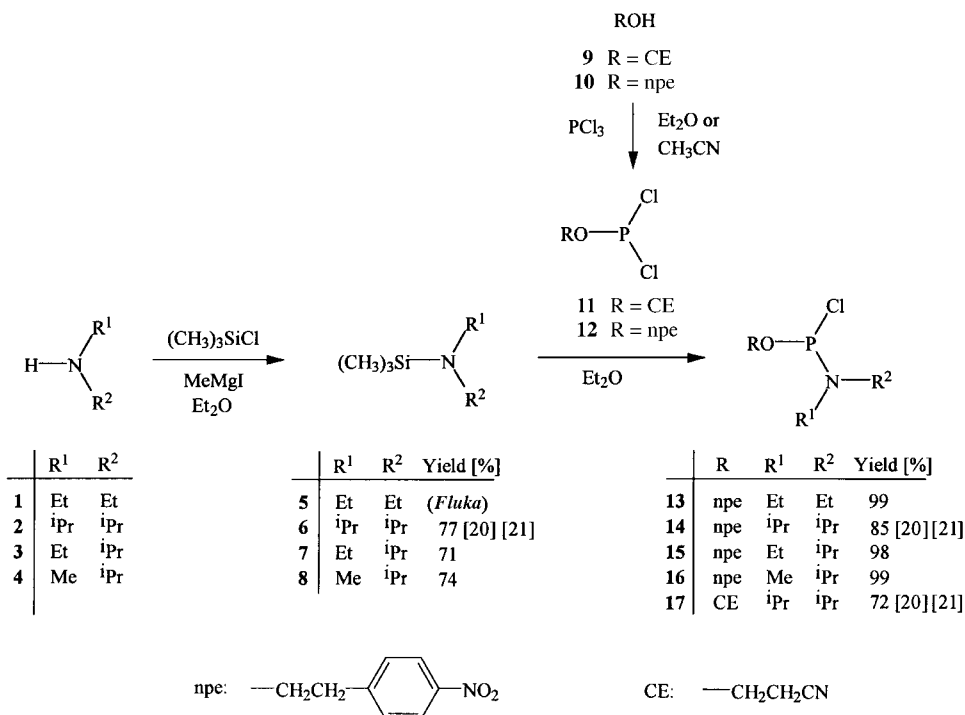
2. Syntheses. – All phosphoramidites were synthesized by phosphitylation using alkyl dialkylphosphoramidochloridites (= alkoxy(chloro)(dialkylamino)phosphanes) which were preferred over alkyl tetraalkylphosphorodiamidites (= alkoxybis(dialkylamino)phosphanes) because of their higher stability when a less bulky *N,N*-dialkyl moiety is used [16] and because of the easy removal of excess phosphitylating reagent by reaction with propan-2-ol (ⁱPrOH) [17]. If the reaction is not stopped with ⁱPrOH, excess phosphitylation reagent hydrolyzes during the workup procedure. Hydrolyzed phosphitylation reagent cannot be removed easily from the product, because it has almost the same chromatographic mobility on silica gel as the phosphoramidite itself. By converting the excess alkyl dialkylphosphoramidochloridite into its alkyl isopropyl diester, separation from the phosphoramidite is achieved easily by silica-gel column chromatography.

The alkyl dialkylphosphoramidochloridites **13–17** were prepared by condensing the appropriate *N,N*-dialkyl-1,1,1-trimethylsilanamine **5–9** with the alkyl phosphorodichloridite **11** or **12** (Scheme 1). *N,N*-Diethyl-1,1,1-trimethylsilanamine (**5**) is commercially available. The other *N,N*-dialkyl-1,1,1-trimethylsilanamines were synthesized by the procedure of *Transjö* [18] in yields ranging from 71 to 77%. The 2-cyanoethyl phosphorodichloridite (**11**) and the 2-(4-nitrophenyl)ethyl phosphorodichloridite (**12**) were prepared by reaction of the appropriate alcohol with excess phosphorus trichloride according to the published procedures [13][19]. Distillation of **12** is not recommended due to possible decomposition. The 2-cyanoethyl diisopropylphosphoramidochloridite (**17**) could be distilled under high vacuum whereas the 2-(4-nitrophenyl)ethyl dialkylphosphoramidochloridites **13–16** decomposed during anticipated distillation and were, therefore, used without further purification [20][21].

For the synthesis of the 2'- and 3'-*O*-methylnucleoside phosphoramidites **27–51** and **56–59**, the appropriate protected nucleosides **18–26** and **52–55** [22] were treated with the alkyl dialkylphosphoramidochloridites **13–17** in the presence of *Hünig's* base (Schemes 2 and 3). Yields after workup and silica-gel flash chromatography were between 70 and 90%. As solvent for the phosphitylation reaction, CH₂Cl₂, which takes up moisture less easily, was usually preferred over tetrahydrofuran (THF) since additional H₂O led to more hydrolyzed phosphitylation reagent.

The phosphitylation reaction of 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)-*N*²-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine (**25**) gave rise to a by-product which, according to the ¹H- and ³¹P-NMR spectra, was caused by an additional phosphitylation at the N(2) position of the base in spite of protection with the 2-(4-nitrophenyl)ethoxycarbonyl group. In THF, considerably less by-product was formed than in CH₂Cl₂. For this reason, the guanosine phosphoramidites were synthesized

Scheme 1

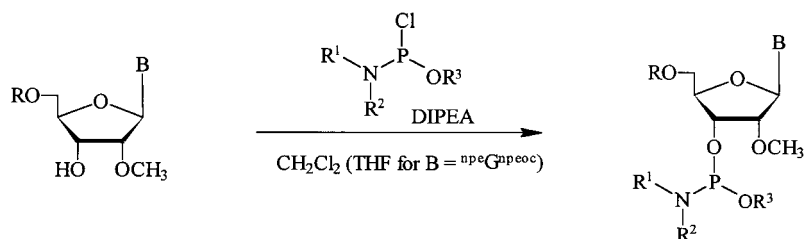


in THF, with the exception of 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)-*N*²-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**48**) because of the poor solubility of the phosphitylating reagent 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (**14**) in THF.

The phosphoramidites generally were purified by silica-gel column chromatography and a petroleum ether/AcOEt gradient. With this solvent mixture, the phosphoramidite was eluted prior to the hydrolyzed phosphitylating reagent, except in the case of **28**, **35**, and **41** which were separated from the hydrolyzed phosphitylating reagent by a petroleum ether/acetone gradient eluting the H-phosphonate prior to the phosphoramidite. Purification of the npe/npeoc-protected guanosine phosphoramidites was not problematic because of their higher *R_f* values in comparison to the hydrolyzed phosphitylating reagent.

Solid-phase oligonucleotide synthesis was performed by analogy to published procedures [2–4][23]. Solid-phase synthesis *via* the npe/npeoc approach requires a DBU-stable linkage of the starting nucleoside through a spacer molecule to a glass-bead support [10][24][25]. Therefore, each of the eight succinylated nucleosides **60–67** were synthesized by reaction of the appropriate protected nucleoside **18**, **21**, **23**, **25**, and **52–55** with succinic anhydride and 4-(dimethylamino)pyridine in CH_2Cl_2 [26] in almost quantitative yields (Scheme 4). The succinylated nucleosides **60–67** were then reacted with LCMAA-CPG (= long-chain (methylamino)alkyl controlled-pore glass, 500 Å;

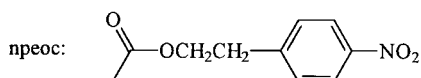
Scheme 2



	Base	R
18	Ura	MeOTr
19	Ura	(MeO) ₂ Tr
20	Ura ^{An}	MeOTr
21	Cyt ^{npeoc}	MeOTr
22	Cyt ^{npeoc}	(MeO) ₂ Tr
23	Ade ^{npeoc}	MeOTr
24	Ade ^{npeoc}	(MeO) ₂ Tr
25	^{npe} Gua ^{npeoc}	MeOTr
26	^{npe} Gua ^{npeoc}	(MeO) ₂ Tr

	Base	R	R ¹	R ²	R ³	Yield [%]
27	Ura	MeOTr	Et	Et	npe	83
28	Ura	(MeO) ₂ Tr	Et	Et	npe	82
29	Ura	MeOTr	iPr	iPr	npe	73
30	Ura	MeOTr	Et	iPr	npe	81
31	Ura	MeOTr	Me	iPr	npe	82
32	Ura	MeOTr	iPr	iPr	CE	85
33	Ura ^{An}	MeOTr	Et	Et	npe	81
34	Cyt ^{npeoc}	MeOTr	Et	Et	npe	89
35	Cyt ^{npeoc}	(MeO) ₂ Tr	Et	Et	npe	84
36	Cyt ^{npeoc}	MeOTr	iPr	iPr	npe	72
37	Cyt ^{npeoc}	MeOTr	Et	iPr	npe	90
38	Cyt ^{npeoc}	MeOTr	Me	iPr	npe	83
39	Cyt ^{npeoc}	MeOTr	iPr	iPr	CE	87
40	Ade ^{npeoc}	MeOTr	Et	Et	npe	88
41	Ade ^{npeoc}	(MeO) ₂ Tr	Et	Et	npe	80
42	Ade ^{npeoc}	MeOTr	iPr	iPr	npe	72
43	Ade ^{npeoc}	MeOTr	Et	iPr	npe	84
44	Ade ^{npeoc}	MeOTr	Me	iPr	npe	80
45	Ade ^{npeoc}	MeOTr	iPr	iPr	CE	81
46	^{npe} Gua ^{npeoc}	MeOTr	Et	Et	npe	83
47	^{npe} Gua ^{npeoc}	(MeO) ₂ Tr	Et	Et	npe	80
48	^{npe} Gua ^{npeoc}	MeOTr	iPr	iPr	npe	72
49	^{npe} Gua ^{npeoc}	MeOTr	Et	iPr	npe	81
50	^{npe} Gua ^{npeoc}	MeOTr	Me	iPr	npe	82
51	^{npe} Gua ^{npeoc}	MeOTr	iPr	iPr	CE	80

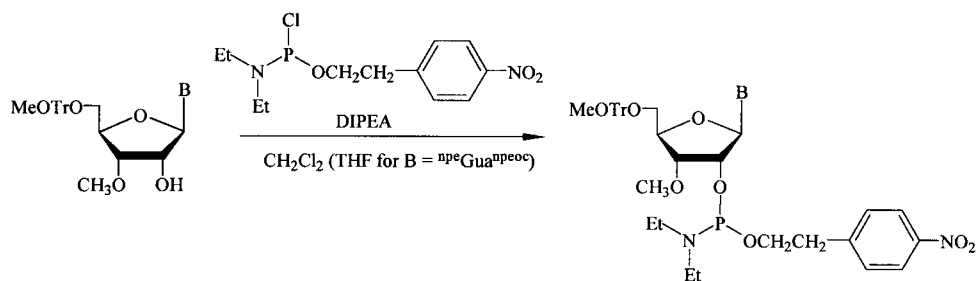
CE, npe: see Scheme 1

An: 4-MeOC₆H₄

68) with the coupling reagent 2-[[2-(2-cyanoethoxy)-2-oxoethylidene]amino]-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU) and 4-methylmorpholine in MeCN, followed by a capping process with Ac₂O and 4-(dimethylamino)pyridine in pyridine to give the solid supports **69**–**76** in loadings of 23–38 μmol/g. Loadings were determined according to Atkinson and Smith [27].

For the assembly of the oligonucleotides, an automated DNA synthesizer was used. A synthesis column filled with the desired starting nucleoside **69**–**76** was attached to the synthesizer. The subsequent oligonucleotide synthesis consisted of a programmed repetitive cycle of chemical reactions such as deprotection of the terminal trityl group with CF₃COOH, coupling with a 0.1M solution of nucleoside phosphoramidite **27**–**51**

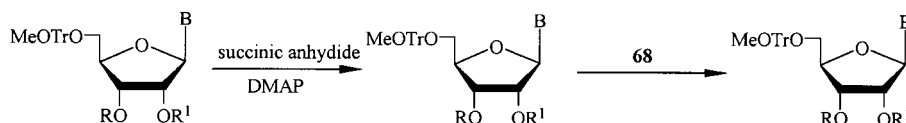
Scheme 3



	Base
52	Ura
53	Cyt ^{npeoc}
54	Ade ^{npeoc}
55	npeGua ^{npeoc}

	Base	Yield [%]
56	Ura	78
57	Cyt ^{npeoc}	79
58	Ade ^{npeoc}	77
59	npeGua ^{npeoc}	75

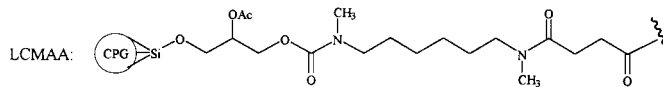
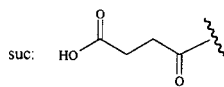
Scheme 4



	Base	R	R'
18	Ura	H	OMe
21	Cyt ^{npeoc}	H	OMe
23	Ade ^{npeoc}	H	OMe
25	npeGua ^{npeoc}	H	OMe
52	Ura	OMe	H
53	Cyt ^{npeoc}	OMe	H
54	Ade ^{npeoc}	OMe	H
55	npeGua ^{npeoc}	OMe	H

	Base	R	R'
60	Ura	suc	OMe
61	Cyt ^{npeoc}	suc	OMe
62	Ade ^{npeoc}	suc	OMe
63	npeGua ^{npeoc}	suc	OMe
64	Ura	OMe	suc
65	Cyt ^{npeoc}	OMe	suc
66	Ade ^{npeoc}	OMe	suc
67	npeGua ^{npeoc}	OMe	suc

	Base	R	R'
69	Ura	LCMAA	OMe
70	Cyt ^{npeoc}	LCMAA	OMe
71	Ade ^{npeoc}	LCMAA	OMe
72	npeGua ^{npeoc}	LCMAA	OMe
73	Ura	OMe	LCMAA
74	Cyt ^{npeoc}	OMe	LCMAA
75	Ade ^{npeoc}	OMe	LCMAA
76	npeGua ^{npeoc}	OMe	LCMAA



or **56**–**59**, and 1*H*-tetrazole, capping of any unreacted OH functions by acetylation with Ac₂O, and oxidation of the phosphite triester with I₂ as well as excessive washing steps. The coupling efficiency of each condensation was determined by absorption measurement of the released trityl solutions. The last synthesis cycle was ended after the detritylation step to give a 'trityl-off' product. Because no further purification was anticipated, there is no need for a 'trityl-on' oligonucleotide. To remove all npe and npeoc protecting groups, the support was treated with 1M DBU in dry MeCN for 10 h. Thereafter, the fully deblocked oligonucleotide was cleaved from the support by treatment with concentrated NH₃ solution for 2 h. Finally, the products were

lyophilized in a *Speed-vac* concentrator, and the quality of the crude 2'- and 3'-*O*-methyloligoribonucleotides was analyzed by reversed-phase HPLC.

3. Discussion. – The stability of the phosphoramidites in solution was tested using decoupled ^{31}P -NMR spectroscopy. Thus, 20 mg of the phosphoramidite were dissolved in 0.4 ml of CDCl_3 in a NMR tube which was flushed with Ar and stoppered. A first spectrum was measured immediately after dissolution and a second one after 2 weeks at room temperature. Some of the CDCl_3 evaporated thereby and was re-added. The stability of phosphoramidite **27** in CDCl_3 was compared to its stability in CD_3CN showing that more degradation products were formed in the latter solvent within the same period of time (*Fig. 1*).

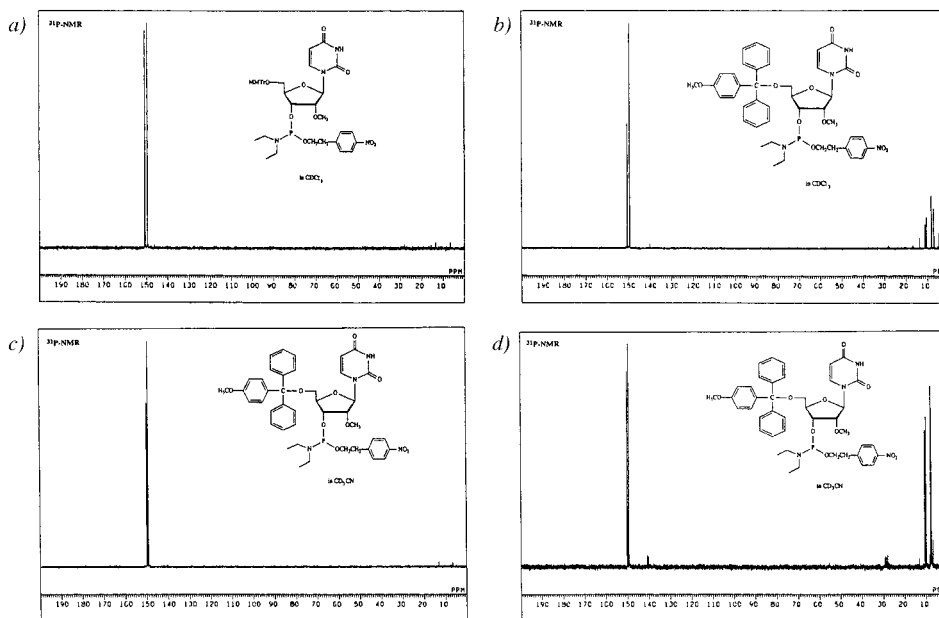


Fig. 1. Stability of 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)uridine 3'-[2-(4-nitrophenyl)ethyl]diethylphosphoramidite (**27**) at room temperature in CDCl_3 and in CD_3CN as determined by ^{31}P -NMR spectroscopy: a) c) spectra after dissolving in CDCl_3 and CD_3CN , respectively, and b) d) spectra after 14 days in CDCl_3 and CD_3CN solution, respectively

The optimal condensation time for each of the phosphoramidites was determined by synthesizing the short homologous oligonucleotides **77–90** using a 1- μmol RNA cycle for the synthesis on a 0.6- μmol scale and a 0.2- μmol RNA cycle for the 0.2- μmol scale. Coupling times were altered from 600 to 40 s. The trityl values and the purity of the raw products were taken into consideration for the determination of the average stepwise yield (ASWY) of the different phosphoramidites at different coupling times. When using 5'-(monomethoxytrityl)-2'-*O*-methyluridine 3'-[2-(4-nitrophenyl)ethyl]diethylphosphoramidite (**27**), 5'-(monomethoxytrityl)-2'-*O*-methyluridine 3'-[2-(4-nitrophenyl)ethyl]ethyl(isopropyl)phosphoramidite (**30**), 5'-(monomethoxytrityl)-2'-*O*-methyluridine 3'-[2-(4-nitrophenyl)ethyl]isopropyl(methyl)phosphoramidite (**31**),

or 5'-(monomethoxytrityl)-2'-*O*-methyluridine 3'-(2-cyanoethyl diisopropylphosphoramidite) (**32**), coupling times of 40 s were already sufficient to give ASWYs of > 99% for the synthesis of the decakis(2'-*O*-methyluridylylate) (**77**), whereas the same synthesis with 5'-(monomethoxytrityl)-2'-*O*-methyluridine 3'-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**29**) and a coupling time of 120 s gave only a ASWY below 98% (*Table*). Similar results were obtained for the corresponding adenosine phosphoramidites ($\rightarrow$ **81–83**) whereas the cytidine and guanosine derivatives showed less reactivity ($\rightarrow$ **79** and **80**, and **84–86**, resp.).

Thus, a coupling time of 300 s was needed for an almost quantitative condensation of cytidine phosphoramidites **36**, **37**, and **38** ($\rightarrow$ **80**), whereas a coupling time of 120 s gave only an ASWY of ca. 97% (see *Fig. 2*), except in the case of 2'-*O*-methyl-5'-(monomethoxytrityl)-*N*⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3'-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**36**) which showed only an ASWY of 92.1% under the same conditions (*Table*).

The 2'-*O*-methyl-5'-*O*-(monomethoxytrityl)-*N*²-[2-(4-nitrophenyl)ethoxycarbonyl]-*O*⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**48**) was again less reactive than all other tested guanosine phosphoramidites and gave only a ASWY of 97.6% when coupled for 300 s ($\rightarrow$ **84**) in comparison to a $\geq$ 99% ASWY for phosphoramidites **46** and **51** ($\rightarrow$ **84–86**).

No differences in the quality of synthesized oligonucleotides were found when changing from the 1.0- μ mol RNA cycle for the synthesis on the 0.6- μ mol scale to the 0.2- μ mol RNA cycle and 0.2- μ mol scale, maintaining otherwise the same conditions. As discussed above, 2-(4-nitrophenyl)ethyl phosphoramidites derived from different bases each bearing two corresponding alkyl groups did not show the same reactivity. Therefore, coupling times in correlation to the base (A, U shorter, G and C longer) as described by *Lyttle et al.* for oligoribonucleotide synthesis [9] could reduce the time needed for a 2'-*O*-methyloligoribonucleotide synthesis without loss of product purity.

As expected, the exchange of the 2-cyanoethyl by the 2-(4-nitrophenyl)ethyl group stabilized the phosphoramidite. At the same time, this made the phosphoramidite less reactive in contrast to the reported findings of *Stengele* who claimed equivalent reactivity for 2'-*O*-[2-(4-nitrophenyl)ethyl]sulfonylribonucleoside 3'-phosphoramidites [10]. The 2'-*O*-methylnucleoside 3'-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidites] are very stable, and they did not show any detectable degradation after 14 days in comparison with a 5 to 10% degradation of the 2-cyanoethyl diisopropylphosphoramidites under the same conditions. But as shown by the synthesis of homo-oligonucleotides, it turned out that the 2-(4-nitrophenyl)ethyl diisopropylphosphoramidites needed longer coupling times for the condensation reaction than the equivalent cyanoethyl diisopropylphosphoramidites.

4. Conclusion. – The exchange of the 2-cyanoethyl by the 2-(4-nitrophenyl)ethyl group in 2-cyanoethyl diisopropylphosphoramidites makes the phosphoramidites more stable towards decomposition but less reactive. To obtain equally reactive phosphoramidites the diisopropylamino moiety can be replaced by a diethylamino, isopropyl-(methyl)amino, as well as an ethyl(isopropyl)amino moiety. All those phosphoramidites show about the same reactivity in oligonucleotide synthesis and the same stability in CHCl_3 solution. An advantage of the 2-(4-nitrophenyl)ethyl phosphoramidites over

Table. *Synthesized 2'- and 3'-O-Methyloligoribonucleotides*

Sequence	Amidite	Scale [μmol]	Coupling time [s]	ASWY [%] ^{a)}	Yield [OD_{260}]
5'-(U _m) ₁₀ -3' (77)	27	0.6	600	100	46
	27	0.6	300	100	42
	32	0.6	300	100	46
	27	0.6	120	99.5	43
	29	0.6	120	97.6	40
	30	0.6	120	99.1	45
	31	0.6	120	100	41
	32	0.6	60	100	45
	32	0.2	60	100	20
	27	0.2	40	100	15
	30	0.2	40	100	14
	31	0.2	40	99.1	14
	32	0.2	40	99.2	13
5'-(U _m) ₁₈ -3' (78)	31	0.6	120	100	40
	33	0.5	120	98.1	79 ^{b)}
5'-(C _m) ₆ -3' (79)	39	0.6	300	100	21
5'-(C _m) ₁₀ -3' (80)	34	0.6	600	99.9	38
	36	0.6	600	100	53
	37	0.6	600	99.5	45
	38	0.6	600	100	36
	36	0.6	300	100	47
	37	0.6	300	100	42
	38	0.6	300	100	49
	34	0.2	120	97.3	8
	36	0.2	120	92.1	10.6
	37	0.2	120	96.4	8.5
	38	0.2	120	97.3	9
	39	0.2	120	97.0	10
	39	0.6	120	97.1	42
5'-(A _m) ₅ -3' (81)	44	0.2	40	100	10
5'-(A _m) ₁₀ -3' (82)	42	0.6	120	98.5	57
	44	0.2	120	99.9	18
5'-(A _m) ₁₈ -3' (83)	43	0.2	120	99.7	30
5'-(G _m) ₆ -3' (84)	48	0.2	300	97.6	17
	51	0.2	300	99.0	17
5'-(G _m) ₈ -3' (85)	51	0.2	300	99.1	23
	51	0.2	120	97.8	22
5'-(G _m) ₁₀ -3' (86)	46	0.6	600	99.4	66
	46	0.6	300	99.4	52
5'-(U _m) ₁₀ -2' (87)	56	0.6	300	99.9	34
5'-(C _m) ₁₀ -2' (88)	57	0.6	300	95.6	30
5'-(A _m) ₁₀ -2' (89)	58	0.2	120	100	21
5'-(G _m) ₁₀ -2' (90)	59	0.2	120	95.5	17

^{a)} ASWY: average stepwise yield. ^{b)} Raised *OD* value due to contamination with anisamide.

the 2-cyanoethyl phosphoramidite is the possible TLC detection by UV light of hydrolyzed phosphitylating agent during their synthesis. This makes the separation of hydrolyzed phosphitylating agent from the target phosphoramidite during the purification by column chromatography (silica gel) easier.

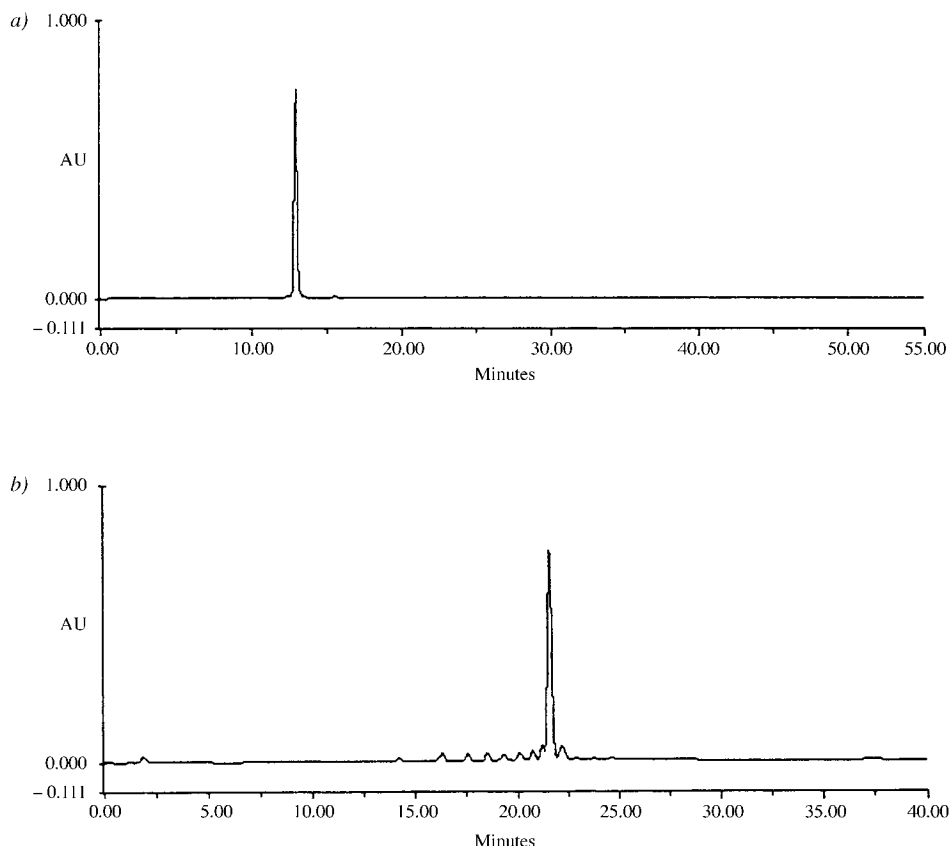


Fig. 2. Reversed-phase HPLC of 5'-(C_m)₁₀-3' (**80**), with **37** as phosphoramidite: a) coupling time 300 s and b) coupling time 120 s. Column: LiChrospher 100 RP-18, 5 μ m, 4 $\times$ 125 mm (Merck); gradient: 2.5% MeCN in 0.1M (Et_3NH)Ac (pH 7) for 2 min and then 2.5–20% MeCN in 0.1M (Et_3NH)Ac (pH 7) within 30 min; flow 1 ml/min.

5. Physical Data. – The structural assignments of the newly synthesized compounds are based on elemental analyses, UV, ^1H -NMR, and ^{31}P -NMR spectra.

The silylamines **5–8** do not have a chromophore and, therefore, do not show a characteristic UV spectrum. Due to their volatility, no elemental analysis data were obtained either. The UV and ^1H -NMR spectra of the 2-(4-nitrophenyl)ethyl dialkylphosphoramidochloridites **13–16** are dominated by the 2-(4-nitrophenyl)ethyl group. The phosphoramidites **27–51** and **56–59** show similar UV spectra as their starting nucleosides. The chemical shifts of the ^1H -NMR spectra do not show much change in comparison to the starting nucleosides. Additional signals can be seen from the phosphoramidite's alkyl groups. The introduction of the phosphorus(III) center leads to two diastereoisomeric products. This results in a doubling of most of the ^1H -NMR signals and makes the interpretation difficult, especially in the range from 2.5 to 5 ppm. The ^{31}P -NMR spectra show with how many P-containing products the phosphoramidite is contaminated. The phosphoramidites exhibit two characteristic

signals (diastereoisomers) with chemical shifts at *ca.* 150 ppm, whereas degradation products from the phosphoramidite as well as the phosphitylating reagent have chemical shifts in the range of 0 to 35 ppm.

Experimental Part

General: Products were dried under high vacuum. TLC: precoated silica-gel thin-layer sheets *F1500 LS 254* from *Schleicher & Schuell* or 60 *F₂₅₄* from *Merck*. Flash chromatography (FC): silica gel (*Baker*, 30–60 μm); 0.2–0.3 bar. M.p.: *Gallenkamp* or *Büchi-510* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer, Lambda 15*; λ_{max} in nm (log ϵ). $^1\text{H-NMR}$: *Bruker AC 250*; in ppm rel. to SiMe_4 or CDCl_3 ((D_6) DMSO, D_2O) as internal standard. $^{31}\text{P-NMR}$: *Joel 400 MHz*; in ppm rel. to H_3PO_4 .

1. *N-Ethyl-N-isopropyl-1,1,1-trimethylsilanamine (7)*. To Mg chips (5.47 g, 0.225 mol) suspended in anhyd. Et_2O (60 ml), MeI (14.1 ml, 32 g, 0.225 mol) in anhyd. Et_2O (30 ml) was added dropwise with stirring within *ca.* 40 min so that the soln. was refluxed slightly. The mixture was refluxed for an additional 50 min, and then *N-ethyl-N-isopropylamine (3)* (25 ml, 18 g, 0.21 mol) was added dropwise. The mixture was refluxed for 1 h, and after cooling to 0° , trimethylsilyl chloride (Me_3SiCl ; 28.6 ml, 24.4 g, 0.225 mol) was added within 10 min under vigorous stirring. After refluxing for 20 h, the mixture was allowed to cool. The colorless liquid was decanted from the brownish residue. The residue was washed with anhyd. Et_2O (3×30 ml), and the combined liquids were evaporated. The remaining colorless liquid was fractionally distilled *in vacuo* under ice-cooling of the receiving flasks: 23.4 g (71%) of **7**. Colorless liquid. B.p. $27^\circ/8$ Torr. $^1\text{H-NMR}$ (CDCl_3): 3.20 (*sept.*, Me_2CH); 2.72 (*q*, MeCH_2); 1.02 (*d*, Me_2CH); 0.92 (*t*, MeCH_2); 0.02 (*s*, Me_3Si).

2. *N-Isopropyl-N,1,1,1-tetramethylsilanamine (8)*. As described for **7**, with Mg chips (7.3 g, 0.3 mol), Et_2O (80 ml), MeI (18.8 ml, 43 g, 0.3 mol), and Et_2O (40 ml; reflux for an additional 60 min), *N-methylisopropylamine (4)*; 28.7 ml, 20 g, 0.275 mol, and Me_3SiCl (38.1 ml, 32.6 g, 0.3 mol; added within 20 min); 29.3 g (73%) of **8**. Colorless liquid. B.p. $22^\circ/11$ Torr or $122^\circ/760$ Torr. $^1\text{H-NMR}$ (CDCl_3): 3.24 (*sept.*, Me_2CH); 2.30 (*s*, Me); 1.00 (*d*, Me_2CH); 0.01 (*s*, Me_3Si).

3. *2-(4-Nitrophenyl)ethyl Diethylphosphoramidochloridite (13)* [10]. To a soln. of crude, slightly yellow 2-(4-nitrophenyl)ethyl phosphorodichloridite (**12**) [13][20][21] (synthesized from PCl_5 and 2-(4-nitrophenyl)ethanol according to [20][21]; 26.7 g, 100 mmol) in anhyd. Et_2O (150 ml) at 0° , *N,N*-diethyl-1,1,1-trimethylsilanamine (**5**) (20.8 ml, 16.0 g, 110 mmol) in anhyd. Et_2O (100 ml) were added dropwise within 60 min. After stirring for 2 h at r.t., the Et_2O and excess amine were evaporated. The remaining yellowish oil was dried for 5 h under high vacuum: 30.17 g (99%) of **13**. UV (MeOH): 271 (3.98), 212 (3.86), 202 (4.01). $^1\text{H-NMR}$ (CDCl_3): 8.17 (*d*, 2 H *o* to NO_2); 7.41 (*d*, 2 H *m* to NO_2); 4.15 (*q*, $\text{CH}_2\text{CH}_2\text{O}$); 3.19–2.98 (*m*, $\text{CH}_2\text{CH}_2\text{O}$, 2 MeCH_2); 1.10 (*t*, 2 MeCH_2). $^{31}\text{P-NMR}$ (CDCl_3): 177.55.

4. *2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidochloridite (15)*. As described for **13**, with **12** (12.26 g, 45 mmol), Et_2O (75 ml), and *N-ethyl-N-isopropyl-1,1,1-trimethylsilanamine (7)*; 7.97 g, 50 mmol) in Et_2O (50 ml; added within 20 min): 14.05 g (98%) of **15**. UV (MeOH): 272 (3.99), 213 (3.87), 203 (4.03). $^1\text{H-NMR}$ (CD_3CN): 8.11 (*d*, 2 H *o* to NO_2); 7.45 (*d*, 2 H *m* to NO_2); 4.12 (*q*, $\text{CH}_2\text{CH}_2\text{O}$); 3.63 (*m*, Me_2CH); 3.11–2.95 (*m*, $\text{CH}_2\text{CH}_2\text{O}$, MeCH_2); 1.12 (*d*, Me_2CH); 1.03 (*t*, MeCH_2). $^{31}\text{P-NMR}$ (CD_3CN): 176.80. Anal. calc. for $\text{C}_{13}\text{H}_{20}\text{ClN}_2\text{O}_3\text{P}$ (318.74): C 48.99, H 6.32, N 8.79; found: C 49.31, H 6.54, N 9.00.

5. *2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidochloridite (16)*. As described for **13**, with **12** (14.10 g, 52 mmol) Et_2O (75 ml), and *N-isopropyl-N-1,1,1-tetramethylsilanamine (8)*; 8.28 g, 57 mmol) in Et_2O (50 ml; added within 20 min): 15.7 g (99%) of **16**. UV (MeOH): 270 (3.98), 212 (3.85), 202 (3.99). $^1\text{H-NMR}$ (CD_3CN): 8.11 (*d*, 2 H *o* to NO_2); 7.45 (*d*, 2 H *m* to NO_2); 4.11 (*q*, $\text{CH}_2\text{CH}_2\text{O}$); 3.70 (*sept.*, Me_2CH); 3.07 (*t*, $\text{CH}_2\text{CH}_2\text{O}$); 2.48 (*d*, MeN); 1.09 (*d*, Me_2CH). $^{31}\text{P-NMR}$ (CD_3CN): 181.70. Anal. calc. for $\text{C}_{12}\text{H}_{18}\text{ClN}_2\text{O}_3\text{P}$ (304.71): C 47.30, H 5.95, N 9.19; found: C 47.43, H 6.11, N 9.00.

6. *2'-O-Methyl-5'-O-(monomethoxytrityl)uridine 3'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (27)*. To a soln of 2'-O-methyl-5'-O-(monomethoxytrityl)uridine (**18**) [22] (531 mg, 1.0 mmol) in dry CH_2Cl_2 (4 ml), abs. (^iPr) Et_3N (0.6 ml, 3.5 mmol) and **13** (488 mg, 1.6 mmol) were added. The mixture was stirred under Ar for 50 min at r.t. and then quenched with dry $^i\text{PrOH}$ (200 μl). After stirring for another 15 min, the mixture was poured on CH_2Cl_2 /phosphate buffer (pH 7) 1:1 (40 ml). The aq. layer was extracted twice with CH_2Cl_2 (20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue taken up in CH_2Cl_2 /petroleum ether 1:1 and purified by FC (silica gel (10 g), 11×2 cm, petroleum ether/ AcOEt 1:1 (30 ml), 1:2 (30 ml), and 1:3 (30 ml), all with 1%) of Et_3N : 667 mg (83%) of **27**. Colorless foam. TLC (SiO_2 , petroleum ether/ AcOEt / Et_3N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.31), 229 (sh, 4.29), 204 (4.87). $^1\text{H-NMR}$ (CDCl_3): 8.98 (br., NH); 8.16–

7.97 (*m*, H–C(6), 2 H *o* to NO₂); 7.42–7.21 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.87–6.80 (*m*, 2 H *o* to MeO); 6.02, 5.96 (2*d*, H–C(1')); 5.20 (*d*, H–C(5)); 4.58, 4.41 (2*m*, H–C(3')); 4.20 (*m*, H–C(2')); 4.00–3.67 (*m*, CH₂CH₂O); 3.80, 3.79 (2*s*, MeOTr); 3.73 (*m*, H–C(4')); 3.63 (*dd*, 1 H–C(5')); 3.55, 3.53 (2*s*, MeO–C(2)); 3.43 (*m*, 1 H–C(5')); 3.14–2.79 (*m*, CH₂CH₂O, 2 MeCH₂); 1.02, 0.92 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.70, 149.52. Anal. calc. for C₄₂H₄₇N₄O₁₀P·0.5 H₂O (807.84): C 62.45, H 5.99, N 6.94; found: C 62.25, H 5.98, N 6.86.

7. 5'-O-(Dimethoxytrityl)-2'-O-methyluridine 3'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**28**). As described in *Exper.* 6, with 5'-O-(dimethoxytrityl)-2'-O-methyluridine (**19**) [22] (561 mg, 1.0 mmol): 676 mg (82%) of **28**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.30), 234 (4.39), 204 (4.87). ¹H-NMR (CDCl₃): 9.60 (br., NH); 8.18–8.02 (*m*, H–C(6), 2 H *o* to NO₂); 7.41–7.23 (*m*, 12 H, (MeO)₂Tr, 2 H *m* to NO₂); 6.86–6.79 (*m*, 4 H *o* to MeO); 6.04, 5.98 (2*d*, H–C(1')); 5.24 (*d*, H–C(5)); 4.57, 4.41 (2*m*, H–C(3')); 4.21 (*m*, H–C(2')); 4.01–3.70 (*m*, CH₂CH₂O, H–C(4')); 3.80 (*s*, 3 H, (MeO)₂Tr); 3.79, 3.78 (2*s*, 3 H, (MeO)₂Tr); 3.64 (*dd*, 1 H–C(5')); 3.56, 3.54 (2*s*, MeO–C(2')); 3.42 (*m*, 1 H–C(5')); 3.15–2.79 (*m*, CH₂CH₂O, 2 MeCH₂); 1.02, 0.92 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.75, 149.51. Anal. calc. for C₄₃H₄₉N₄O₁₁P·0.5 H₂O (837.86): C 61.64, H 6.02, N 6.69; found: C 61.41, H 6.02, N 6.44.

8. 2'-O-Methyl-5'-O-(monomethoxytrityl)uridine 3'-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite] (**29**). As described in *Exper.* 6, with 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (**14**; 765 mg, 2.3 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1:1, then petroleum ether/acetone 7:2 (100 ml), 3:1 (50 ml), 2:1 (50 ml), and 1:1 (50 ml), all with 1% of Et₃N): 604 mg (73%) of **29**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.68. UV (MeOH): 265 (4.29), 230 (sh, 4.27), 205 (4.82). ¹H-NMR (CDCl₃): 8.11, 7.99 (2*d*, 2 H *o* to NO₂); 7.76, 7.64 (2*d*, H–C(6)); 7.47–7.25 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.86 (*dd*, 2 H *o* to MeO); 5.85 (2*d*, H–C(1')); 5.25 (*t*, H–C(5)); 4.42, 4.32 (2*m*, H–C(3')); 4.06 (*m*, H–C(2')); 3.85 (*m*, CH₂CH₂O); 3.79 (*m*, H–C(4')); 3.75 (2*s*, MeOTr); 3.58–3.28 (*m*, 2 H–C(5'), 2 Me₂CH); 3.42 (2*s*, MeO–C(2')); 3.00, 2.86 (2*t*, 2 H, CH₂CH₂O); 1.14–0.97 (*m*, 2 Me₂CH). ³¹P-NMR (CDCl₃): 150.53, 149.66. ³¹P-NMR (CD₃CN): 150.02, 149.62. Anal. calc. for C₄₄H₅₁N₄O₁₀P (826.88): C 63.91, H 6.22, N 6.78; found: C 63.58, H 6.52, N 6.64.

9. 2'-O-Methyl-5'-O-(monomethoxytrityl)uridine 3'-[2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidite] (**30**). As described in *Exper.* 6, with **15** (510 mg, 1.6 mmol): 667 mg (81%) of **30**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.66. UV (MeOH): 265 (4.31), 230 (4.34), 206 (4.82). ¹H-NMR (CDCl₃): 8.83 (br., NH); 8.16–7.98 (*m*, H–C(6), 2 H *o* to NO₂); 7.42–7.22 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.84, 6.83 (2*d*, 2 H *o* to MeO); 6.02, 5.97 (2*d*, H–C(1')); 5.21, 5.18 (2*d*, H–C(5)); 4.56, 4.42 (2*m*, H–C(3')); 4.20 (*m*, H–C(2')); 4.02–3.40 (*m*, H–C(4'), CH₂CH₂O, Me₂CH, 2 H–C(5')); 3.80, 3.78 (2*s*, MeOTr); 3.55, 3.53 (2*s*, MeO–C(2')); 3.11–2.70 (*m*, CH₂CH₂O, MeCH₂); 1.17–0.91 (*m*, MeCH₂, Me₂CH). ³¹P-NMR (CDCl₃): 151.86, 150.90. Anal. calc. for C₄₃H₄₉N₄O₁₀P·0.5 H₂O (821.86): C 62.84, H 6.13, N 6.82; found: C 62.56, H 6.11, N 6.87.

10. 2'-O-Methyl-5'-O-(monomethoxytrityl)uridine 3'-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite] (**31**). As described in *Exper.* 6, with 2-(4-nitrophenyl)ethyl isopropyl(methyl)phosphoramidochloridite (**16**; 488 mg, 1.6 mmol): 657 mg (82%) of **31**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.64. UV (MeOH): 265 (4.29), 230 (sh, 4.29), 206 (4.83). ¹H-NMR (CDCl₃): 8.92 (br., NH); 8.15–8.00 (*m*, H–C(6), 2 H *o* to NO₂); 7.41–7.21 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.83 (2*d*, 2 H *o* to MeO); 6.01, 5.95 (2*d*, H–C(1')); 5.21, 5.19 (2*d*, H–C(5)); 4.56, 4.41 (2*m*, H–C(3')); 4.19 (*m*, H–C(2')); 4.00–3.38 (*m*, H–C(4'), CH₂CH₂O, Me₂CH, 2 H–C(5')); 3.80, 3.79 (2*s*, MeOTr); 3.55, 3.54 (2*s*, MeO–C(2')); 3.01–2.89 (2*t*, CH₂CH₂O); 2.42–2.24 (2*t*, MeN); 1.09–1.02 (2*d*, Me₂CH). ³¹P-NMR (CDCl₃): 149.63, 148.23. Anal. calc. for C₄₂H₄₇N₄O₁₀P·0.5 H₂O (807.84): C 62.45, H 5.99, N 6.94; found: C 62.07, H 6.13, N 6.77.

11. 2'-O-Methyl-5'-O-(monomethoxytrityl)uridine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**32**) [17]. As described in *Exper.* 6, with 2-cyanoethyl diisopropyl phosphoramidochloridite (**17**) (379 mg, 1.6 mmol): 621 mg (85%) of **32**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.63. UV (MeOH): 262 (4.03), 230 (sh, 4.24), 206 (4.74). ¹H-NMR (CDCl₃): 8.65 (br., 1 NH); 8.08, 7.96 (2*d*, H–C(6)); 7.44–7.23 (*m*, 12 H, MeOTr); 6.89–6.82 (*m*, 2 H *o* to MeO); 6.02, 5.97 (2*d*, H–C(1')); 5.20 (*t*, H–C(5)); 4.63, 4.50 (2*m*, H–C(3')); 4.22 (*m*, H–C(2')); 3.95–3.78 (*m*, H–C(4'), CH₂CH₂O); 3.81, 3.80 (2*s*, MeOTr); 3.70–3.43 (*m*, 2 Me₂CH, 2 H–C(5')); 3.58 (2*s*, MeO–C(2')); 2.65, 2.42 (2*t*, CH₂CH₂O); 1.21–1.02 (*m*, 2 Me₂CH). ³¹P-NMR (CDCl₃): 151.30, 150.87. Anal. calc. for C₃₉H₄₇N₄O₈P·0.5 H₂O (739.81): C 63.32, H 6.54, N 7.57; found: C 63.44, H 6.59, N 7.63.

12. N³-(4-Methoxybenzoyl)-2'-O-methyl-5'-O-(monomethoxytrityl)uridine 3'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**33**). As described in *Exper.* 6, with N³-(4-methoxybenzoyl)-2'-O-methyl-5'-O-(mono-

methoxytrityl)uridine (**20**) [22] (665 mg, 1.0 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 3:2 (50 ml), and 1:1 (50 ml), all with 1% of Et₃N): 755 mg (81%) of **33**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 3:7:1): *R_f* 0.74. UV (MeOH): 276(4.51), 224(4.50), 204(4.90). ¹H-NMR (CDCl₃): 8.23–8.04 (*m*, H–C(6), 2 H *o* to NO₂); 7.90 (*m*, 2 H *o* to CO); 7.43–7.24 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.94 (*d*, 2 H *o* to MeO of An); 6.87–6.83 (*m*, 2 H *o* to MeO of MeOTr); 5.96, 5.91 (2*s*, H–C(1′)); 5.27 (*d*, H–C(5)); 4.62, 4.45 (2*m*, H–C(3′)); 4.23 (*m*, H–C(2′)); 3.99–3.44 (*m*, H–C(4′), CH₂CH₂O, 2 H–C(5′)); 3.86 (*s*, MeO of An); 3.81, 3.79 (2*s*, MeOTr); 3.53, 3.51 (2*s*, Me–C(2′)); 3.15–2.80 (*m*, CH₂CH₂O, 2 MeCH₂); 1.03, 0.93 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.84, 149.59. Anal. calc. for C₃₀H₃₃N₄O₁₂P (932.96): C 64.37, H 5.73, N 6.00; found: C 63.83, H 5.85, N 5.62.

13. 2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**34**). As described in *Exper. 6*, with 2′-O-methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**21**) [22] (723 mg, 1.0 mmol): 882 mg (89%) of **34**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): *R_f* 0.60. UV (MeOH): 274(4.39), 235(4.46), 205(4.94). ¹H-NMR (CDCl₃): 8.57 (*m*, H–C(6)); 8.17 (*d*, 2 H *o* to NO₂); 8.11, 8.05 (2*d*, 2 H *o* to NO₂); 7.44–7.25 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.84 (*m*, 2 H *o* to MeO); 6.73 (*m*, H–C(5)); 5.98 (*d*, H–C(1′)); 4.51–4.25 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH₂CH₂OCO); 3.96–3.64 (*m*, CH₂CH₂OP, H–C(4′)); 3.81, 3.79 (2*s*, MeOTr); 3.67, 3.65 (2*s*, MeO–C(2′)); 3.44 (*m*, 2 H–C(5′)); 3.11 (*t*, CH₂CH₂O), 3.04–2.80 (*m*, CH₂CH₂O, 2 MeCH₂); 0.98, 0.89 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.55, 149.74. Anal. calc. for C₅₁H₅₃N₆O₁₃P (991.01): C 61.81, H 5.59, N 8.48; found: C 61.21, H 5.54, N 8.24.

14. 5′-O-(Dimethoxytrityl)-2′-O-methyl-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**35**). As described in *Exper. 6*, with 5′-O-(dimethoxytrityl)-2′-O-methyl-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**22**) [22] (753 mg, 1.0 mmol): 858 mg (84%) of **35**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): *R_f* 0.60. UV (MeOH): 274(4.42), 236(4.56), 204(4.96). ¹H-NMR (CDCl₃): 8.60 (*m*, H–C(6)); 8.17 (*d*, 2 H *o* to NO₂); 8.11, 8.05 (2*d*, 2 H *o* to NO₂); 7.41–7.25 (*m*, 14 H, (MeO)₂Tr, 2 × 2 H *m* to NO₂); 6.84 (*m*, 4 H *o* to MeO); 6.74 (*m*, H–C(5)); 5.98 (*d*, H–C(1′)); 4.56–4.25 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH₂CH₂OCO); 3.96–3.64 (*m*, CH₂CH₂OP, H–C(4′)); 3.81, 3.79 (2*s*, (MeO)₂Tr); 3.66, 3.64 (2*s*, MeO–C(2′)); 3.44 (*m*, 2 H–C(5′)); 3.11 (*t*, CH₂CH₂O); 3.04–2.76 (*m*, CH₂CH₂O, 2 MeCH₂); 0.99, 0.90 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.47, 149.31. Anal. calc. for C₅₂H₅₇N₆O₁₄P · 0.5 H₂O (1030.04): C 60.64, H 5.68, N 8.16; found: C 60.22, H 5.84, N 7.87.

15. 2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite] (**36**). As described in *Exper. 6*, with **21** (723 mg, 1.0 mmol) and **14** (765 mg, 2.3 mmol). Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1:1, then petroleum ether/acetone 7:2 (100 ml), 3:1 (50 ml), 2:1 (50 ml), and 1:1 (50 ml), all with 1% of Et₃N): 732 mg (72%) of **36**. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): 0.64. Colorless foam. UV (MeOH): 274(4.42), 235(4.48), 205(4.93). ¹H-NMR (CDCl₃): 8.57 (*m*, 1 H, H–C(6)); 8.18 (*d*, 2 H *o* to NO₂); 8.11, 8.01 (2*d*, 2 H *o* to NO₂); 7.45–7.24 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.83 (*m*, 2 H *o* to MeO); 6.69 (*m*, H–C(5)); 6.00 (*d*, H–C(1′)); 4.51–4.22 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH₂CH₂OCO); 3.96–3.41 (*m*, CH₂CH₂OP, H–C(4′), 2 Me₂CH, 2 H–C(5′)); 3.81–3.79 (2*s*, MeOTr); 3.66, 3.63 (2*s*, MeO–C(2′)); 3.11 (*t*, CH₂CH₂O); 2.98, 2.83 (2*t*, CH₂CH₂O); 1.21–0.95 (*m*, 2 Me₂CH). ³¹P-NMR (CDCl₃): 149.98, 149.57. Anal. calc. for C₅₃H₅₉N₆O₁₃P · 0.5 H₂O (1026.06): C 62.04, H 5.89, N 8.19; found: C 61.79, H 5.90, N 8.36.

16. 2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidite] (**37**). As described in *Exper. 6*, with **21** [22] (723 mg, 1.0 mmol) and **15** (510 mg, 1.6 mmol): 905 mg (90%) of **37**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): *R_f* 0.62. UV (MeOH): 274(4.42), 233(4.50), 205(4.96). ¹H-NMR (CDCl₃): 8.57 (*m*, H–C(6)); 8.18 (*d*, 2 H *o* to NO₂); 8.11, 8.03 (2*d*, 2 H *o* to NO₂); 7.44–7.25 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.84 (*m*, 2 H *o* to MeO); 6.71 (*m*, H–C(5)); 5.98 (*d*, H–C(1′)); 4.51–4.24 (*m*, H–C(3′), H–C(2′)); 4.43 (*t*, CH₂CH₂OCO); 3.96–3.40 (*m*, CH₂CH₂OP, H–C(4′), Me₂CH, 2 H–C(5′)); 3.81, 3.79 (2*s*, MeOTr); 3.66, 3.64 (2*s*, MeO–C(2′)); 3.11 (*t*, CH₂CH₂O); 3.04–2.83 (*m*, CH₂CH₂O, MeCH₂); 1.17–0.89 (*m*, Me₂CH, MeCH₂). ³¹P-NMR (CDCl₃): 151.44, 150.70. Anal. calc. for C₅₂H₅₇N₆O₁₃P · 0.5 H₂O (1014.04): C 61.59, H 5.77, N 8.29; found: C 61.21, H 5.79, N 8.27.

17. 2′-O-Methyl-5′-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3′-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite] (**38**). As described in *Exper. 6*, with **21** [22] (723 mg, 1.0 mmol) and **16** (488 mg, 1.6 mmol): 823 mg (83%) of **38**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): *R_f* 0.60. UV (MeOH): 274(4.42), 234(4.52), 205(4.93). ¹H-NMR (CDCl₃): 8.57 (*m*, H–C(6)); 8.18 (*d*, 2 H *o* to NO₂); 8.11, 8.05 (2*d*, 2 H *o* to NO₂); 7.44–7.25 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂);

6.84 (*m*, 2 H *o* to MeO); 6.74 (*m*, H–C(5)); 5.97 (*d*, H–C(1')); 4.51–4.25 (*m*, H–C(3'), H–C(2')); 4.43 (*t*, CH₂CH₂OCO); 3.96–3.58 (*m*, CH₂CH₂OP, H–C(4'), Me₂CH); 3.81–3.79 (2*s*, MeOTr); 3.66, 3.64 (2*s*, MeO–C(2')); 3.44 (*m*, 2 H–C(5')); 3.10 (*t*, CH₂CH₂O); 2.98, 2.88 (2*t*, CH₂CH₂O); 2.40, 2.22 (2*d*, MeN); 1.09–0.98 (*m*, Me₂CH). ³¹P-NMR (CDCl₃): 149.30, 147.94. Anal. calc. for C₅₁H₅₅N₆O₁₃P·0.5 H₂O (1000.02): C 61.25, H 5.64, N 8.40; found: C 60.84, H 5.66, N 8.52.

18. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3'-[2-(2-Cyanoethyl Diisopropylphosphoramidite)] (**39**). As described in *Exper.* 6, with **21** [22] (723 mg, 1.0 mmol) and **17** (379 mg, 1.6 mmol): 803 mg (87%) of **39**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.59 (0.56, 0.62). UV (MeOH): 281(4.18), 234(4.45), 206(4.84). ¹H-NMR (CDCl₃): 8.59, 8.49 (2*d*, H–C(6)); 8.18 (*d*, 2 H *o* to NO₂); 7.68 (br., NH); 7.45–7.25 (*m*, 14 H, MeO–Tr, 2 H *m* to NO₂); 6.87, 6.84 (2*d*, 2 H *o* to MeO); 6.72, 6.65 (2*d*, H–C(5)); 6.01 (*d*, H–C(1')); 4.58–4.38 (*m*, H–C(3'), CH₂CH₂OCO); 4.27 (*m*, H–C(2')); 3.90 (*m*, H–C(4')); 3.86–3.38 (*m*, CH₂CH₂OP, 2 Me₂CH, 2 H–C(5')); 3.81 (*s*, MeOTr); 3.67 (*s*, Me–C(2')); 3.09 (*t*, CH₂CH₂O); 2.61, 2.39 (2*t*, CH₂CH₂O); 1.26–0.98 (*m*, 2 Me₂CH). ³¹P-NMR (CDCl₃): 151.10, 150.78. Anal. calc. for C₄₈H₅₅N₆O₁₁P·0.5 H₂O (931.98): C 61.86, H 5.95, N 9.02; found: C 61.56, H 6.08, N 8.91.

19. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**40**). As described in *Exper.* 6, with 2'-O-methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**23**) [22] (747 mg, 1.0 mmol): 893 mg (88%) of **40**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.68. UV (MeOH): 267(4.60), 232(sh, 4.40), 204(4.96). ¹H-NMR (CDCl₃): 8.66 (*s*, H–C(8)); 8.31 (br., NH); 8.20–8.05 (*m*, H–C(2), 2 × 2 H *o* to NO₂); 7.44–7.18 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.80 (*m*, 2 H *o* to MeO); 6.14 (*t*, H–C(1')); 4.65–4.50 (*m*, H–C(3'), H–C(2')); 4.53 (*t*, CH₂CH₂OCO); 4.37 (*m*, H–C(4')); 4.02–3.68 (*m*, CH₂CH₂OP); 3.77, 3.76 (2*s*, MeOTr); 3.50 (*dd*, 1 H–C(5')); 3.44, 3.43 (2*s*, MeO–C(2')); 3.33 (*m*, 1 H–C(5')); 3.17–2.84 (*m*, 2 CH₂CH₂O, 2 MeCH₂); 1.01, 0.94 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.63, 149.85. Anal. calc. for C₅₂H₅₅N₈O₁₂P (1015.03): C 61.53, H 5.46, N 11.04; found: C 60.93, H 5.40, N 10.69.

20. 5'-O-(Dimethoxytrityl)-2'-O-methyl-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**41**). As described in *Exper.* 6, with 5'-O-(dimethoxytrityl)-2'-O-methyl-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**24**) [22] (777 mg, 1.0 mmol): 836 mg (80%) of **41**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.68. UV (MeOH): 267(4.59), 236(4.47), 204(4.98). ¹H-NMR (CDCl₃): 8.68 (*s*, H–C(8)); 8.47 (br., NH); 8.18–8.05 (*m*, H–C(2), 2 × 2 H *o* to NO₂); 7.43–7.21 (*m*, 14 H, (MeO)₂Tr, 2 × 2 H *m* to NO₂); 6.80 (*d*, 4 H *o* to MeO); 6.15 (*t*, H–C(1')); 4.70–4.50 (*m*, H–C(3'), H–C(2')); 4.53 (*t*, CH₂OCO); 4.37, 4.32 (2*m*, H–C(4')); 4.06–3.80 (*m*, CH₂CH₂OP); 3.77, 3.76 (2*s*, (MeO)₂Tr); 3.50 (*dd*, 1 H–C(5')); 3.45, 3.44 (2*s*, MeO–C(2')); 3.33 (*m*, 1 H–C(5')); 3.14 (*t*, CH₂CH₂O); 3.07–2.85 (*m*, CH₂CH₂O, 2 MeCH₂); 1.02, 0.95 (2*t*, 2 MeCH₂). ³¹P-NMR (CDCl₃): 150.66, 149.80. Anal. calc. for C₅₃H₅₇N₈O₁₃P·0.5 H₂O (1054.07): C 60.39, H 5.55, N 10.63; found: C 60.07, H 5.67, N 10.30.

21. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite] (**42**). As described in *Exper.* 6, with **23** [22] (747 mg, 1.0 mmol) and **14** (765 mg, 2.3 mmol). Purification by FC (silica gel, 10 g, 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1:1, then petroleum ether/acetone 7:2 (100 ml), 3:1 (50 ml), 2:1 (50 ml), and 1:1 (50 ml), all with 1% of Et₃N): 751 mg (72%) of **42**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.72. UV (MeOH): 267(4.62), 233(4.40), 206(4.95). ¹H-NMR (CDCl₃): 8.67 (*s*, H–C(8)); 8.33 (br., NH); 8.19–8.01 (*m*, H–C(2), 2 × 2 H *o* to NO₂); 7.44–7.18 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.79 (*d*, 2 H *o* to MeO); 6.16, 6.12 (2*d*, H–C(1')); 4.64–4.50 (*m*, H–C(3'), H–C(2')); 4.53 (*t*, CH₂CH₂OCO); 4.39–4.34 (*m*, H–C(4')); 3.77, 3.76 (2*s*, MeOTr); 4.05–3.28 (*m*, CH₂CH₂OP, 2 Me₂CH, 2 H–C(5')); 3.45, 3.43 (2*s*, MeO–C(2')); 3.14 (*t*, CH₂CH₂O); 3.02, 2.83 (2*t*, CH₂CH₂O); 1.19–1.03 (*m*, 2 Me₂CH). ³¹P-NMR (CDCl₃): 150.64, 149.76. Anal. calc. for C₅₄H₅₉N₈O₁₂P·0.5 H₂O (1052.09): C 61.65, H 5.70, N 10.74; found: C 61.40, H 5.74, N 10.58.

22. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-[2-(4-Nitrophenyl)ethylphosphoramidite] (**43**). As described in *Exper.* 6, with **23** [22] (747 mg, 1.0 mmol) and **15** (510 mg, 1.6 mmol): 864 mg (84%) of **43**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.70. UV (MeOH): 267(4.57), 235(4.36), 204(4.94). ¹H-NMR (CDCl₃): 8.66 (*s*, H–C(8)); 8.34 (*s*, NH); 8.19–8.04 (*m*, H–C(2), 2 × 2 H *o* to NO₂); 7.44–7.21 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.80 (*m*, 2 H *o* to MeO); 6.15, 6.13 (2*d*, H–C(1')); 4.66–4.50 (*m*, H–C(3'), H–C(2')); 4.53 (*t*, CH₂CH₂OCO); 4.35 (*m*, H–C(4')); 4.04–3.68 (*m*, CH₂CH₂OP); 3.77, 3.76 (2*s*, MeOTr); 3.52 (*dd*, 1 H–C(5'), Me₂CH); 3.44, 3.43 (2*s*, MeO–C(2')); 3.33 (*dd*, 1 H–C(5')); 3.14 (*t*, CH₂CH₂O); 3.04–2.83 (*m*, CH₂CH₂O, MeCH₂); 1.16–0.96 (*m*, Me₂CH, MeCH₂).

^{31}P -NMR (CDCl_3): 151.94, 151.14. Anal. calc. for $\text{C}_{53}\text{H}_{57}\text{N}_8\text{O}_{12}\text{P}$ (1029.06): C 61.86, H 5.58, N 10.89; found: C 61.39, H 5.73, N 10.76.

23. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-[2-(4-nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite] (**44**). As described in *Exper.* 6, with **23** [22] (747 mg, 1.0 mmol) and **16** (488 mg, 1.6 mmol): 812 mg (80%) of **44**. Colorless foam. TLC (SiO_2 , petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.68. UV (MeOH): 267 (4.58), 234 (sh, 4.38), 206 (4.93). ^1H -NMR (CDCl_3): 8.67 (s, H-C(8)); 8.41 (br., NH); 8.18–8.05 (m, H-C(2), 2 $\times$ 2 H *o* to NO_2); 7.44–7.18 (m, 16 H, MeOTr, 2 $\times$ 2 H *m* to NO_2); 6.80, 6.79 (2d, 2 H *o* to MeO); 6.16, 6.14 (2d, H-C(1')); 4.68–4.50 (m, H-C(3'), H-C(2')); 4.53 (t, CH_2 , CH_2OCO); 4.38, 4.32 (2m, H-C(4')); 3.99–3.48 (m, $\text{CH}_2\text{CH}_2\text{OP}$, Me_2CH); 3.77, 3.76 (2s, MeOTr); 3.50 (dd, 1 H-C(5')); 3.45, 3.44 (2s, Me-C(2')); 3.33 (m, 1 H-C(5')); 3.14 (t, $\text{CH}_2\text{CH}_2\text{O}$); 3.02, 2.88 (2t, $\text{CH}_2\text{CH}_2\text{O}$); 2.41, 2.32 (2d, MeN); 1.10–0.98 (m, Me_2CH). ^{31}P -NMR (CDCl_3): 149.48, 148.75. Anal. calc. for $\text{C}_{52}\text{H}_{55}\text{N}_8\text{O}_{12}\text{P} \cdot 0.5 \text{H}_2\text{O}$ (1024.04): C 61.65, H 5.70, N 10.74; found: C 61.40, H 5.74, N 10.58.

24. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-[2-(2-cyanoethyl) diisopropylphosphoramidite] (**45**). As described in *Exper.* 6, with **23** [22] (747 mg, 1.0 mmol) and **17** (379 mg, 1.6 mmol): 768 mg (81%) of **45**. Colorless foam. TLC (SiO_2 , petroleum ether/AcOEt/Et₃N 1:9:1): R_f 0.66 (0.63, 0.69). UV (MeOH): 267 (4.44), 234 (4.29), 204 (4.90). ^1H -NMR (CDCl_3): 8.68, 8.65 (2s, H-C(8)); 8.49 (br, NH); 8.21–8.13 (m, H-C(2), 2 H *o* to NO_2); 7.47–7.19 (m, 14 H, MeOTr, 2 H *m* to NO_2); 6.80 (m, 2 H *o* to MeO); 6.15, 6.14 (2d, H-C(1')); 4.70 (m, H-C(3')); 4.60 (m, H-C(2')); 4.52 (t, $\text{CH}_2\text{CH}_2\text{OCO}$); 4.42, 4.36 (2m, H-C(4')); 3.96–3.82 (m, $\text{CH}_2\text{CH}_2\text{OP}$); 3.78, 3.77 (2s, MeOTr); 3.71–3.51 (m, 2 Me_2CH , 1 H-C(5')); 3.48 (s, MeO-C(2')); 3.36 (m, 1 H-C(5')); 3.13 (t, $\text{ArCH}_2\text{CH}_2\text{O}$); 2.65, 2.37 (2t, $\text{NCCCH}_2\text{CH}_2\text{O}$); 1.22–1.06 (m, Me_2CH). ^{31}P -NMR (CDCl_3): 151.65, 150.93. Anal. calc. for $\text{C}_{49}\text{H}_{55}\text{N}_8\text{O}_{10}\text{P} \cdot \text{H}_2\text{O}$ (965.02): C 60.99, H 5.95, N 11.61; found: C 61.04, H 5.93, N 11.61.

25. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-[2-(4-nitrophenyl)ethyl Diethylphosphoramidite] (**46**). As described in *Exper.* 6, with 2'-O-methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**25**) [22] (912 mg, 1.0 mmol) and dry THF as solvent. Purification by FC (silica gel (10 g), 11 $\times$ 2 cm, soln. in CH_2Cl_2 /petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et₃N): 980 mg (83%) of **46**. Colorless foam. TLC (SiO_2 , petroleum ether/AcOEt/Et₃N 3:7:1): R_f 0.70. UV (MeOH): 269 (4.64), 236 (4.41), 205 (4.95). ^1H -NMR (CDCl_3): 8.17–7.97 (m, 3 $\times$ 2 H *o* to NO_2 , H-C(8), NH); 7.54–7.22 (m, 18 H, MeOTr, 3 $\times$ 2 H *m* to NO_2); 6.78 (d, 2 H *o* to MeO); 6.07 (2d, H-C(1')); 4.82 (t, $\text{CH}_2\text{CH}_2\text{O}$); 4.61–4.47 (m, H-C(3'), H-C(2')); 4.41 (t, $\text{CH}_2\text{CH}_2\text{OCO}$); 4.30, 4.26 (2m, H-C(4')); 4.06–3.70 (m, $\text{CH}_2\text{CH}_2\text{OP}$); 3.70 (s, MeOTr); 3.44 (s, MeO-C(2'), 1 H-C(5')); 3.32 (t, $\text{CH}_2\text{CH}_2\text{O}$, 1 H-C(5')); 3.10–2.82 (m, 2 $\text{CH}_2\text{CH}_2\text{O}$, 2 MeCH_2); 1.14, 1.00 (2t, 2 MeCH_2). ^{31}P -NMR (CDCl_3): 150.48, 149.94. Anal. calc. for $\text{C}_{60}\text{H}_{62}\text{N}_9\text{O}_{15}\text{P} \cdot \text{H}_2\text{O}$ (1198.20): C 60.15, H 5.38, N 10.52; found: C 59.61, H 5.37, N 10.17.

26. 5'-O-(Dimethoxytrityl)-2'-O-methyl-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-[2-(4-nitrophenyl)ethyl Diethylphosphoramidite] (**47**). As described in *Exper.* 6, with 5'-O-(dimethoxytrityl)-2'-O-methyl-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**26**) [22] (942 mg, 1.0 mmol) and dry THF as solvent. Purification by FC (silica gel (10 g), 11 $\times$ 2 cm, soln. in CH_2Cl_2 /petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et₃N): 968 mg (80%) of **47**. Colorless foam. TLC (SiO_2 , petroleum ether/AcOEt/Et₃N 3:7:1): R_f 0.70. UV (MeOH): 269 (4.66), 236 (4.52), 204 (5.01). ^1H -NMR (CDCl_3): 8.17–7.97 (m, 3 $\times$ 2 H *o* to NO_2 , H-C(8)); 7.54–7.22 (m, 16 H, (MeO)₂Tr, 3 $\times$ 2 H *m* to NO_2); 6.78 (d, 4 H *o* to MeO); 6.07 (m, H-C(1')); 4.82 (t, $\text{CH}_2\text{CH}_2\text{O}$); 4.61–4.47 (m, H-C(3'), H-C(2')); 4.41 (t, $\text{CH}_2\text{CH}_2\text{OCO}$); 4.30, 4.26 (2m, H-C(4')); 4.06–3.70 (m, $\text{CH}_2\text{CH}_2\text{OP}$); 3.77, 3.75 (2s, (MeO)₂Tr); 3.44 (s, 2' MeO-C(2'), 1 H-C(5')); 3.32 (t, $\text{CH}_2\text{CH}_2\text{O}$, 1 H-C(5')); 3.10–2.82 (m, 2 $\text{CH}_2\text{CH}_2\text{O}$, 2 MeCH_2); 1.14, 1.00 (2t, 2 MeCH_2). ^{31}P -NMR (CDCl_3): 150.50, 149.90. Anal. calc. for $\text{C}_{61}\text{H}_{64}\text{N}_9\text{O}_{16}\text{P} \cdot 0.5 \text{H}_2\text{O}$ (1219.21): C 60.09, H 5.29, N 10.34; found: C 59.70, H 5.40, N 9.81.

27. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine 3'-[2-(4-nitrophenyl)ethyl Diisopropylphosphoramidite] (**48**). As described in *Exper.* 6, with **25** [22] (912 mg, 1.0 mmol) and **14** (765 mg, 2.3 mmol). Purification by FC (silica gel (10 g), 11 $\times$ 2 cm, soln. in CH_2Cl_2 /petroleum ether 1:1, then petroleum ether/AcOEt 2:1 (30 ml), 3:2 (30 ml), 1:1 (50 ml), and 1:2 (50 ml), all with 1% of Et₃N): 870 mg (72%) of **48**. Colorless foam. TLC (SiO_2 , petroleum ether/AcOEt/Et₃N 3:7:1): R_f 0.74. UV (MeOH): 269 (4.65), 236 (4.43), 206 (4.92). ^1H -NMR (CDCl_3): 8.19–7.97 (m, 3 $\times$ 2 H *o* to NO_2 , H-C(8)); 7.55–7.17 (m, 18 H, MeOTr, 3 $\times$ 2 H *m* to NO_2); 6.82–6.76 (m, 2 H *o* to MeO); 6.09, 6.03 (2d, H-C(1')); 4.83 (t, $\text{CH}_2\text{CH}_2\text{O}$); 4.59–4.48 (m, H-C(3'), H-C(2')); 4.40 (t, $\text{CH}_2\text{CH}_2\text{OCO}$); 4.33, 4.29 (2m, H-C(4')); 4.04–3.69 (m, $\text{CH}_2\text{CH}_2\text{OP}$); 3.77, 3.75 (2s, MeOTr); 3.65–3.27 (m, 2 Me_2CH , 2 H-C(5'));

3.44 (s, MeO–C(2')); 3.33 (t, CH₂CH₂O); 3.04–2.79 (m, CH₂CH₂O); 1.17–0.98 (m, 2 Me₂CH). ³¹P-NMR (CDCl₃): 150.49, 149.91. Anal. calc. for C₆₂H₆₆N₉O₁₅P·H₂O (1226.25): C 60.72, H 5.59, N 10.28; found: C 60.65, H 5.59, N 10.06.

28. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 3'-[2-(4-Nitrophenyl)ethyl Ethyl(isopropyl)phosphoramidite] (**49**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol), **15** (510 mg, 1.6 mmol), and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1 : 1, then petroleum ether/AcOEt 2 : 1 (30 ml), 1 : 1 (30 ml), and 1 : 2 (70 ml), all with 1% of Et₃N): 967 mg (81%) of **49**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 3 : 7 : 1): R_f 0.74. UV (MeOH): 269(4.64), 236(4.42), 204(4.97). ¹H-NMR (CDCl₃): 8.18–7.99 (m, 3 × 2 H *o* to NO₂, H–C(8)); 7.54–7.18 (m, 18 H, MeOTr, 3 × 2 H *m* to NO₂); 6.79 (m, 2 H *o* to MeO); 6.09, 6.04 (2d, H–C(1')); 4.83 (t, CH₂CH₂O); 4.60–4.47 (m, H–C(3'), H–C(2')); 4.40 (t, CH₂CH₂O–CO); 4.32, 4.28 (2m, H–C(4')); 4.04–3.65 (m, CH₂CH₂OP); 3.77, 3.75 (2s, MeOTr); 3.63–3.46 (m, Me₂CH, 1 H–C(5')); 3.44 (s, MeO–C(2')); 3.32 (t, CH₂CH₂O, 1 H–C(5')); 3.10–2.80 (m, 2 CH₂CH₂O, MeCH₂); 1.15–0.93 (m, Me₂CH, MeCH₂). ³¹P-NMR (CDCl₃): 151.80, 151.27. Anal. calc. for C₆₁H₆₄N₉O₁₅P (1194.21): C 61.35, H 5.40, N 10.56; found: C 60.81, H 5.41, N 10.25.

29. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 3'-[2-(4-Nitrophenyl)ethyl Isopropyl(methyl)phosphoramidite] (**50**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol), **16** (488 mg, 1.6 mmol), and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1 : 1, then petroleum ether/AcOEt 2 : 1 (30 ml), 1 : 1 (30 ml), and 1 : 2 (70 ml), all with 1% of Et₃N): 968 mg (82%) of **50**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 3 : 7 : 1): R_f 0.70. UV (MeOH): 269(4.62), 236(4.38), 204(4.94). ¹H-NMR (CDCl₃): 8.18–7.99 (m, 3 × 2 H *o* to NO₂, H–C(8)); 7.58–7.20 (m, 18 H, MeOTr, 3 × 2 H *m* to NO₂); 6.79 (m, 2 H *o* to MeO); 6.09, 6.05 (2d, H–C(1')); 4.83 (t, CH₂CH₂O); 4.60 (m, H–C(3')); 4.52–4.38 (m, H–C(2'), CH₂CH₂O–CO); 4.32, 4.26 (2m, H–C(4')); 4.01–3.42 (m, CH₂CH₂OP, Me₂CH, 1 H–C(5')); 3.77, 3.76 (2s, MeOTr); 3.44 (s, MeO–C(2')); 3.32 (t, CH₂OCH₂, 1 H–C(5')); 3.10–2.85 (m, 2CH₂CH₂O); 2.39, 2.29 (2d, MeN); 1.09–0.94 (m, Me₂CH). ³¹P-NMR (CDCl₃): 149.33, 148.88. Anal. calc. for C₆₀H₆₂N₉O₁₅P (1180.18): C 61.06, H 5.30, N 10.68; found: C 60.95, H 5.34, N 10.37.

30. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**51**). As described in *Exper. 6*, with **25** [22] (912 mg, 1.0 mmol), **17** (379 mg, 1.6 mmol), and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1 : 1, then petroleum ether/AcOEt 2 : 1 (30 ml), petroleum ether/AcOEt 1 : 1 (30 ml), and petroleum ether/AcOEt 1 : 2 (70 ml), all with 1% of Et₃N): 890 mg (80%) of **51**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 3 : 7 : 1): R_f 0.68 (0.72, 0.65). UV (MeOH): 269(4.62), 235(4.40), 206(4.92). ¹H-NMR (CDCl₃): 8.16 (d, 2 × 2 H *o* to NO₂); 8.02, 7.98 (2s, H–C(8)); 7.55–7.18 (m, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.82–6.78 (m, 2 H *o* to MeO); 6.08, 6.03 (2d, H–C(1')); 4.82 (t, CH₂CH₂O); 4.62–4.53 (m, H–C(3'), H–C(2')); 4.41 (t, CH₂CH₂OCO); 4.29 (m, H–C(4')); 3.96–3.84 (m, CH₂CH₂OP); 3.77 (2s, MeOTr); 3.66–3.36 (m, 2 Me₂CH, 2 H–C(5')); 3.47 (s, MeO–C(2')); 3.32 (t, ArCH₂CH₂O); 3.07 (t, ArCH₂CH₂O); 2.68, 2.34 (2t, NCCCH₂CH₂O); 1.20–1.02 (m, 2Me₂CH). ³¹P-NMR (CDCl₃): 151.38, 151.04. Anal. calc. for C₅₇H₆₂N₉O₁₃P·H₂O (1130.16): C 60.57, H 5.71, N 11.15; found: C 60.64, H 5.75, N 11.16.

31. 3'-O-Methyl-5'-O-(monomethoxytrityl)uridine 2'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**56**). As described in *Exper. 6*, with 3'-O-Methyl-5'-O-(monomethoxy)trityluridine (**52**) [22] (531 mg, 1.0 mmol): 623 mg (78%) of **56**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1 : 9 : 1): R_f 0.64. UV (MeOH): 265(4.33), 229 (sh, 4.33), 206(4.85). ¹H-NMR (CDCl₃): 8.12 (d, 2 H *o* to NO₂); 8.02 (t, H–C(6)); 7.41–7.23 (m, 14 H, MeOTr, 2 H *m* to NO₂); 6.86 (dd, 2 H *o* to MeO); 5.98, 5.92 (2d, H–C(1')); 5.31 (2d, H–C(5)); 4.35–4.42 (m, H–C(2')); 4.18 (m, H–C(4')); 4.01–3.86 (m, H–C(3'), CH₂CH₂OP); 3.81 (2s, MeOTr); 3.56 (m, 1 H–C(5')); 3.46 (m, 1 H–C(5')); 3.41 (2s, MeO–C(3')); 3.12–2.93 (m, CH₂CH₂O, 2 MeCH₂); 1.02 (m, 2 MeCH₂). ³¹P-NMR (CDCl₃): 152.01, 151.12. Anal. calc. for C₄₂H₄₇N₄O₁₀P·0.5 H₂O (807.84): C 62.45, H 5.99, N 6.94; found: C 61.94, H 6.02, N 6.33.

32. 3'-O-Methyl-5'-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 2'-[2-(4-Nitrophenyl)ethyl Diethylphosphoramidite] (**57**). As described in *Exper. 6*, with 3'-O-methyl-5'-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine (**53**) [22] (723 mg, 1.0 mmol): 783 mg (79%) of **57**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1 : 9 : 1): R_f 0.60. UV (MeOH): 274(4.39), 236(4.46), 205(4.90). ¹H-NMR (CD₃CN): 8.30 (m, H–C(6)); 8.13 (m, 2 × 2 H *o* to NO₂); 7.53–7.28 (m, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.88 (dd, 2 H *o* to MeO); 6.78 (m, H–C(5)); 5.78 (d, H–C(1')); 4.57, 4.47 (2m, H–C(2')); 4.40 (t, CH₂CH₂OCO); 4.11–3.86 (m, CH₂CH₂OP, H–C(3'), H–C(4')); 3.76 (2s, MeOTr);

3.37 (*s*, 2 H–C(5')); 3.32 (2*s*, MeO–C(3')); 3.09–2.97 (*m*, 2CH₂CH₂O, 2 MeCH₂); 0.98 (*m*, 2 MeCH₂). ³¹P-NMR (CD₃CN): 152.40, 151.32. Anal. calc. for C₅₁H₅₅N₆O₁₃P·0.5 H₂O (1000.02): C 61.25, H 5.64, N 8.40; found: C 61.08, H 5.63, N 8.11.

33. 3'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2'-[2-(4-nitrophenyl)ethyl Diethylphosphoramidite] (**58**). As described in *Exper.* 6, with 3'-O-methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**54**) [22] (747 mg, 1.0 mmol): 782 mg (77%) of **58**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 1:9:1): *R_f* 0.68. UV (MeOH): 267 (4.60), 232 (sh, 4.40), 206 (4.93). ¹H-NMR (CDCl₃): 8.65 (*s*, H–C(8)); 8.23–8.01 (*m*, NH, H–C(2), 2 × 2 H *o* to NO₂); 7.46–7.19 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.81 (*d*, 2 H *o* to MeO); 6.14, 6.11 (2*d*, H–C(1')); 5.30–5.07 (*m*, H–C(2')); 4.53 (*t*, CH₂CH₂OCO); 4.32 (*m*, H–C(4')); 4.11, 4.04 (2*t*, H–C(3')); 3.96–3.32 (*m*, CH₂CH₂OP, 2 H–C(5')); 3.78 (*s*, MeOTr); 3.42 (*s*, MeO–C(3')); 3.15 (*t*, CH₂CH₂O); 3.03–2.75 (*m*, 6 H, CH₂CH₂O, 2 MeCH₂); 0.97 (*t*, MeCH₂); 0.86 (*t*, MeCH₂). ³¹P-NMR (CDCl₃): 150.78, 150.04. Anal. calc. for C₅₂H₅₅N₈O₁₂P·0.5 H₂O (1024.04): C 60.99, H 5.51, N 10.94; found: C 60.94, H 5.58, N 10.86.

34. 3'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine 2'-[2-(4-nitrophenyl)ethyl Diethylphosphoramidite] (**59**). As described in *Exper.* 6, with 3'-O-methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**55**) [22] (912 mg, 1.0 mmol) and dry THF as solvent. Purification by FC (silica gel (10 g), 11 × 2 cm, soln. in CH₂Cl₂/petroleum ether 1:1, then 2:1 (30 ml), 1:1 (30 ml), and 1:2 (70 ml), all with 1% of Et₃N): 885 mg (75%) of **59**. Colorless foam. TLC (SiO₂, petroleum ether/AcOEt/Et₃N 3:7:1): *R_f* 0.70. UV (MeOH): 269 (4.62), 235 (4.40), 204 (4.95). ¹H-NMR (CD₃CN): 8.25 (br., NH); 8.10–7.86 (*m*, 3 × 2 H *o* to NO₂, H–C(8)); 7.53–7.15 (*m*, 18 H, MeOTr, 3 × 2 H *m* to NO₂); 6.71 (*d*, 2 H *o* to MeO); 5.93, 5.88 (2*d*, H–C(1')); 5.13 (*m*, H–C(2')); 4.76 (*m*, CH₂CH₂O); 4.42 (*m*, H–C(4')); 4.34 (*m*, CH₂CH₂OCO); 4.07 (*d*, H–C(3')); 3.83, 3.63 (2*t*, CH₂CH₂OP); 3.69 (*s*, MeOTr); 3.37, 3.36 (2*s*, MeO–C(3')); 3.39–3.20 (*m*, 2 H–C(5')); 3.27 (*t*, CH₂CH₂O); 3.04–2.69 (*m*, 2CH₂CH₂O, 2 MeCH₂); 0.94 (*t*, MeCH₂); 0.75 (*t*, MeCH₂). ³¹P-NMR (CD₃CN): 150.70, 149.62. Anal. calc. for: C₆₀H₆₂N₉O₁₅P·H₂O (1198.20): C 60.15, H 5.38, N 10.52; found: C 59.92, H 5.39, N 10.30.

35. 2'-O-Methyl-5'-O-(monomethoxytrityl)uridine 3'-(Hydrogen Butanedioate) (**60**). To a soln. of 2'-O-methyl-5'-O-(monomethoxytrityl)uridine (**18**) (265 mg, 0.50 mmol) in CH₂Cl₂ (1 ml), succinic anhydride (= dihydrofuran-2,5-dione; 100 mg, 1.0 mmol) and DMAP (= 4-(dimethylamino)pyridine; 80 mg, 0.65 mmol) were added, and the soln. was stirred for 1 h at r.t. The mixture was diluted with CH₂Cl₂ (20 ml) and washed with sat. NaHCO₃ soln. (20 ml). The aq. phase was extracted twice with CH₂Cl₂ (10 ml), the combined org. phase washed with sat. NaHCO₃ soln. (20 ml), and the aq. phase extracted back with CH₂Cl₂ (10 ml). After a final wash with 10% citric acid (25 ml), the combined org. phase was dried (Na₂SO₄) and evaporated and the residue dried for 24 h at 40°: 312 mg (99%) of **60**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): *R_f* 0.47. UV (MeOH): 261 (4.03), 231 (4.22), 205 (4.78). ¹H-NMR (CDCl₃): 10.51 (*s*, NH); 8.08 (*d*, H–C(6)); 7.39–7.25 (*m*, 12 H, MeOTr); 6.84 (*d*, 2 H *o* to MeO); 5.91 (*s*, H–C(1')); 5.30 (*m*, H–C(3'), H–C(5)); 4.30 (*d*, H–C(2')); 3.97 (*m*, H–C(4')); 3.80 (*s*, MeOTr); 3.66 (*dd*, 1 H–C(5')); 3.56 (*s*, MeO–C(2')); 3.41 (*dd*, 1 H–C(5')); 2.82–2.52 (*m*, CH₂CH₂). Anal. calc. for: C₃₄H₃₄N₂O₁₀ (630.65): C 64.75, H 5.43, N 4.44; found: C 64.32, H 5.59, N 4.21.

36. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 3'-(Hydrogen Butanedioate) (**61**). As described in *Exper.* 35, with **21** (361 mg, 0.50 mmol): 406 mg (99%) of **61**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): *R_f* 0.43. UV (MeOH): 280 (4.21), 235 (4.44), 205 (4.84). ¹H-NMR (CDCl₃): 8.57 (*d*, H–C(6)); 8.16 (*d*, 2 H *o* to NO₂); 7.10–7.26 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.95 (*d*, 1 H–C(5)); 6.85 (*d*, 2 H *o* to MeO); 5.98 (*s*, H–C(1')); 5.35 (*m*, H–C(3')); 4.35 (*m*, H–C(4'), CH₂CH₂OCO); 3.97 (*d*, H–C(2')); 3.80 (*s*, MeOTr); 3.66 (*s*, MeO–C(2'), 1 H–C(5')); 3.38 (*dd*, 1 H–C(5')); 3.04 (*t*, CH₂CH₂O); 2.91–2.49 (*m*, CH₂CH₂). Anal. calc. for C₄₃H₄₂N₄O₁₃ (822.82): C 62.77, H 5.15, N 6.64; found: C 62.62, H 5.29, N 6.64.

37. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 3'-(Hydrogen Butanedioate) (**62**). As described in *Exper.* 35, with **23** (373 mg, 0.50 mmol): 408 mg (96%) of **62**. Colorless foam. TLC (SiO₂, CH₂CO₂/MeOH 20:1): *R_f* 0.48. UV (MeOH): 266 (4.46), 234 (4.31), 205 (4.87). ¹H-NMR (CDCl₃): 8.64 (*s*, H–C(8)); 8.16 (*d*, H–C(2), 2 H *o* to NO₂); 7.45–7.19 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.81 (*d*, 2 H *o* to MeO); 6.12 (*d*, H–C(1')); 5.54 (*t*, H–C(3')); 4.77 (*m*, H–C(2')); 4.51 (*t*, CH₂CH₂OCO); 4.36 (*m*, H–C(4')); 3.77 (*s*, MeOTr); 3.54 (*dd*, 1 H–C(5')); 3.43 (*dd*, 1 H–C(5')); 3.36 (*s*, MeO–C(2')); 3.14 (*t*, CH₂CH₂O); 2.73 (*s*, CH₂CH₂). Anal. calc. for C₄₄H₄₂N₆O₁₂ (846.85): C 62.41, H 5.00, N 9.92; found: C 61.92, H 5.11, N 9.68.

38. 2'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 3'-(Hydrogen Butanedioate) (**63**). As described in *Exper.* 35 with **25** (456 mg, 0.5 mmol): 491 mg (97%) of **63**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): *R*_f 0.51. UV (MeOH): 269(4.56), 234(4.38), 205(4.85). ¹H-NMR (CDCl₃): 8.15 (*dd*, 2 × 2 H *o* to NO₂); 7.97 (*s*, H-C(8)); 7.51–7.21 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.80 (*d*, 2 H *o* to MeO); 6.10 (*d*, H-C(1')); 5.54 (*m*, H-C(3')); 4.80 (*t*, CH₂CH₂O); 4.65 (*dd*, H-C(2')); 4.42 (*t*, CH₂CH₂OCO); 4.30 (*m*, H-C(4')); 3.77 (*s*, MeOTr); 3.48 (*dd*, 1 H-C(5')); 3.41 (*dd*, 1 H-C(5')); 3.34 (*s*, MeO-C(2')); 3.30 (*t*, CH₂CH₂O); 3.07 (*t*, CH₂CH₂O); 2.72 (*m*, CH₂CH₂). Anal. calc. for C₅₂H₄₉N₇O₁₅ · H₂O (1030.02): C 60.64, H 4.99, N 9.52; found: C 60.35, H 4.91, N 9.39.

39. 3'-O-Methyl-5'-O-(monomethoxytrityl)uridine 2'-(Hydrogen Butanedioate) (**64**). As described in *Exper.* 35 with **52** (265 mg, 0.50 mmol): 493 mg (99%) of **64**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): *R*_f 0.43. UV (MeOH): 260(4.00), 230(4.21), 204(4.73). ¹H-NMR (CDCl₃): 10.26 (*s*, NH); 7.86 (*d*, H-C(6)); 7.41–7.18 (*m*, 12 H, MeOTr); 6.86 (*d*, 2 H *o* to MeO); 6.10 (*d*, H-C(1')); 5.47 (*m*, H-C(2')); 5.40 (*d*, H-C(5)); 4.12 (*m*, H-C(3'), H-C(4')); 3.80 (*s*, MeOTr); 3.55 (*d*, 1 H-C(5')); 3.42 (*dd*, 1 H-C(5')); 3.39 (*s*, MeO-C(3')); 2.71 (*m*, CH₂CH₂). Anal. calc. for: C₃₄H₃₄N₂O₁₀ (630.65); C 64.75, H 5.43, N 4.44; found: C 64.29, H 5.52, N 4.29.

40. 3'-O-Methyl-5'-O-(monomethoxytrityl)-N⁴-[2-(4-nitrophenyl)ethoxycarbonyl]cytidine 2'-(Hydrogen Butanedioate) (**65**). As described in *Exper.* 35, with **53** (361 mg, 0.50 mmol): 393 mg (96%) of **65**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): 0.40. UV (MeOH): 280(4.19), 235(4.43), 204(4.84). ¹H-NMR (CDCl₃): 8.34 (*d*, H-C(6)); 8.16 (*d*, 2 H *o* to NO₂); 7.43–7.21 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.98 (*d*, H-C(5)); 6.85 (*d*, 2 H *o* to MeO); 6.02 (*s*, H-C(1')); 5.52 (*m*, H-C(2')); 4.38 (*t*, CH₂CH₂OCO); 4.12 (*s*, H-C(3'), H-C(4')); 3.80 (*s*, MeOTr); 3.57 (*d*, 1 H-C(5')); 3.40 (*d*, 1 H-C(5')); 3.35 (*s*, MeO-C(3')); 3.07 (*t*, CH₂CH₂O); 2.72 (*m*, CH₂CH₂). Anal. calc. for C₄₃H₄₂N₄O₁₃ · H₂O (840.84): C 61.42, H 5.27, N 6.66; found: C 61.58, H 5.23, N 6.56.

41. 3'-O-Methyl-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine 2'-(Hydrogen Butanedioate) (**66**). As described in *Exper.* 35, with **54** (373 mg, 0.50 mmol): 412 mg (97%) of **66**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): *R*_f 0.45. UV (MeOH): 266(4.46), 233(4.30), 206(4.85). ¹H-NMR (CDCl₃): 9.32 (*br.*, NH); 8.65 (*s*, H-C(8)); 8.18 (*s*, H-C(2)); 8.12 (*d*, 2 H *o* to NO₂); 7.41–7.16 (*m*, 14 H, MeOTr, 2 H *m* to NO₂); 6.79 (*d*, 2 H *o* to MeO); 6.23 (*d*, H-C(1')); 5.98 (*m*, H-C(2')); 4.48–4.38 (*m*, CH₂CH₂OCO, H-C(3')); 4.29 (*m*, H-C(4')); 3.75 (*s*, MeOTr); 3.50 (*m*, H-C(5')); 3.39 (*s*, MeO-C(3')); 3.36 (*m*, 1 H-C(5')); 3.09 (*t*, CH₂CH₂O); 2.67 (*s*, CH₂CH₂). Anal. calc. for C₄₄H₄₂N₆O₁₂ · H₂O (864.87): C 61.10, H 5.13, N 9.72; found: C 60.78, H 5.10, N 9.85.

42. 3'-O-Methyl-5'-O-(monomethoxytrityl)-N²-[2-(4-nitrophenyl)ethoxycarbonyl]-O⁶-[2-(4-nitrophenyl)-ethyl]guanosine 2'-(Hydrogen Butanedioate) (**67**). As described in *Exper.* 35, with **55** (228 mg, 0.25 mmol), succinic anhydride (50 mg, 0.5 mmol), and DMAP (40 mg, 0.33 mmol): 244 mg (96%) of **67**. Colorless foam. TLC (SiO₂, CH₂Cl₂/MeOH 20:1): *R*_f 0.43. UV (MeOH): 269(4.54), 235(4.36), 206(4.86). ¹H-NMR (CDCl₃): 8.17–8.12 (*m*, 2 × 2 H *o* to NO₂); 8.04 (*s*, H-C(8)); 7.50–7.15 (*m*, 16 H, MeOTr, 2 × 2 H *m* to NO₂); 6.77 (*d*, 2 H *o* to MeO); 6.14 (*d*, H-C(1')); 5.85 (*m*, H-C(2')); 4.76 (*t*, CH₂CH₂O); 4.59 (*t*, H-C(3')); 4.41 (*t*, CH₂CH₂OCO); 4.18 (*m*, H-C(4')); 3.76 (*s*, MeOTr); 3.49 (*dd*, 1 H-C(5')); 3.40 (*s*, 3 MeO-C(3')); 3.39 (*dd*, 1 H-C(5')); 3.27 (*t*, CH₂CH₂O); 3.05 (*t*, CH₂CH₂O); 2.69 (*m*, CH₂CH₂). Anal. calc. for C₅₂H₄₉N₇O₁₅ (1012.00): C 61.17, H 4.88, N 9.69; found: C 61.43, H 5.11, N 9.39.

43. Long-Chain (Methylamino)alkyl Controlled-Pore Glass (LCMAA-CPG; **68**). To a soln. of 1.1'-Carbonylbis[1*H*-diimidazole] (5 g, 62 mmol) in abs. CH₂Cl₂ (150 ml) under Ar, glyceryl-CPG 500 Å (*Bioran, Schott*; 5 g, dried for several hours under high vacuum) was added and shaken at r.t. for 6 h. Then, the material was collected in a glass suction filter and washed with CH₂Cl₂ (4 ×) by taking up the CPG in CH₂Cl₂ (50 ml) and suction-filtering. Thereafter, the CPG material was taken up in CH₂Cl₂ (50 ml), *N,N*'-dimethylhexane-1,6-diamine (5 ml, 57 mmol) was added, and the mixture was shaken for 18 h at r.t. The LCMAA-CPG (**68**) was isolated in a glass-frit suction funnel, washed with MeOH, DMF, pyridine, H₂O, MeOH, acetone, and Et₂O, and dried at 40° under high vacuum.

44. Derivatives **69–76** of LCMAA-CPG 500 Å (**68**) and Nucleoside 3'-or 2'-(Hydrogen Butanedioate) **60–67**. To a soln. of 18 μmol of the nucleoside 3'- or 2'-hydrogen butanedioate (11.3 mg of **60** or **64**; 14.8 mg of **61** or **65**; 15.2 mg of **62** or **66**; 18.5 mg of **63** or **67**) in abs. MeCN (2 ml), LCMAA-CPG (**68**; 250 mg), 2-[(2-(cyanoethoxy)-2-oxoethylidene]amino]-1,1,3,3-tetramethyl]uronium tetrafluoroborate (TOTU; 8 mg, 24 mmol), and methylmorpholine (5 mg, 45 mmol) were added. After shaking for 4 h, the CPG material was collected in a glass-frit suction funnel and washed with MeOH, DMF, MeOH, acetone and Et₂O. For the capping procedure, the nucleoside-functionalized CPG, abs. pyridine (5 ml), Ac₂O (100 ml, 1.06 mmol) and DMAP (5 mg, 0.04 mmol) were kept at r.t. for 30 min. The nucleoside-functionalized CPG (**69–76**) was isolated in a

glass suction filter, washed with MeOH, DMF, H₂O, MeOH, acetone, and Et₂O, and dried at 40° under high vacuum. Determination of loading: A defined amount of **69–76** (3–7 mg) was weighed into a 10-ml measuring flask. After addition of 10 ml of 0.2M TsOH in MeCN, the absorption of the trityl cation was measured at 478 nm against 0.2M TsOH in MeCN. The loading was calculated by the formula $L [\mu\text{mol/g}] = 14.4 \cdot A/m$ (L = loading, A = absorbance at 478 nm, m = weighed CPG **69–76** in mg): **69**, L = 26.7 $\mu\text{mol/g}$; **70**, L = 38.0 $\mu\text{mol/g}$; **71**, L = 28.7 $\mu\text{mol/g}$; **72**, L = 23.3 $\mu\text{mol/g}$; **73**, L = 30.9 $\mu\text{mol/g}$; **74**, L = 28.4 $\mu\text{mol/g}$; **75**, L = 28.4 $\mu\text{mol/g}$; **76**, L = 24.9 $\mu\text{mol/g}$.

45. *Assembly of Oligonucleotides 77–90.* The syntheses were carried out with an *Applied Biosystems 380B* or *392* DNA synthesizer. Nucleoside-functionalized CPG material **69–76** (0.6 or 0.2 μmol) was packed into a 1-mmol *ABI* crimp column. Cycles of nucleotide addition were carried out by a programmed series of reagent and solvent washes based on recommended procedures with the following main steps: 1) 5'-*O*-MeOTr Deprotection: 5% CCl₃COOH (for (MeO)₂Tr deprotection, 3% CCl₃COOH) in CH₂Cl₂, delivered in 3 15-s and 2 10-s bursts with intermediate 4-s block flushes (*ABI 380*), 4 6-s bursts with intermediate 5-s trityl flushes (*ABI 392*); the eluate from this step was collected and the absorbance at 478 nm (498 nm for (MeO)₂Tr) measured to determine the condensation yields. 2) Coupling: 0.1M phosphoramidite and 0.5M 1*H*-tetrazole in dry MeCN, delivered in alternating reagent pulses with a subsequent waiting time of 40 to 600 s. 3) Capping: Ac₂O/2,6-dimethylpyridine/THF 1:1:8 and 1-methyl-1*H*-imidazole/THF 16:84, delivered in one 15-s burst with a subsequent wait time of 10 s (*ABI 380*), 10-s burst and wait time of 5 s (*ABI 392*). 4) Oxidation: 0.05M I₂ in THF/H₂O/pyridine 7:2:1, delivered in one 15-s burst with a subsequent wait time of 15 s (*ABI 380*), 8-s burst and wait time of 15 s (*ABI 392*).

Deprotection and cleavage program: 1) npe/npeoc Deprotection for the *ABI 380B*: 1M DBU in MeCN delivered in a 100-s burst with a consecutive waiting time of 900 s and then 9 more 60-s bursts followed by waiting times of 900 s, 1800 s, 2 × 3600 s, and 5 × 5400 s (total waiting time 10.5 h). *ABI 392*: burst times reduced to 30 s for the first and to 25 s for the consecutive ones. To assure complete removal of all DBU, the column was additionally washed with CF₃COOH for trityl-off products in a detritylation step before final cleavage. 2) Cleavage from the support for the *ABI 380B*: conc. NH₃ delivered in a 22-s burst with a consecutive waiting time of 1800 s repeated 3 times (total waiting time 2 h). *ABI 392*: burst times reduced to 11 s.

The NH₃ soln. was collected and lyophilized in a *Speed-vac* concentrator under high vacuum. The isolated amount of oligonucleotide was determined by measuring the absorbance at 260 nm.

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Design of New Mesogenic Block Molecules: Formation of Columnar Mesophases by Calamitic Bolaamphiphiles with Lateral Lipophilic Substituents**

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The investigation of the driving forces of molecular self-organization is a present topic of chemical research. During the last decades the formation of supramolecular structures based on the microphase separation of incompatible subunits which are covalently linked within macromolecules (block copolymers) has been studied intensively.^[1] Such segregation effects are not limited to polymers, but also play an important role in biological systems as well as in the self-organization of low molecular weight materials, especially for the formation of positionally ordered (smectic, columnar, and cubic) liquid crystalline phases.^[2] Here, the ordered assemblies often result from the combined action of rigidity and microsegregation. In most cases these two driving forces act in the same direction, enhancing each other. If, however, microsegregation and rigidity compete with each other, novel low molecular weight

block molecules should result which could be able to form new supramolecular structures.

Recently we reported on the liquid crystalline compound **1**, which can be regarded as a block molecule containing three incompatible portions: a rigid, rodlike terphenyl unit; two flexible, lipophilic chains; and a lateral hydrophilic group (Figure 1).^[3] The large lateral substituent disturbs the parallel

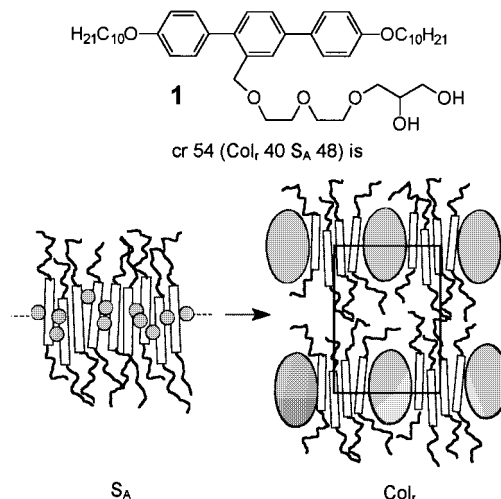


Figure 1. Structure of compound **1** and models of its organization in the mesophases (abbreviations: cr = crystalline phase, S_A = smectic A phase, Col_r = rectangular columnar phase, is = isotropic liquid).

alignment of the rigid cores, and in this way makes the formation of smectic layer structures difficult. However, it did not completely suppress the formation of mesophases, but gave rise to a rectangular columnar mesophase (Col_r). The formation of this columnar phase was explained by the microsegregation of the lateral polar chains from the rigid terphenyl cores into cylindrical domains with a locally enhanced concentration of the flexible and polar polyether chains. This leads to a fragmentation of the smectic layer structure into ribbonlike segments, which organize into the rectangular two-dimensional lattice of the columnar mesophase.^[3]

In an attempt to generalize the conclusions made for the self-organization of **1**, we have now synthesized the novel low molecular weight triblock molecules **3–6**. The topology of the incompatible moieties is the reverse of that in **1** (Table 1).^[4] Whereas in **1** the lipophilic chains are grafted in terminal positions and the hydrophilic group is attached in a lateral

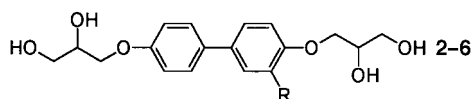


Table 1. Transition temperatures for **2–6**, as determined by polarized light microscopy.^[a]

Compd	R	T [°C]
2	H	cr 245 S _A 294 is ^[7c]
3	CH ₃	cr 104 S _A 231 is
4	<i>n</i> -C ₃ H ₇	cr ? ^[b] S _A 149 is
5	<i>n</i> -C ₉ H ₁₉	cr 67 Col 98 is
6	<i>n</i> -C ₁₁ H ₂₃	cr 84 Col, 116 is

[a] Abbreviations are explained in the legend to Figure 1. [b] No crystalline phase has been obtained yet.

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position at the rigid terphenyl unit, compounds **3–6** contain hydrophilic terminal groups and a lipophilic lateral group connected with a biphenyl unit. Bearing hydrophilic groups at both ends of a hydrophobic molecular part, these molecules belong to the class of bolaamphiphiles.^[5]

Bolaamphiphiles are of great interest, because such lipids form stable monolayer membranes and represent a main constituent of the cell membranes of archae bacteria. Many bolaamphiphiles can also form highly stable smectic liquid crystalline phases.^[6–8] These mesophases can be further stabilized if rigid calamitic segments, such as biphenyl units, are introduced into the hydrophobic central part of the molecules (**2**, Table 1).^[7c] The presence of small lateral groups at the biphenyl core of such calamitic bolaamphiphilic tetraols (**3** and **4**) leads to a drastic lowering of the melting temperatures. Also the stability of the liquid crystalline phases is reduced considerably, but **5** and **6** still exhibit enantiotropic mesomorphism despite their very long lateral alkyl groups. The liquid crystalline phases of **3** and **4** with small lateral substituents are S_A monolayer structures (S_{A1}), as expected. For the S_{A1} phase of **3** a layer thickness of 1.8 nm (0.86 molecular lengths) was determined by X-ray diffraction.

However, the mesophases formed by the long-chain compounds **5** and **6** are different from those of **3** and **4**. This was first indicated by polarized light optical microscopy between crossed polarizers. On cooling of **5** and **6** from their isotropic liquid states, the formation of fernlike dendritic textures can be observed at 98 °C and 116 °C, respectively; these coalesce to mosaiclike textures with spherulitic domains (Figure 2). Remarkably, the mesophase of **6**, with the longer lateral chain, is more stable than that of **5**. This is in contrast to the observations usually made on elongation of lateral chains of nonamphiphilic calamitic liquid crystals.

To clarify the structures of these phases, X-ray diffraction investigations were carried out with well-developed monodomains of the mesophase of **6**. The outer diffuse scattering, which excludes a crystalline phase or a highly ordered smectic low-temperature mesophase, forms a closed ring, indicating strong deviations of the molecular long axes from a preferred direction. The small-angle region exhibits a lot of spotlike reflections, confirming a rectangular columnar 2D lattice. The lattice parameters are $a = 5.4$ nm and $b = 5.8$ nm (Figure 3 and Table 2). The length of the molecules **6** in their most extended conformation from head group to head group is only 2.1 nm (CPK models). Thus, both lattice parameters are significantly larger than twice the molecular dimensions. To deduce a suitable model for the arrangement of the molecules in this columnar mesophase, we have to take into account the following considerations:

- The large lateral alkyl groups should disturb the parallel organization of the rigid cores. However, the strong attractive interactions provided by hydrogen bonding at both ends of the bolaamphiphilic molecules^[7] should inhibit the complete collapse of the smectic layers.
- Because of the position of the alkyl chains close to one of the diol head groups, the two head groups become non-equivalent. The ability to form intermolecular hydrogen bonds should be reduced for the diol groups neighboring the alkyl chains in comparison with the other diol groups.

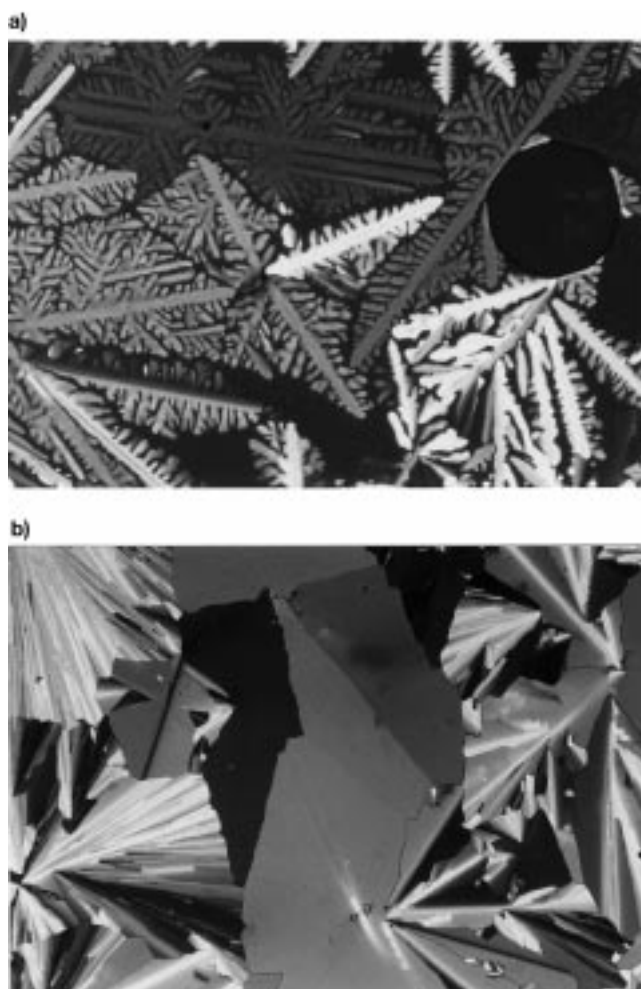


Figure 2. Polarized light optical micrographs of the mesophase of **6**; a) fernlike dendritic texture at 116 °C; b) mosaiclike texture with spherulitic domains at 115.5 °C.

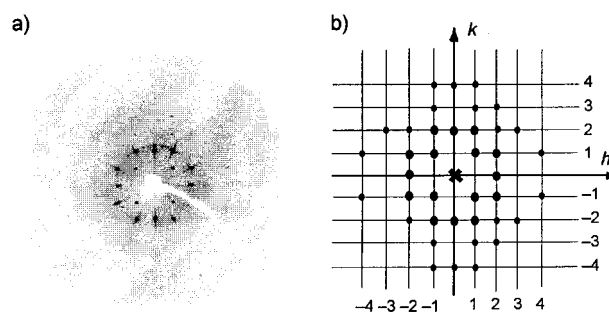


Figure 3. a) Small-angle X-ray diffraction pattern of an aligned sample of **6** at 113 °C (2D detector); b) sketch of the diffraction pattern.

- The lipophilic and flexible alkyl chains are incompatible with both the polar diol groups and the rigid biphenyl units, and have a tendency to segregate and to form distinct regions with a locally enhanced concentration of lipophilic chains.

Based on these considerations a tentative model for the columnar mesophase of **6** can be deduced: Accordingly, the molecules should organize in such a way that their lipophilic chains can concentrate with formation of separate regions. This could be achieved if the molecules in neighboring layers

Table 2. Bragg reflections and Miller indices for the mesophase of **6** at $T = 113^\circ\text{C}$.

hk	$\theta_{\text{obs.}} [^\circ]$	$\theta_{\text{calcd}} [^\circ]$
11	1.14	1.12
02	1.52	1.52
20	1.63	1.63
12	1.82	1.73
21	1.85	1.80
13	2.42	2.42
23	2.76	2.80
32	2.88	2.88
14	3.13	3.15
41	3.34	3.35

align on average antiparallel, whereas molecules in the same layer arrange parallel as shown in Figure 4b. Since the hydrogen-bonding ability of the diol groups next to the alkyl chains is reduced for steric reasons, their intermolecular

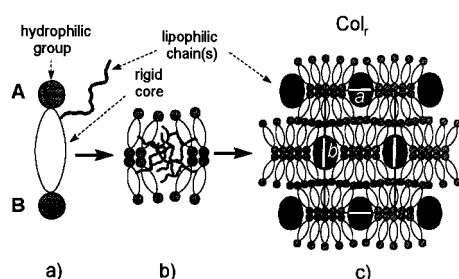


Figure 4. Arrangement suggested for the molecules of **6** in the rectangular columnar mesophase.

hydrogen-bonding networks should preferably collapse, whereas those of the diol groups at the opposite end of the molecules largely remain intact.

The lipophilic regions formed by microsegregation of the alkyl chains should therefore interrupt the hydrogen-bonding networks of the diol groups neighboring the alkyl chains (A in Figure 4), whereas the others should remain. This could lead to the coexistence of two different types of hydrogen-bonding networks in the system. One type, formed by the head groups neighboring the alkyl chains, is continuous in only one dimension; that is, this network is located within the ribbons. The second type is formed by the diol groups at the opposite termini of the biphenyl cores (B in Figure 4) and should be continuous in two dimensions; that is, this network represents undulated layers connecting the ribbons. In other words, every second hydrogen-bonding network is disrupted by the cylindrical domains of the alkyl chains. Thus, less space is available for the diol groups within the ribbons than for those in the layers, and therefore the degree of interdigitation should be different in these two regions. An optimum space filling could be ensured by a nonintercalated head-to-head arrangement of the polar head groups in the ribbons, whereas the head groups in the layers should be strongly intercalated (Figure 4c).

On the basis of these assumptions one of the lattice parameters should correspond to twice the length of a (hypothetical) dimer that is intercalated in the head group region ($2 \times 3.6 \text{ nm} = 7.2 \text{ nm}$). Taking into account the tilting of

the molecules at the edges of the ribbons which is necessary to obtain efficient space filling (see Figure 4c), the lattice parameter $b = 5.8 \text{ nm}$ most likely corresponds to twice the thickness of the ribbons. Then, the parameter $a = 5.4 \text{ nm}$ should correspond to the lateral distance between the ribbons. Under this assumption in average about five molecules should be arranged side by side across the diameter of each ribbon. Thus, the proposed model seems reasonable, because it is in accordance with the molecular dimensions and explains the experimental findings.

Figure 5a shows another representation of this arrangement: The incompatible hydrophilic and rigid aromatic units separate from each other by forming layers, and every second

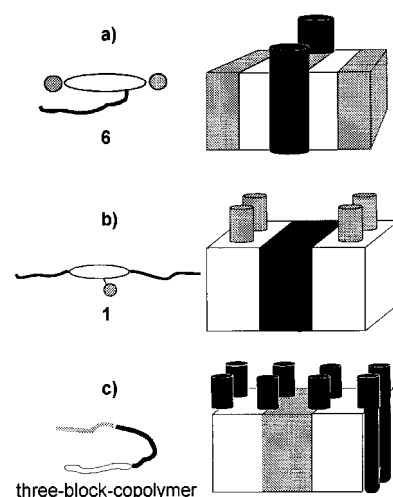


Figure 5. Comparison of the structures of columnar phases of a) **6**, b) **1**, and c) a triblock copolymer. In (a) and (b) gray = hydrophilic groups, white = aromatic cores, black = lipophilic chains; in (c) the different shading distinguishes the incompatible blocks.

polar layer is interrupted in regular intervals by columns with an enhanced concentration of lipophilic chains.

This model describes a novel type of thermotropic columnar structure which is in many respects different from those known for dislike molecules or conventional amphiphiles. However, there are remarkable similarities to the model proposed for the columnar mesophase of the lateral amphiphile **1** (Figure 5b). In the rectangular columnar phase of **1** the lipophilic alkyl chains form layers, whereas the lateral hydrophilic groups segregate from the terphenyl rigid cores and form columns between the aromatic regions. Although the self-organization of these two classes of mesogens is unique among low molecular weight materials, there is a close relationship to the supermolecular structures of some linear triblock copolymers. For such compounds microphase separation can lead to formation of columnar structures like that shown in Figure 5c.^[1, 9]

This work shows that highly smectogenic bolaamphiphiles with rigid cores can be forced to self-assemble to columnar mesophases if their smectic order is disturbed by large lateral lipophilic substituents. Their phase structure results from the combination of microsegregation and rigidity. It is imaginable that the application of this structural concept to macro-

molecular systems could lead to new phase structures with useful macroscopic properties.

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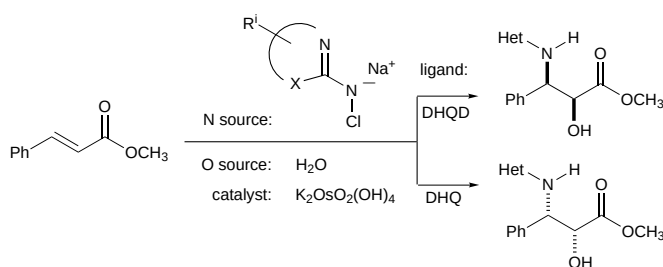
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Catalytic Asymmetric Aminohydroxylation with Amino-Substituted Heterocycles as Nitrogen Sources**

Lukas J. Goossen, Hong Liu, K. Ruprecht Dress, and K. Barry Sharpless*

Dedicated to Professor Theodore Cohen on the occasion of his 70th birthday

The β -amino alcohol moiety appears in numerous biologically active compounds,^[1] and the osmium-catalyzed asymmetric aminohydroxylation (AA) of olefins provides an efficient method for the enantioselective creation of this important functionality.^[2] Sulfonamides,^[2a, 3] amides,^[4] and carbamates^[5] have been utilized as nitrogen sources for the AA, and there has been rapid improvement in both scope and selectivity since the initial report.^[2a] The *N*-protected amino alcohols obtained were usually converted into free amino alcohols, so that the development of a set of orthogonally cleavable protecting groups had been our major concern.^[2–5] Current developments in combinatorial chemistry stimulated us to exploit this reaction for the direct introduction of biomedically relevant heterocyclic substructures to olefins.^[6] We now report an enantioselective procedure for the vicinal addition of a hydroxyl group and amino-substituted heterocycles to olefins (Scheme 1).



Scheme 1. Aminohydroxylation with amino-substituted heterocycles. R^1 indicates the remaining portion of the heterocycle. Het = heterocycle, DHQ = dihydroquinine, DHQD = dihydroquinidine.

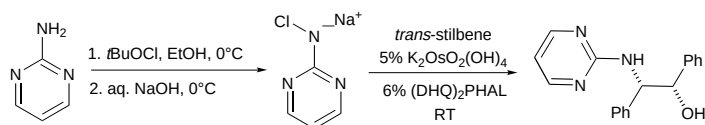
Attempts to extend the scope of the nitrogen sources in the AA to heterocyclic substituted amines had long been frustrated by very poor turnover and side reactions (e.g. ring chlorinations)^[7] until Jerina et al. achieved a breakthrough with the use of an adenine derivative as nitrogen source in the aminohydroxylation of a unique olefin.^[8] Then, in collabo-

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ration with the Jerina group, we developed a general procedure for this kind of transformation;^[9] however, no asymmetric induction was achieved. We reasoned that the size and particular placement of the heteroatoms in the adenine moiety favor “second cycle turnover”,^[3] precluding the desired chirality transfer from the alkaloid ligand. Fortunately, we have since found that simple aminopyrimidines and aminotriazines function as excellent reagents for the AA. With a few modifications to the original procedure, stilbene is converted into either enantiomer of the corresponding amino alcohol with high enantiomeric excesses with 2-aminopyrimidine as the nitrogen source (Scheme 2).

Scheme 2



Scheme 2. Aminohydroxylation of *trans*-stilbene with 2-aminopyrimidine. (DHQ)₂PHAL = 1,4-bis(dihydroquininyl)phthalazine.

It is imperative that the N-chlorination be performed in the absence of water to retard the electrophilic aromatic substitution. Deprotonation with aqueous NaOH affords the chloramine salts, which are relatively stable, especially under an inert atmosphere. A slight deficit of NaOH ensured the absence of excess OH[−] even if some chloramine had been consumed by side reactions. The only suitable solvent systems proved to be mixtures of primary alcohols and water (approx. 2:1 ratio). No turnover was observed for mixtures of water and acetonitrile or *tert*-butyl alcohol. The use of highly polar

protic solvents appears crucial to facilitate the rate of hydrolysis of the relatively electron rich osmium azaglycolate intermediate.

Stilbene was chosen as an ideal test olefin for probing the reactivity of other heterocycles because of the mediocre enantioselectivities observed with standard nitrogen sources. The results are documented in Table 1.

The enantiomeric excesses of 56 to 97% observed with these heterocyclic nitrogen sources are quite impressive when compared to those obtained with earlier nitrogen sources for the AA.^[10] Obviously, the *ee* value depends on both steric and electronic characteristics of the heterocycles. Smaller nitrogen sources seem to provide higher enantiomeric excesses than the more sterically demanding systems (entries 1, 2 versus 3, 5). There seems to be a modest *ee* advantage for less electron withdrawing substituents on nitrogen (entries 1, 3 versus 2, 5). The chemical yields are reasonable to good, with ring-chlorinated amino alcohols as the major contaminants (entry 1).^[11]

To map out the scope of this reaction on the olefin side, we screened a number of representative olefins with 2-amino-4,6-dimethyl-1,3,5-triazine as nitrogen source, for the simple reason that it had shown midrange enantioselectivity with stilbene (Table 1, entry 3). A remarkably broad range of olefins gave good yields and enantioselectivities when subjected to the reaction conditions (Table 2). Cinnamate and fumarate (entries 1 and 2) are among the best substrates, whereas the enantioselectivity for the trisubstituted olefin remains poor (entry 5). In contrast to the highly regioselective adenine derivatives,^[9] the simple heterocyclic nitrogen sources give modest regioselectivities, comparable to that of the earlier versions of the AA. For all unsymmetrical olefins, a strong preference for the isomer with nitrogen in the benzylic position was observed; isopropyl cinnamate gave only a single

Table 1. Aminohydroxylation of stilbene with various amino-substituted heterocycles.

Entry	Substrate	Product ^[a]	Yield [%] ^[b]	% <i>ee</i> ^[c]	M.p. [°C]	[α] _D ²⁰ ^[d]
1			45	97	132 (decomp)	− 0.7 (<i>c</i> = 0.59)
2			50	88	190 (decomp)	− 0.7 (<i>c</i> = 1.14)
3			86	87	— ^[e]	+ 19.8 (<i>c</i> = 0.98)
4			60	56	119 (decomp)	− 11.2 (<i>c</i> = 0.13)
5			65	67	130 (decomp)	− 3.2 (<i>c</i> = 0.29)

[a] Major product from the reaction using (DHQ)₂PHAL. [b] Yields of isolated pure products after chromatography on silica gel. [c] Determined by chiral HPLC. [d] Concentration in grams per 100 mL of EtOH/CHCl₃ (1/1). [e] Not applicable.

Table 2. Aminohydroxylation of various olefins with 2-amino-4,6-dimethyl-1,3,5-triazine (TriazNH₂).

Entry	Substrate	Product ^[a]	Regioselectivity ^[b]	% ee ^[c]		Yield [%] ^[d]
				(DHQ) ₂ PHAL	(DHQD) ₂ PHAL	
1			> 20:1	99	98	97
2			–	92	81	94
3			–	87	87	86
4			–	79	71	92
5			> 20:1	41	37	60
6			5:1	86	80	74
7			6:1	95	95	79
8			6:1	98	96	80
9			2:1	46	48	75
10			7:1	45	38	81

[a] Major product from the reaction using (DHQ)₂PHAL. [b] Determined by ¹H NMR spectroscopy. [c] Determined by HPLC on a chiral stationary phase. [d] Yields of isolated products from the reaction using the (DHQ)₂PHAL ligand, where regioisomers are possible, of a mixture of both regioisomers. Similar yields and regioselectivities were obtained with the (DHQD)₂PHAL ligand.

regioisomer. The regioisomers were separable by preparative thin-layer chromatography (TLC).

In summary, this new generation of nitrogen sources greatly extends the scope of the osmium-catalyzed AA. Of additional interest, the AA can now be considered as a means for introducing complex heterocyclic fragments into hydrocarbon backbones when appropriate olefinic functionality is present.

Experimental Section

Representative procedure for the aminohydroxylations: reaction of 2-aminopyrimidine with *trans*-stilbene: All solvents were deoxygenated by three freeze–pump–thaw cycles under nitrogen; all reactions were performed under an argon atmosphere. 2-Aminopyrimidine (166 mg, 1.75 mmol) was dissolved in dry ethanol (20.0 mL). The solution was cooled in an ice bath. *tert*-Butyl hypochlorite (190 mg, 1.75 mmol) was added. The solution was allowed to warm up to room temperature (RT) and stirred for 30 min. Then it was cooled again to 0 °C, and aqueous NaOH

(1.00 M, 1.50 mL, 1.50 mmol) was added. The solution was again warmed to RT in a water bath, and water (8.5 mL) was added. Finely divided *trans*-stilbene (90.1 mg, 0.50 mmol), (DHQ)₂PHAL (23.3 mg, 30 μmol, 6 mol %), and K₂OsO₂(OH)₄ (9.2 mg, 25 μmol, 5 mol %) were added. The mixture was stirred at RT for 12 h. A saturated solution of NaHSO₃ (4 mL) and water (10 mL) was added, and the mixture was stirred for 1 h at RT. The mixture was then placed in a separatory funnel, and EtOAc (80 mL) was added. The organic phase was separated and washed with water (40 mL) and brine (40 mL). After the mixture was dried over MgSO₄, the solvent was evaporated to leave a brownish residue. The product was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH, 1/99–5/95) yielding 66 mg (45 %) of product as an off-white solid. As a second band, small quantities of the corresponding ring-chlorinated product were isolated. M.p. 132 °C (decomp); HR-MS (FAB) for C₁₈H₁₇N₃O: calcd for MH⁺: 292.1450, found: 292.1460; ¹H NMR (400 MHz, CDCl₃, 25 °C, TMS): δ = 8.12 (d, ³J(H,H) = 8 Hz, 2H, N=CH), 7.37–7.21 (m, 10H, Ph), 6.45 (t, ³J(H,H) = 8 Hz, 1H, py), 6.28 (brd, ³J(H,H) = 13 Hz, 1H, NH), 5.29 (dd, ³J(H,H) = 13, 7 Hz, 1H, CHN), 5.07 (d, ³J(H,H) = 7 Hz, 2H, CHO), 4.08 (s, 1H, OH); ¹³C NMR (100 MHz, CDCl₃): δ = 162.2, 157.9, 141.4, 140.5, 128.5, 128.1, 127.5, 127.4, 127.1, 126.4, 111.0, 78.0, 61.7; [α]_D²⁰ = –0.7 (c = 0.59, in EtOH/CHCl₃ (1/1),

$\alpha = -0.4^\circ$); HPLC (Chiralpak AS, *i*PrOH/hexane 3/7, 1 mL min⁻¹): $t_r = 5.1$ (1*S*,2*S*), 7.2 min (1*R*,2*R*).

Assignment of the absolute configuration: (1*S*,2*S*)-2-Amino-1,2-diphenylethanol was converted into an authentic sample of the 1*S*,2*S* enantiomer mentioned above by reaction with 2-bromopyrimidine.^[12]

All other compounds were produced similarly. Regioisomers were usually separable by preparative TLC. It is advisable to keep the solvent polarity as high as possible; however, for some nonpolar olefins the addition of a small quantity of *n*-propanol was necessary to ensure the formation of homogeneous mixtures. A catalyst loading of 5% is sufficient for converting even heterocycles prone to electrophilic aromatic substitution. In most cases, however, it is possible to reduce the catalyst content to 1% without significant loss of *ee*; for example isopropyl cinnamate (Table 2, entry 1) gave 96% *ee* and 51% yield after 24 h. It is also usually possible to reduce the excess of the chlorinated salt of the heterocyclic amine to two equivalents.

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High-Resolution Calorimetry: New Perspectives for the Study of Phase Transitions**

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New developments in the area of microcalorimetry permit the thermal investigation of materials in sub- μ g quantities.^[1] The thermal investigation of masses in the picogram range is also of interest, for example, for the analysis of surfaces, and still represents a large experimental challenge. King and co-workers recently reported methods to perform the thermal analysis of adsorption phenomena,^[2] but practical limits still exist that prevent these high-resolution methods, with a sensitivity in the nanojoule range, from having a wide application under simple laboratory conditions.

Presented herein is a calorimeter whose efficiency enables the thermal analysis of substances on the nano- and picogram scale and with a picojoule sensitivity by using simple experimental equipment. The calorimeter^[3] is based on techniques well known in scanning probe microscopy and formed on a concept that was used to prove a surface reaction under high vacuum conditions.^[4] We were able to quantify a solid–solid phase transition in *n*-alkanes in air. Only 7 pg of substance were needed. This corresponds to a released heat of a mere 500 pJ. The calorimeter furthermore has a time resolution of 0.5 ms, and dynamic effects can also be studied. This enables us to determine differences in the solid–solid phase transition of the *n*-alkanes while different phases are being formed. Differences in the phase dynamics of odd and even-numbered *n*-alkanes were also studied.

The essential item of the calorimeter (Figure 1) is a micro-fabricated composite cantilever in the form of a bimetallic

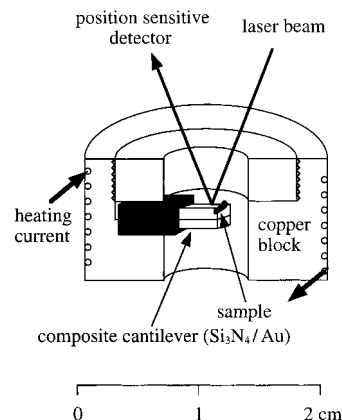


Figure 1. Schematic arrangement of the calorimeter. The heating block with the bimetallic cantilever are accommodated in a closed housing to avoid any disturbing air turbulence.

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strip. The deflection of the cantilever caused by a change in temperature is detected by using the optical deflection method.^[3] The bimetallic cantilever is manually loaded with the *n*-alkane. The mass of the alkane is measured by analyzing the shift in the resonance frequency of the cantilever with a spectrum analyzer. This means that the bimetallic cantilever not only represents a very sensitive thermometer but also an exact balance for weighing small quantities of substance.^[5]

The heating and cooling mode for a bimetallic cantilever carrying 15 ng tetracosane ($C_{24}H_{50}$) is given in Figures 2a and 2b, respectively. The deviations from the baseline arise from a

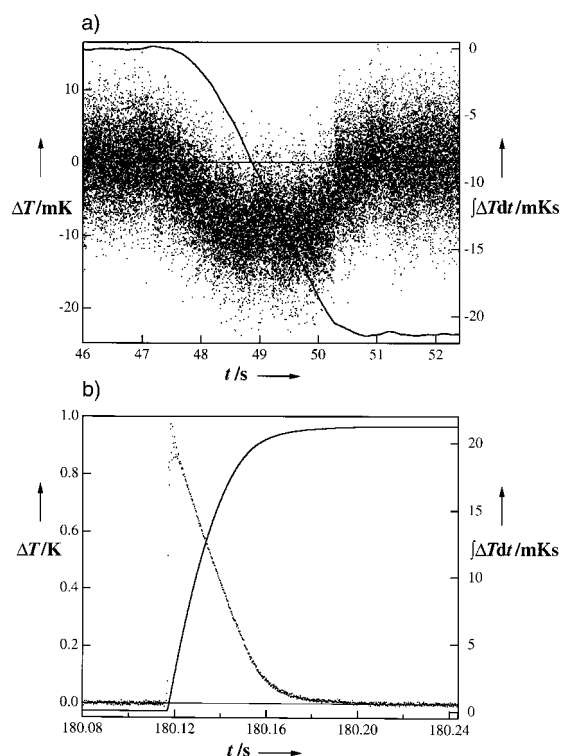


Figure 2. Section of the heating and cooling mode of a bimetallic cantilever that was loaded with a 15 ng tetracosane flake. The data represent the change in the cantilever temperature ΔT during the formation of the rotator phase (a) and the crystalline phase (b). The solid line corresponds to the time dependent integration $\int \Delta T dt$ of the temperature change. The latter result is proportional to the consumed (a) or produced heat (b).

solid–solid phase transition.^[6] The phase transition from the crystalline into the rotator phase is shown in Figure 2a. This phase transition occurs at a cantilever temperature of 47.5–48.0 °C. The formation of the crystalline phase from the rotator phase is given in Figure 2b. The latter phase transition occurs at a remarkably lower cantilever temperature of 41.0 °C, which results from a supercooling of the rotator phase. It is clear that the rotator phase forms over a period of 3–4 s, while the formation of the crystalline phase from the supercooled rotator phase takes place within 40 ms. The temporal course differs too: the cantilever temperature is close to constant during the formation of the rotator phase as expected for a phase transition. The temperature of the cantilever rises abruptly and subsequently decreases smoothly during the growth of the crystalline phase from the supercooled rotator phase. Solid–solid phase transitions can

exhibit remarkably different dynamics, depending on whether a rotator or a crystalline phase is formed. The differences in the phase transitions can be observed because the response time of the calorimeter is in the order of milliseconds.

The energy that is released or consumed during the phase transitions can be quantified by an integration of the areas in Figures 2a and 2b. The solid line represents the integral. Both phase transitions yield identical areas and, therefore, similar heats, although the time dependence of the phase transitions varies significantly. The data of the processes can be reproduced with an error of 10% for the rotator phase, and with an error of 5% for the formation of the crystalline phase. In an independent second experiment, with an identically constructed cantilever, we found the apparatus constant of the calorimeter to be $6.5 \times 10^{-5} \text{ WK}^{-1}$. Therefore, the integrated areas in Figures 2a and 2b of $21 \times 10^{-3} \text{ Ks}$ correspond to a transition heat of 1.36 μJ in both cases. With a sample mass of 15 ng this gives an enthalpy change for the particular tetracosane flake of 30.6 kJ mol^{-1} (literature value:^[6] 31.3 kJ mol^{-1}). This demonstrates that the new calorimeter serves as a very sensitive tool for thermal analysis.

Besides tetracosane, we have also studied tricosane ($C_{23}H_{48}$) with the nanocalorimeter. Tricosane flakes of different masses showed the expected solid–solid phase transition. We again observed different kinetics during the formation of the rotator phase and the crystalline phase. On closer observation it was evident that tricosane and tetracosane form the crystalline phase in a different manner. While in the tricosane case even with a sample mass of more than 100 ng the increase of the cantilever temperature for the phase transition was limited to $\Delta T \approx 0.5 \text{ K}$, tetracosane did not show this saturation effect for comparable sample masses ($\Delta T > 1.5 \text{ K}$). It might be concluded from this observation that different kinetics exist for the formation of the crystalline phase in tetracosane (even number of carbon atoms) and in tricosane (odd number of carbon atoms).

For a sample loading of more than one nanogram the dynamics of the calorimeter is determined by the heat capacity of the sample mass, whereas in cases with a sample loading of significantly less than one nanogram, the dynamics of the calorimeter is only determined by the thermal properties of the bimetallic cantilever. The very small, but finite, mass of the cantilever represents the natural limit for the smallest detectable heat by the calorimeter, as well as for the time resolution of the experiment. To demonstrate the heat sensitivity and time resolution of the calorimeter, the bimetallic cantilever was loaded with a tricosane flake of 40 pg. The latter flake had a diameter of approximately 2–3 μm , and was still visible with an optical microscope. The resulting phase transition from the supercooled rotator phase is shown in Figure 3. The change in the cantilever temperature caused by the phase transition is clearly detected. The area under the peak of the first data set corresponds to a heat of 3.1 nJ or an enthalpy of formation of 25 kJ mol^{-1} . This fits the literature data of 21.8 kJ mol^{-1} very well, especially if one considers that the error in weighing the sample in air amounts to 25%. With such small masses it is impossible to differentiate the signal for the formation of the rotator phase, which has to be collected over a time of 3–4 s, from the noise.

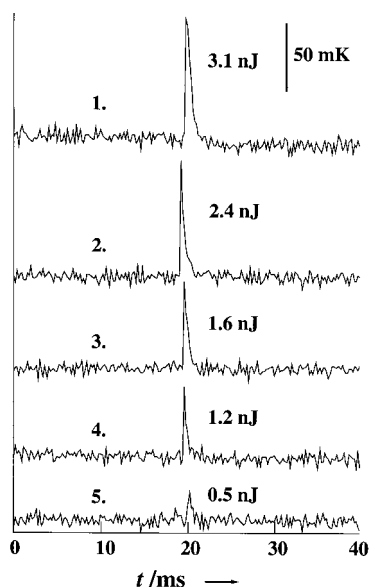


Figure 3. The recorded change in temperature of the cantilever ΔT , using the same 40 pg tricosane flake during the formation of the crystalline phase over five successive runs. The reduction of the transition heat is a result of the loss of substance by sublimation.

The phase transition from the supercooled rotator phase is well resolved because the crystalline phase forms rapidly. The area under the peak decreases smoothly on repeated runs as a result of partial sublimation of the sample. However, the formation of the crystalline phase is still detectable after five runs, and the area under the peak now corresponds to a transition heat of 500 pJ with a sample mass of about 7 pg. Although the amount of substance and the resulting heat of transition decreases with each run, the time constant of about 0.5 ms for the decrease of the cantilever temperature remains constant. In this case, the time constant is only defined by the intrinsic time constant of the bimetallic cantilever and not by the sample mass. The dynamics of phase transitions that are faster than 0.5 ms can, therefore, not be studied with this particular cantilever.

Besides analyzing phase transitions in solids, we have used an identically constructed calorimeter that was suitable for the application under high vacuum conditions for the thermal analysis of small metallic clusters in the gas phase.^[7] So far, the calorimeter is limited to the thermal analyses of solids or reactions on solids. Future work will be focused on the fabrication of bimetallic cantilevers with integrated moulds for storing liquids and then carrying out thermal analysis. In fact, the thermal analysis can only be performed over an interval of approximately $\Delta T \approx 50^\circ\text{C}$, but we hope to surpass this limit by the application of modified sensors. Samples in the nanogram range have already been analyzed thermogravimetrically up to 300°C with piezo-resistive cantilevers.^[8] In addition, special true differential experiments will rise the heat sensitivity. This also will enable the study of phase transitions over an expanded time scale.

Experimental Section

The calorimeter is schematically shown in Figure 1. Tetracosane and tricosane are commercially available (Fluka, p.a., purity $\geq 99.5\%$). The

composite cantilevers can be obtained from Park Scientific.^[9] The bimetallic cantilever is coated with a 50 nm gold layer on a $550\text{ nm Si}_3\text{N}_4$ base. The cantilever was typically $200\text{ }\mu\text{m}$ long and $18\text{ }\mu\text{m}$ wide. The alkane flakes were placed manually on the cantilever. The adhesion of the alkane flake was monitored with an optical microscope. The flake must be put on the free side of the cantilever, otherwise an exact determination of the flake's mass with the aid of the spectrum analyzer becomes impossible. Next, the loaded cantilever was placed in the heater, and finally it was checked with the optical microscope that the laser beam had focused on the free gold-coated end of the cantilever. The reflected laser beam is directed by a mirror onto the position-sensitive detector. The copper block is heated at $2\text{--}3\text{ W}$ (this results in a heating rate of approximately 0.02 K s^{-1}). The signal of the heater's thermocouple and the deflection of the bimetallic cantilever are recorded in parallel with 5000 data points per second. The small dimensions of the cantilevers allow fast thermal equilibration of the cantilever and the copper block. Therefore, the recorded heating block temperature equals the cantilever's temperature. The apparatus constant of the calorimeter is determined in an independent run with an identically constructed bimetallic cantilever. The calculation of absolute values for the transition heat at the phase transitions can now be carried out by using the temperature changes of the cantilever.

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Homoleptic Lanthanide Complexes of Chelating Phosphanamides—An Experimental and Theoretical Study**

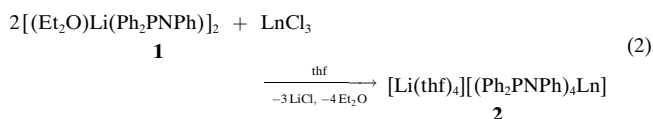
Thomas G. Wetzel, Stefanie Dehnen, and Peter W. Roesky*

Phosphanamides (phosphazanes), phosphanimides (phosphazenes), and their cyclic analogues have long been known and have attracted the attention of experimentalists and theorists alike because of their comparatively short P–N bonds.^[1] If a phosphanamine of the general formula $R_2PN(H)R'$ or a phosphanimine of the general formula $R_2(H)P=NR'$ is deprotonated, the resulting anion could in principle be described as phosphanamide **A** or iminophosphide **B** [Eq. (1)].^[2] Theoretical studies showed that the anion



has a shortened P–N bond, but nevertheless basically corresponds to a phosphanamide with the negative charge mainly located on the nitrogen atom.^[1b] Phosphanamides are known to act as both bridging and terminal ligands for middle and late transition metals.^[3, 4]

Herein we report on the first phosphanamide complexes of Group 3 metals and the lanthanides. We were mainly interested in the coordination of phosphanamides (chelating ligands which feature a hard and a soft donor atom close together) to the hard lanthanide(III) ions. Transmetalation of **1** with anhydrous LnCl_3 ($\text{Ln} = \text{Y}, \text{Yb}, \text{Lu}$) in THF leads to products **2** ($\text{Ln} = \text{Y}$ (**2a**), Yb (**2b**), and Lu (**2c**)) [Eq. (2)].



Even with an excess of LnCl_3 **2** was the only product which could be isolated. The new complexes have been characterized by standard analytical and spectroscopic techniques, and the solid-state structures of **2b** and **2c** (Figure 1) as well as of the neutral ligand $\text{Ph}_2\text{PN}(\text{H})\text{Ph}$ (**3**)^[5] were established by single-crystal X-ray diffraction.^[6] Crystals of **2b** and **2c** that consist of an ion pair composed of a $[\text{Li}(\text{thf})_4]^+$ cation and a $[(\text{Ph}_2\text{PNPh})_4\text{Ln}]^-$ anion are isomorphous. The ligands of the anions show η^2 coordination, thus forming four three-membered rings (azaphosphametallacyclopropanes). Whereas a

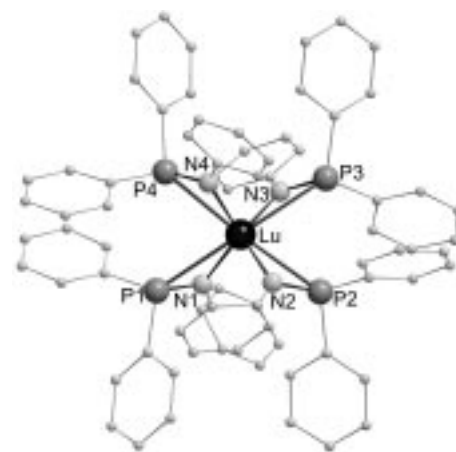


Figure 1. Solid-state structure of $[(\text{Ph}_2\text{PNPh})_4\text{Lu}]^-$ (without hydrogen atoms, SCHAKAL drawing). Selected bond lengths [pm] and angles $^\circ$ (also given for the isostructural $[(\text{Ph}_2\text{PNPh})_4\text{Yb}]^-$): Lu–N 225.2(7)–227.2(8), Yb–N 226.2(7)–227.9(7), Lu–P 288.6(3)–304.0(3), Yb–P 288.5(2)–303.1(2); N–Lu–N 100.7(3)–125.0(3), N–Yb–N 100.3(3)–126.4(3).

large number of homoleptic eight-coordinated compounds with four-, five-, and six-membered rings are known, homoleptic three-membered rings are extremely rare and to the best of our knowledge unknown for the lanthanides.^[4, 7]

At a first glance the N_4P_4 coordination polyhedron of the anions looks highly symmetric, and D_2 symmetry is expected. A closer look shows that the polyhedron has no symmetry at all and may be best described as an extremely distorted cube. In fact none of the classical polyhedra known for coordination number eight gives a correct description.^[8] Nevertheless, the anions are chiral, with both enantiomers cocrystallizing in the unit cell.

Not only the positions of the phenyl rings, which disturb the nearly realized C_2 symmetry, but also the strong differences of the $\text{Ln}–\text{P}$ distances are the reasons for the unsymmetry. Whereas the $\text{Ln}–\text{N}$ bond lengths are equal within the limits of error (**2b**: 226.2(7)–227.9(7) pm; **2c**: 225.2(7)–227.2(8) pm), the $\text{Ln}–\text{P}$ bond lengths differ by about 15 pm (**2b**: 288.5(2)–303.1(2) pm; **2c**: 288.6(3)–304.0(3) pm). The geometry of the LnN_4 subunit is a distorted tetrahedron having N–Ln–N angles in the range 100.3(3)–126.4(3) $^\circ$ (**2b**) or 100.7(3)–125.0(3) $^\circ$ (**2c**), whereas the LnP_4 subunit is best described as a twisted rectangle with the metal atom in the center. The two P_2N_2 planes of the coordination polyhedron are distorted to give elongated parallelograms. The P–N–P angles amount to 109.7(7)–112.7(9) $^\circ$ (**2b**) or 109.9(9)–113.9(9) $^\circ$ (**2c**). The N–P–N angles range between 62.3(2) $^\circ$ and 67.5(5) $^\circ$ (**2b**) or between 61.46(2) $^\circ$ and 67.31(6) $^\circ$ (**2c**). The two P_2N_2 parallelograms are staggered, being rotated by about 120 $^\circ$ with respect to one another. Comparable $\text{Ln}–\text{P}$ contacts are reported for the few known $\text{Ln}–\text{PR}_3$ compounds (e.g. 294.1(3) pm for $[(\text{Me}_3\text{C}_5)_2\text{YbCl}(\text{Me}_2\text{PCH}_2\text{PMe}_2)]^{[9]}$ and 288.1(1)–294.7(1) pm for $[(\text{Me}_3\text{SiO})\text{Lu}[\text{C}(\text{PMe}_2)_2(\text{SiMe}_3)_3]^{[10]}]$). In terms of bond lengths and angles, the neutral ligand **3** and the coordinated $[(\text{Ph}_2\text{PNPh})_4\text{Ln}]^-$ anion do not differ significantly. Thus the P–N bond lengths of **2b** (av 167.2(8) pm), **2c** (av 167.3(8) pm), and **3** (169.6(3) pm) all lie in a narrow range.

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To probe further the coordination of the $[\text{Ph}_2\text{PNPh}]^-$ anion to the lanthanides, ^1H and ^{31}P NMR spectra of **2a** and **2c** were recorded in solution. As expected the ^{31}P NMR spectra show only one signal. For **2a** $^2J(\text{P},\text{Y})$ coupling of 5.4 Hz could be observed,^[11] whereas $^1J(\text{P},\text{Y})$ and $^2J(\text{P},\text{P})$ coupling were not detected, even at low temperature (203 K). This may be the result of a rapid isomerization (dissociation/association) of the phosphorus atoms in solution. Therefore, the Ln–P interaction can only be weak.

To better understand the nature of the Ln–P contacts, as well as the reason for the unsymmetry of **2**, theoretical investigations with DFT methods^[12] (DFT = density functional theory) and the MP2 method^[13] (MP2 = Møller–Plesset second-order perturbation theory) of a series of $[(\text{R}_2\text{PNR})_4\text{Y}]^-$ complexes were carried out. The calculations were undertaken with the program system TURBO-MOLE.^[14] To study the steric as well as the electronic influence of the substituent R of the R_2PNR^- ligand, three compounds with R = H, Me, or Ph were computed. For minimization of the computational effort, an idealized C_2 symmetry was assumed for all calculated complexes. The structure optimizations with the DFT method were in all cases followed by a population analysis based on occupation numbers by Erhardt and Ahlrichs.^[15]

It can be seen from the calculations that increasing steric demand of R causes both significant shortening of the Y–P contacts (on average) besides slightly elongated Y–N bonds. Additionally, there is increasing disparity in the two types of Y–P distances, even though all structure optimizations were started with equal Y–P or Y–N bond lengths.^[16] The calculated Y–P distances are larger than the distances in known Y– PR_3 complexes (281.7–304.5 pm)^[17] in all cases, except for one extrapolated value. Since all the other interatomic distances of the computed $[(\text{Ph}_2\text{PNPh})_4\text{Y}]^-$ anion correspond well with the experimental data, the discrepancy concerning the Y–P contacts might indicate a very weak interaction. This would allow packing constraints in the crystal to play a superior role. A comparison of the DFT and the MP2 structural parameters for the Me- or Ph-substituted complexes indicates the mainly dispersive nature of the Y–P contacts.^[18] Whereas the Y–N bonds are shortened by a typical value of only about 3 pm with respect to the DFT bond length, the Y–P distances are more strongly affected (up to 24.5 pm shorter) for the MP2 method. The population analyses^[19] show a significant but unsymmetric tightening of the Y–P interactions when going from R = H to R = Ph. We assume that electronic effects are not very important for the discussed observation, as the tendency of decreasing Y–P distances is independent of the electron-pushing (Me) or electron-pulling nature (Ph) of the substituents.

We suggest that the unsymmetric coordination pattern of the P atoms can be explained by the following effect of the large Ph substituents: On the one hand, the steric demand of the Ph groups in η^1 -coordinated N-bound R_2PNR^- ligands at the periphery of the complex forces the phosphorus atoms to approach the metal center, tending towards η^2 coordination (no Y–P contact is formed in the case of the sterically less demanding H_2PNH^- ligand). On the other hand, the formation of four equally spaced chelating rings is hindered by the

newly arising steric interactions of the hypothetical η^2 coordination.

The single-crystal X-ray and the computed structure of **2** feature the following results. The first phosphoramidate complexes of Group 3 metals and the lanthanides were isolated. These are also the first complexes of Group 3 metals and the lanthanides having four ligands showing near- η^2 coordination, which leads to a distorted structure. According to theoretical investigations the differences in Ln–P bond lengths, which cause the unsymmetric shape of the coordination polyhedron, is best explained by steric effects. Nevertheless, based on the very weak calculated Ln–P binding interactions, packing constraints are suggested to have a further influence on the geometry.

Experimental Section

2a: THF (50 mL) was added at room temperature to YCl_3 (195 mg, 1.0 mmol) and $[(\text{Et}_2\text{O})\text{Li}(\text{Ph}_2\text{PNPh})]_2$ (1.428 g, 2.0 mmol), and the mixture was stirred for 18 h at room temperature. Then the suspension was filtered, and pentane (100 mL) was layered on top of the filtrate. After 2 d colorless crystals were obtained. Yield 260 mg (15%). ^1H NMR ($[\text{D}_8]\text{THF}$, 250 MHz, 25 °C): δ = 1.71–1.79 (m, thf), 3.59–3.66 (m, thf), 6.17–6.25 (m, 4H, Ph), 6.59–6.65 (m, 4H, Ph), 6.72–6.78 (m, 8H, Ph), 6.87–6.93 (m, 4H, Ph), 7.21–7.28 (m, 16H, Ph), 7.44–7.50 (m, 4H, Ph); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_8]\text{THF}$, 101.3 MHz, 25 °C): δ = 9.20 (d, $^2J(\text{Y},\text{P})$ = 5.4 Hz); ^{89}Y NMR ($[\text{D}_8]\text{THF}$, 14.7 MHz, 25 °C): δ = 296.

2b: Yield 190 mg (11 %). IR (KBr): $\tilde{\nu}$ = 3296 (m), 1595 (vs), 1495 (vs), 1433 (s), 1290 (s), 892 (vs), 756 (s), 739 (vs), 695 (vs) cm^{-1} .

2c: Yield 170 mg (10 %). IR (KBr): $\tilde{\nu}$ = 3296 (m), 1593 (vs), 1482 (vs), 1432 (s), 1287 (s), 892 (vs), 740 (s), 739 (vs), 695 (vs) cm^{-1} ; ^1H NMR ($[\text{D}_8]\text{THF}$, 250 MHz, 25 °C): δ = 1.72–1.77 (m, 16H, thf), 3.57–3.61 (m, 16H, thf), 6.70–6.77 (m, 4H, Ph), 6.97–7.12 (m, 16H, Ph), 7.33–7.47 (m, 24H, Ph), 7.86–7.95 (m, 16H, Ph); $^{31}\text{P}\{^1\text{H}\}$ NMR ($[\text{D}_8]\text{THF}$, 101.3 MHz, 25 °C): δ = 16.69.

Methods of the theoretical investigations: The efficient R1DFT program^[20] was used for all the calculations with employment of the Becke–Perdew (B–P) functional^[21] and the grid size m3. Basis sets were of SV(P) quality.^[22] For Y, a relativistically corrected effective core potential with 28 core electrons (ECP-28) was used.^[23] The large Y–P distances for the calculated $[(\text{Ph}_2\text{PNPh})_4\text{Y}]^-$ anion were computed as being about 18 or 19 pm longer than the experimentally observed Lu–P or Yb–P bond lengths in $[(\text{Ph}_2\text{PNPh})_4\text{Yb}]^-$ or $[(\text{Ph}_2\text{PNPh})_4\text{Lu}]^-$. Therefore, MP2 calculations were carried out employing the efficient R1MP2 program.^[24] Basis sets were of SV(P) quality for C and H, TZVP quality for P and N, and TZVPP quality for Y.^[25] For the latter, the ECP-28 was again used. Owing to the huge computational effort of the MP2 calculation, the method was employed only for the two smaller systems $[(\text{H}_2\text{PNH})_4\text{Y}]^-$ and $[(\text{Me}_2\text{PNMe})_4\text{Y}]^-$. The differences in interatomic distances between the DFT and MP2 structures were used to extrapolate to a hypothetically computed Ph-substituted MP2 structure $[(\text{Ph}_2\text{PNPh})_4\text{Y}]^-$ (see the supporting information). The extrapolated values represent an improvement over the DFT results when compared to the X-ray structural parameters. The largest differences again arose at the weak Y–P contacts. The extrapolation provided values that were as much as 6.5 pm too short.

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Solvothermal Synthesis of the Canted Antiferromagnet $\{K_2[CoO_3PCH_2N(CH_2CO_2)_2]\}_6 \cdot xH_2O^{**}$

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Derivatives of phosphonic acid have recently been shown to introduce structural diversity into metal oxide based materials produced both solvothermally and by conventional means.^[1] We are currently investigating the use of solvothermal reactions to construct metal–ligand frameworks and have found that rigid aromatic carboxylic acid derivatives are excellent structural members around which to form infinite lattices.^[2] However, the number of molecular clusters synthesized by using solvothermal techniques is still relatively small^[3] and the factors which promote formation of molecular rather than infinite structures are poorly understood. We report herein the results of the extension of our efforts to the synthesis of large molecular species based on flexible chelating ligands.

When Co^{II} nitrate is treated with the potassium salt of the ligand (phosphonomethyl)iminodiacetic acid under hydrothermal conditions only blue-purple solutions are obtained. When the solvent is changed to methanol, the purple crystalline product **1** is formed in good yield. X-ray structure



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analysis^[4] shows the material to be based on a simple structural unit in which the cobalt(II) ions have a trigonal-bipyramidal coordination geometry (Figure 1). The nitrogen atom lies at one apex and the three arms of the ligand coordinate equatorially. This ML unit is the monomer from which hexameric rings are formed. The second apical

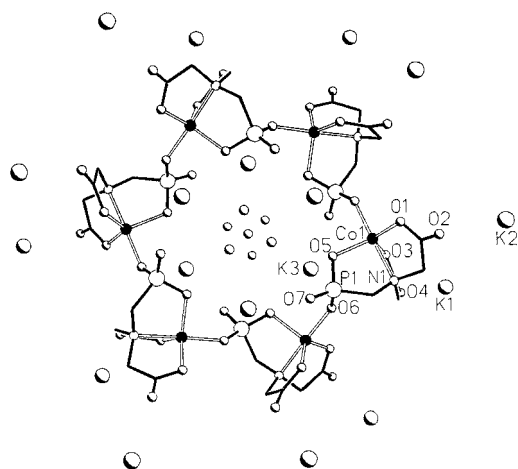


Figure 1. View of a monomeric unit showing the atom numbering scheme. Unlabeled shaded spheres in the center of the ring represent the positions of the disordered lattice water molecules. Selected bond lengths [Å] and angles [°]: Co–O1 2.027(6), Co–O3 1.996(7), Co–O5 2.030(6), Co–O6A 1.967(6), Co–N1 2.169(7); O6A–Co–N1 177.6(3), O1–Co–O3 125.8(3), O1–Co–O5 112.9(3), O3–Co–O5 115.8(3).

coordination site is occupied by a phosphonate oxygen from a neighboring monomer such that oligomers are formed in a head-to-tail fashion. This is in contrast to the behavior observed for related tetradentate ligands and ions such as Fe^{III} where head-to-head bridging generates dimeric six-coordinate species.^[5] Hence the backbone of the rings consists of M₆P₆O₁₂ rings in which every other atom is an oxygen and the metal and phosphorus alternate in the remaining sites. The six-membered ring is generated by a $\bar{3}$ site and is therefore in the chair conformation. The head-to-tail polymerization can take place in either a clockwise or anticlockwise fashion. The symmetry of the space group results in all of the rings in one layer (parallel to the *ab* plane) having one “handedness”, whilst those in the adjacent layers are all of the opposite type. Within each layer the topology of the cobalt atoms is that of a puckered hexagonal sheet which approximates to the gray arsenic^[6] structure (Figure 2). The Co–Co distances are 5.516(2) within the rings and 5.507(2) Å between adjacent units. The rings pack together in such a way as to generate infinite channels parallel to the *c* axis.

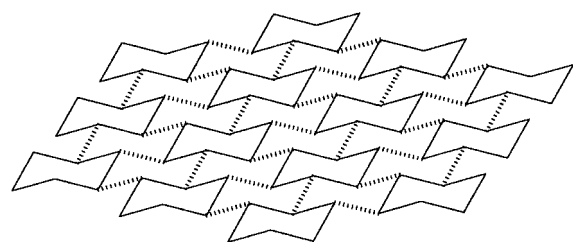


Figure 2. View of the topology of cobalt atoms in a layer parallel to the *ab* plane. Solid lines represent Co–Co vectors within the ring, dashed lines between rings.

An investigation of the magnetic behavior of **1** (Figure 3) revealed that the room temperature moment is consistent with noninteracting *S* = 3/2 centers and the sample obeys the Curie–Weiss law above 100 K with θ = –14 K. At 14 K there

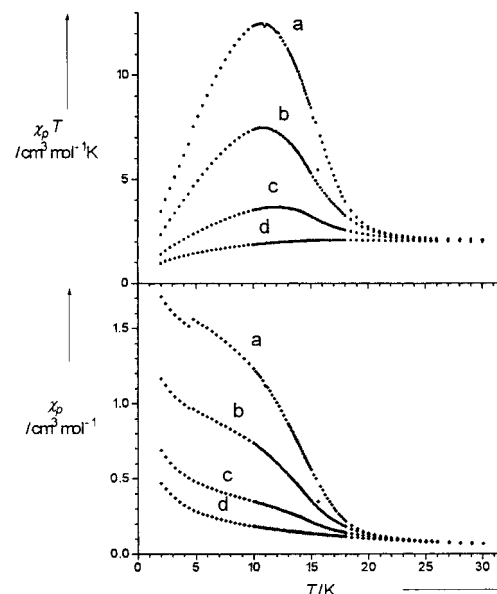


Figure 3. Plot of the temperature dependence of χT (top) and χ (bottom) versus magnetization in fields of a) 10, b) 100, c) 1000, and d) 10000 G.

is a magnetic phase transition marked by a sharp increase in the magnetization to a maximum value at 2 K of 115 G cm³ mol^{–1} (for a field of 100 G). This is equivalent to 0.7% of that expected for ferromagnetic ordering. This behavior is characteristic of canted antiferromagnetism^[7,8] in which a predominantly antiferromagnetic phase possesses a small spontaneous magnetization due to a small deviation from a strictly antiparallel arrangement. A series of temperature scans in various fields (Figure 3) shows a pronounced field dependence of the low-temperature phase confirming our assignment. Furthermore a hysteresis is observed below *T*_c (Figure 4), from which we can directly obtain values (at 5 K) for the coercive field of 370 G and remnant magnetization of

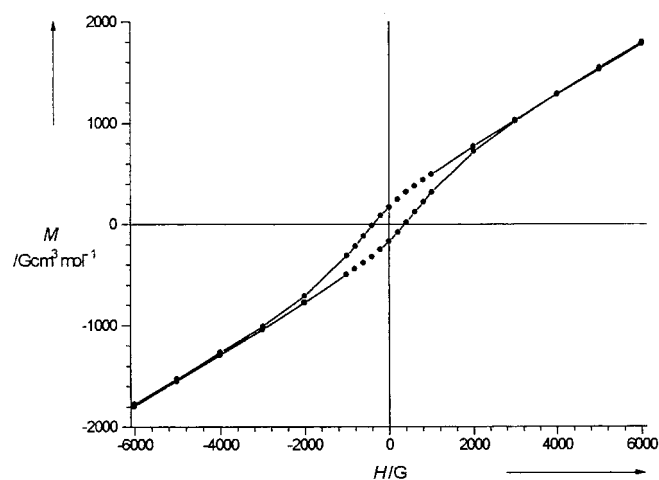


Figure 4. Hysteresis at 5 K.

170 G cm³ mol⁻¹. Fitting the data in the range $\pm(2000-10000)$ G to Equation (1) yields a value for the saturated canted moment of $M_s(5\text{ K}) = 288\text{ G cm}^3\text{ mol}^{-1}$ which corresponds to a canting angle ϕ of $0.98(2)^\circ$. The positions of

$$M(H, T) = M_s(T) + \chi_{\parallel}(T)H \quad (1)$$

spins within the unit cell shows that the stringent symmetry requirements^[7] for canted antiferromagnetism (e.g. no inversion center between coupled spins) are met. That a phase change from paramagnet to canted antiferromagnet occurs without an intermediate antiferromagnetic phase is indicative of the predominant influence of D , the single ion anisotropy over J , the exchange coupling which would have been expected from structural considerations. It is becoming evident that canted antiferromagnetism is surprisingly common in materials with extended structures^[8] but there are few examples of this behavior for molecular materials with the exception of some purely organic radicals.^[9] Hence we believe this to be the first observation of canting for an inorganic molecular compound.

In the last few years solvothermal synthesis has found new applications in coordination chemistry. Despite a great deal of interest and activity, control of product architecture is still elusive. It has been observed that the use of nonaqueous solvothermal conditions encourages the formation of molecular species^[3a] which has also been observed in this work. The ability of Co^{II} to tolerate a range of coordination environments makes it ideal for exploratory studies of this type. Clearly an entirely different lattice would have been observed for a metal requiring octahedral coordination. We have only observed similar structures for Zn^{II}, which also tolerates trigonal-bipyramidal coordination, whilst other divalent metal ions give entirely different structures. In addition the large single-ion anisotropy for trigonal-bipyramidal Co^{II} promotes the formation of the canted magnetic phase.

Experimental Section

A solution of Co(NO₃)₂ · 6H₂O (0.435 g, 1.5 mmol) in methanol (2 mL) was added to a stirred solution of H₂O₃PCH₂N(CH₂CO₂H)₂ (0.341, 1.5 mmol) and KOH (0.337 g, 6 mmol) in methanol (8 mL). The resulting blue slurry was transferred into a 23 mL Teflon-lined autoclave and heated at 200 °C for two days and allowed to cool to room temperature over a period of three hours. The product was filtered and washed with methanol and dried in air. Inspection of the final solid under an optical microscope, revealed that it consisted of blue-purple hexagonal crystals of **1** (yield 0.65 g, 90% based on Co) and colorless crystals of KNO₃ (ca. 10%), which was identified by IR spectroscopy and elemental analysis.

A KNO₃-free sample of **1** was obtained from an identical reaction with Co(OAc)₂ · 4H₂O (0.375 g, 1.5 mmol), H₂O₃PCH₂N(CH₂CO₂H)₂ (0.341, 1.5 mmol), and KOH (0.112 g, 2 mmol).

Elemental analysis calcd for C₅CoH₆K₂NO₇P (%): C 16.25, H 1.89, N 3.97; found: C 15.98, H 1.80, N 3.97.

Magnetic measurements were performed on polycrystalline samples using a SQUID magnetometer in the range 2–300 K.

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C–C Bond Formation between Fischer Carbene Complexes and Allylic Alcohols by a [3,4] Sigmatropic Rearrangement Promoted by a [1,2] M(CO)₅ Shift**

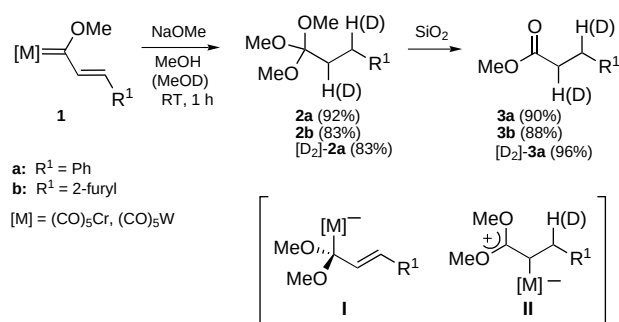
José Barluenga,* Eduardo Rubio,
José A. López-Pelegrín, and Miguel Tomás

*Dedicated to Professor José Elguero
on the occasion of his 65th birthday*

In the last few years Fischer carbene complexes have been developed into useful reagents for selective synthesis of organic molecules.^[1] For instance, the importance of Michael additions of carbon nucleophiles and various cyclization reactions with unsaturated substrates in carbon–carbon bond formation is widely recognized.^[2] However, studies of the reactivity of Fischer carbene complexes towards nucleophiles with heteroatoms, such as alcohols, have mainly focused on physical organic chemistry^[3] or structural modification of the carbene complex.^[4] Moreover, no practical applications of the reactions of alkenylcarbene complexes with oxygen nucleophiles are known, in spite of their superior versatility compared to simple carbenes and even alkynylcarbenes.^[5, 6] We report herein preliminary studies on the reaction of Fischer alkenylcarbene complexes with unsaturated alcohols such as allyl and propargyl derivatives, as well as with simple alcohols such as methanol. These lead to unexpected C–H and C–C bond-forming reactions and might be regarded as a further surprise from Fischer carbene complexes.^[7] Moreover, all reactions were equally effective for both chromium and tungsten carbene complexes.^[8]

Alkenyl(methoxy)carbene complexes **1** (1 mmol) were dissolved in MeOH (5 mL) containing sodium methoxide (0.1 mmol) and stirred for 1 h at room temperature. Conventional chromatographic purification afforded saturated esters **3** in high yields (88–90%). Careful column chromatography of the crude reaction mixture on deactivated silica gel allowed orthoesters **2** to be isolated (83–92%). The reaction of **1a** with NaOMe/MeOD gave the corresponding deuterated methyl ester [D₂]**3a** (96%, 1:1 mixture of diastereoisomers) and the not readily accessible orthoester [D₂]**2a** (83%) (Scheme 1).

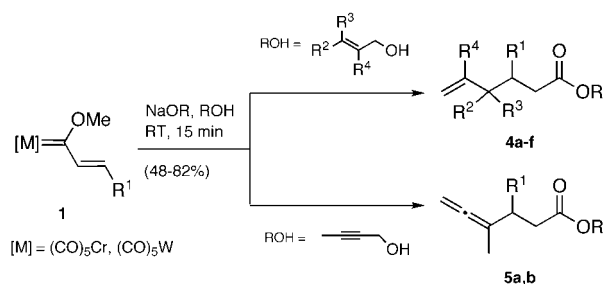
This simple transformation of alkenyl(methoxy)carbene complexes into saturated methyl esters implies the transfer of oxygen (oxidation) and hydrogen (reduction) to the carbene; the sole transfer agent is methanol. Species of types **I** and **II** are likely involved as active intermediates. Intermediate **I**, which arises from 1,2-addition of methoxide ion, was characterized for simple carbene complexes by Bernasconi et al.,^[9]



Scheme 1. Reaction of sodium methoxide/methanol with Fischer alkenylcarbene complexes. Formation of saturated orthoesters **2** and their hydrolysis products **3**.

while species **II** would result from protonation at C-3 promoted by a [1,2] M(CO)₅ shift, a process well known from recent work by us and others.^[10] Formation of **2** would require addition of methanol to **II**, protonation, and reductive elimination of the metal fragment.

On the basis of this mechanistic assumption, we tried to trap **I** with allylic alcohols. The reaction of **1** with allyl alcohol and derivatives thereof in the presence of the corresponding alkoxide ion (0.1 equiv, 20 °C, 15 min) resulted in the transfer of the allyl moiety to the carbene group by selective C–C coupling to give the ester adducts **4** (Scheme 2, Table 1). In



Scheme 2. Reaction of allyl and propargyl alcohols with Fischer alkenylcarbene complexes.

general, the reaction gives satisfactory yields, particularly for unsubstituted ($R^2, R^3, R^4 = H$; entries 1 and 2) and 3-substituted ($R^2 = Pr$; $R^3, R^4 = H$; entry 6) alcohols. Even the prenyl alcohol furnished quite efficiently the adducts **4c, d** ($R^2 = R^3 = Me, R^4 = H$; entries 3 and 4), in which a quaternary carbon center has been created. The C–C coupling occurs with low stereoselectivity (entry 6), but with complete regioselectivity between the γ -C atom of the allyl alcohol and the β -C atom of the carbene complex (entries 3, 4, and 6).^[11] Moreover, the applicability of the method to propargylic alcohols was proven by the reaction of **1** with 2-butyne, which gave the cumulene derivatives **5** in acceptable yields (entries 7 and 8).

A reaction pathway in which a [3,4] sigmatropic rearrangement is promoted by a [1,2] shift of M(CO)₅ is consistent with these results (Scheme 3). The first step involves consecutive methoxy exchange and nucleophilic addition of alkoxide ion to give the tetrahedral intermediate **III**. Probably, **III** then undergoes a [1,2] M(CO)₅ shift followed by an anionic [3,4]

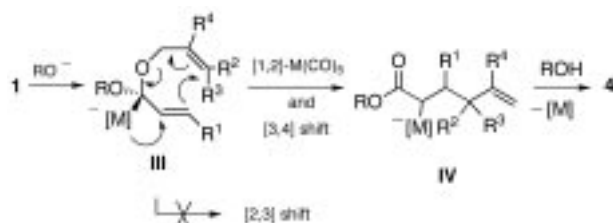
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Table 1. Reaction of carbene complexes with allylic and propargylic alcohols.

Entry	ROH	R ¹	R ²	R ³	R ⁴	Product	Yield [%] ^[a]
1		Ph	H	H	H	4a	82
2		2-Furyl	H	H	H	4b	76
3		Ph	Me	Me	H	4c	48
4		2-Furyl	Me	Me	H	4d	55
5		Ph	H	H	Me	4e	50
6		2-Furyl	Pr	H	H	4f ^[b]	71
7		Ph	–	–	–	5a	62
8		2-Furyl	–	–	–	5b	58

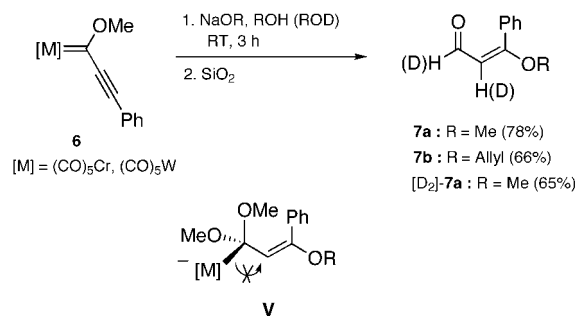
[a] Nonoptimized yields of purified isolated products. All yields refer to reactions with tungsten complexes. [b] Isolated as a 3:2 mixture of diastereoisomers.



Scheme 3. Mechanism proposed for the rearrangement observed in the formation of **4**.

sigmatropic rearrangement to form **IV**, which gives **4** upon protonation and reductive elimination of the metal moiety. The transition metal plays a crucial role in the *peri* selectivity of the process, since diallyl ether lithium species analogous to **III** do not undergo a [3,4] shift, but only a [2,3] sigmatropic rearrangement.^[12]

Support for the above reaction pathways, specifically the 1,2-addition and [1,2] M(CO)₅ shift steps, was gained by studying the alcoholysis of alkynylcarbene complexes (Scheme 4). The reaction of **6** with NaOMe/MeOH and NaOC₃H₇/C₃H₇OH (20°C, 3 h) furnished, after chromatographic purification, **7a** (78%) and **7b** (66%), respectively. Using deuteriated methanol gave [D₂]**7a** (65%). In this case,



Scheme 4. Reaction of alcohols with Fischer alkynylcarbene complexes.

the facile 1,4-addition of the alcohol precedes the 1,2-addition of alkoxide to give intermediate **V**. The electron-releasing nature of the alkoxy group at C-3 of **V** prevents 1,2 migration of the anionic metal complex fragment, and the process continues by conventional reductive metal elimination and hydrolysis.

In summary, we have demonstrated simple, novel applications of chromium and tungsten Fischer carbene complexes based on the [1,2] migration of the pentacarbonylmetal group. For instance, methanol readily transfers hydrogen to the carbon–carbon double bond of alkenylcarbene complexes by double protonation. More importantly, we discovered a new facet of Fischer carbene complexes: C–C bond formation by transfer of an allyl or propargyl group in a novel [3,4] sigmatropic shift.^[13] This rearrangement, which is very rare in aliphatic compounds^[14] and unknown for diallyl ethers,^[12] is facilitated by the above-mentioned [1,2]M(CO)₅ shift.^[15]

Experimental Section

General procedure for the preparation of **4** and **5**: The carbene complex (1 mm) was added to a solution (0.02 M) of the sodium alkoxide in the corresponding alcohol (5 mL). After stirring at room temperature until complete disappearance of the carbene complex (monitored by IR spectroscopy), the solvent was removed at reduced pressure and the crude product subjected to column chromatography (silica gel, hexane/ethyl acetate 40/1). All compounds gave satisfactory analytical data, including elemental analysis and mass and NMR spectra.

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Keywords: carbene complexes • chromium • orthoesters • rearrangements • tungsten

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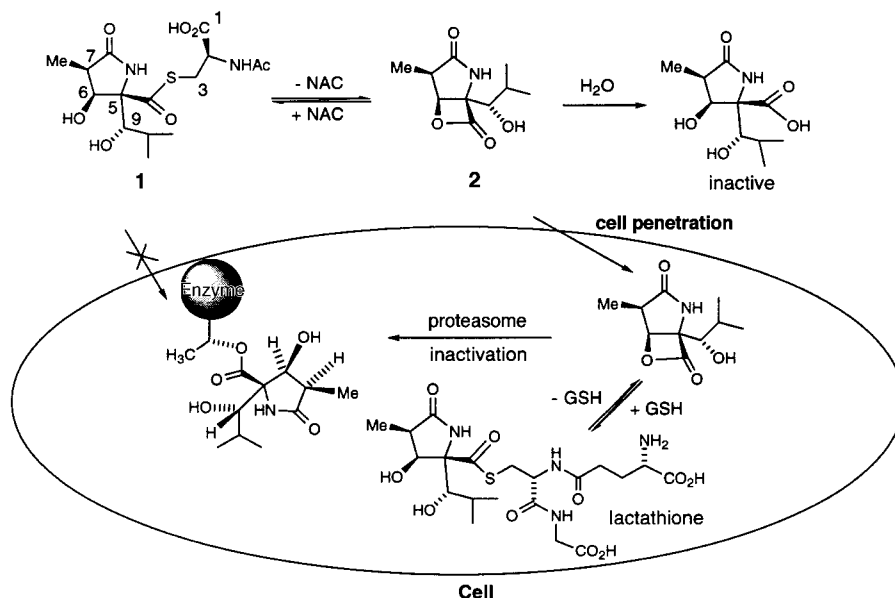


Figure 1. Mechanism of proteasome inhibition by (+)-lactacystin.

Total Synthesis of (+)-Lactacystin**

James S. Panek* and Craig E. Masse

(+)-Lactacystin (**1**) is a metabolite isolated from *Streptomyces* sp. OM-6519 that exhibits significant neurotrophic activity.^[1a] The relative and absolute stereochemistry of (+)-lactacystin has been elucidated by ¹H and ¹³C NMR spectroscopy, and single-crystal X-ray analysis.^[1b] (+)-Lactacystin has been shown to be a potent proteasome inhibitor, and has led to speculation that it may be therapeutically useful in the

treatment of arthritis.^[2] The equally potent β -lactone **2** acts by selective acylation of the N-terminal threonine residue of a protein subunit of the cylindrical 20S proteasome,^[3] a result confirmed by X-ray crystallographic studies at 2.4 Å resolution of the lactacystin inactivated proteasome.^[4] Recent mechanistic studies have shown that lactacystin hydrolyzes to the inactive dihydroxy acid through its β -lactone **2**. It is the β -lactone species that subsequently acylates the proteasome and results in its inactivation (Figure 1).^[3b] The presence of intracellular levels of glutathione (GSH) converts **2** into lactathione, which is believed to act as a “lactone reservoir”.^[3c]

(+)-Lactacystin (**1**) is a unique member of a class of neurotrophic factors since it consists of a nonprotein γ -lactam thioester. Its compact array of five resident stereogenic centers renders (+)-lactacystin a significant target for synthesis; a number of other syntheses of **1** have been reported.^[5] A critical issue relative to the synthesis of lactacystin is the fact that most of the structural features of **1** are essential to maintain its unique biological profile. The C4-carboxylic moiety and the C6-hydroxyl group must be *cis* because of the necessity for β -lactone formation to achieve proteasome inactivation.^[4] The absolute configuration of the C9-hydroxyl and the isopropyl substituents are also essential for biological activity.^[5c] The C7-methyl group is critical to the activity and stability of **1**, although replacement of this group with either ethyl or isopropyl groups does lead to a two- to threefold increase in activity.^[5c]

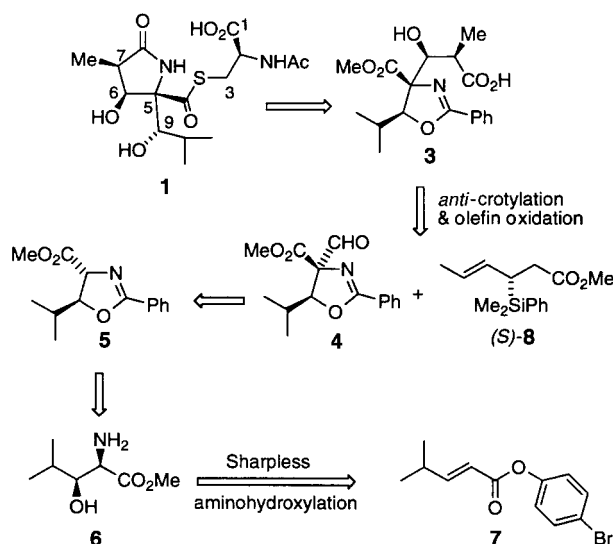
As a consequence of these strict structural and stereochemical requirements any synthesis of **1** must not only be efficient, but also highly selective for the introduction of each of the stereogenic centers. Our approach to lactacystin nicely compliments the synthesis by Smith and co-workers^[5e] through the use of the hydroxyleucine-derived oxazoline **5** to set the stage for the critical *anti*-crotylation reaction for the installation of the C6 and C7 stereocenters.

Retrosynthetic analysis of the lactacystin skeleton (Scheme 1) reveals two key operations: a) stereoselective

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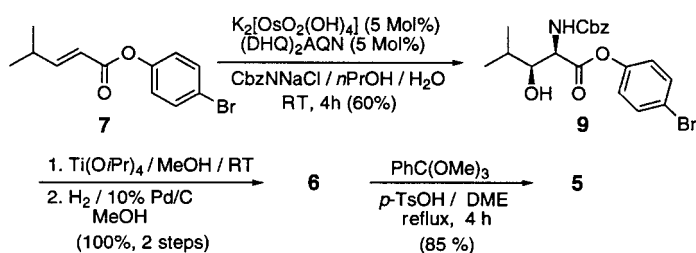


Scheme 1. Retrosynthetic analysis.

generation of the heterocyclic aldehyde **4** derived from a 3-hydroxyleucine derivative and b) an asymmetric crotylsilane addition to aldehyde **4** to generate the fully elaborated stereochemical core of the parent molecule **1**. Earlier efforts in this area^[5e] have established that aldol surrogates such as (*E*)-crotylboron or (*E*)-crotylchromium(II) reagents exhibit only modest levels of diastereoselection for the (6*S*)-isomer (dr = 2:1 → 4:1). We anticipated that the chiral silane reagents should be capable of providing enhanced levels of diastereoselection for this double stereodifferentiating reaction since Lewis acid promoted reactions involving allylsilanes proceed through open transition structures.^[6] These transition states diminish the destabilizing effects associated with the steric congestion of heterocyclic aldehyde **4**. The first stage of the synthesis involved the development of an efficient stereoselective synthesis of heterocyclic aldehyde **4** that contained the C5 and C9 stereocenters of lactacystin. This required an efficient route to oxazoline **5**. Currently, the most efficient approach to oxazoline **5** requires ten steps with an overall yield of 60% from (*E*)-4-methyl-2-penten-1-ol.^[5e]

Our first objective in the development of a more practical synthesis of oxazoline **5** was to devise a concise and stereoselective synthesis of the hydroxyleucine unit. Numerous approaches to the 3-hydroxyleucine synthon **6** have been reported.^[7] However, they either lack the flexibility to prepare the various isomers, require the preparation of a chiral catalyst system, or are prohibitively lengthy and impractical for large scale preparation. Given those considerations, the present synthesis of (2*R*,3*S*)-hydroxyleucine methyl ester (**6**) employs commercially available materials and utilizes an asymmetric catalytic aminohydroxylation of (*p*-bromophenyl)-4-methyl-2-pentenoate.

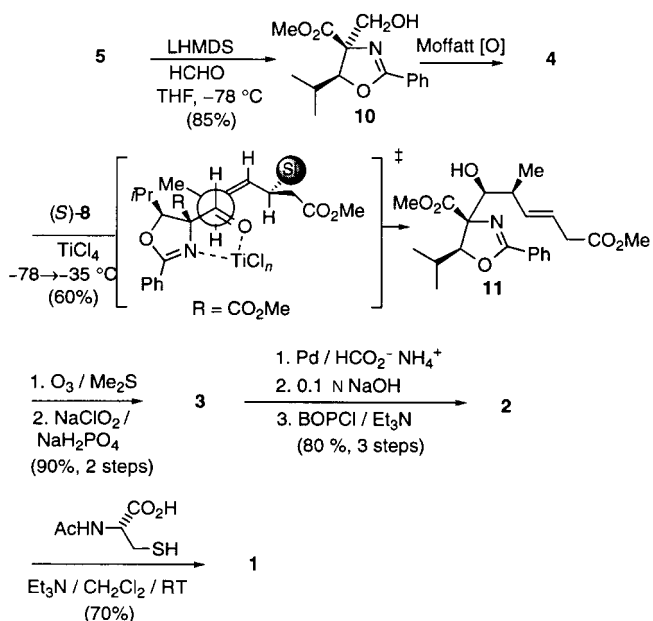
The asymmetric aminohydroxylation (AA) of **7** using the benzylcarbamate-based Sharpless reaction^[8] and 1,4-bis(dihydroquininyl)anthraquinone (DHQ)₂AQN gave **9** with good levels of regioselectivity (7:1) in favor of the α -amino ester and high levels of enantioselectivity (87% *ee*, Scheme 2). The ratio of regioisomers was determined by ¹H NMR analysis of the crude product and the initial *ee* value of 87% could be



Scheme 2. Preparation of oxazoline **5**. Cbz = "carbobenzy" = benzyl-oxycarbonyl, DME = 1,2-dimethoxyethane.

raised to >99% by recrystallization (two times) from EtOH/H₂O (1/1). Subsequent transesterification to the methyl ester in the presence of Ti(O*i*Pr)₄^[9] and removal of the benzyloxycarbonyl group by hydrogenolysis afforded **6**. Finally, treatment of **6** with trimethylorthobenzoate in the presence of *p*-toluenesulfonic acid^[10] provided the *trans*-oxazoline **5**. The virtues of this approach are apparent from the concise nature of the synthetic sequence, mild reaction conditions, and the fact that either enantiomer can be prepared by the proper choice of the alkaloid ligand.

The preparation of the heterocyclic aldehyde **4** was accomplished according to a literature precedent established by Smith et al.^[5e] Oxazoline **5** was subjected to an aldol condensation with formaldehyde according to the Seebach protocol^[11] to afford primary alcohol **10** as a single diastereomer (Scheme 3). The topological bias for this ester enolate was presumably controlled by the chirality of the oxazoline in



Scheme 3. Completion of the synthesis of (+)-lactacystin (**1**). LHMDS = lithium bis(trimethylsilyl)amide.

which the bulky isopropyl group functions as the controller of diastereoselectivity. Oxidation of the primary alcohol using the Moffatt protocol (dicyclohexylcarbodiimide, DMSO, pyridine, trifluoroacetic acid)^[12] provided the desired heterocyclic aldehyde **4**. Any attempt to purify this product resulted in deformylation, so this aldehyde was used without purifica-

tion. The critical *anti*-selective crotylation reaction was then carried out to establish the relative configuration at the C6 and C7 centers. This double stereodifferentiating^[13] reaction was readily accomplished with TiCl₄ to afford homoallylic alcohol **11** with high levels of diastereoselectivity (*anti:syn* > 30:1) and in 50–60% yield. This *anti*-bond construction was presumably achieved through simultaneous coordination of the aldehyde oxygen atom and the nitrogen atom in the oxazoline ring. The 1,3-relationship of the heteroatoms ideally predisposes the more Lewis basic nitrogen atom relative to the aldehyde carbonyl group to generate a 5-membered chelate with TiCl₄ via the illustrated synclinal transition state (Scheme 3).^[14] Oxidative cleavage of (*E*)-olefin **11** under standard ozonolysis conditions and subsequent oxidation with sodium chlorite^[15] furnished carboxylic acid **3**.

The completion of (+)-lactacystin was initiated by catalytic transfer hydrogenation of the oxazoline moiety with Pd-black to give the γ -lactam methyl ester after cyclization. Saponification of the methyl ester under mild conditions afforded the dihydroxy acid, which was directly converted into β -lactone **2** by treatment with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl). We employed the lactone opening strategy developed by Corey et al. to attach the *N*-acetyl-L-cysteine side chain.^[5b] Treatment of **2** with *N*-acetyl-L-cysteine/Et₃N furnished synthetic (+)-**1** identical in all respects to the natural product (¹H and ¹³C NMR, IR spectroscopies, HR-MS, optical rotation, and TLC).^[5b]

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Aerobic Oxidation of Primary Alcohols by a New Mononuclear Cu^{II}-Radical Catalyst**

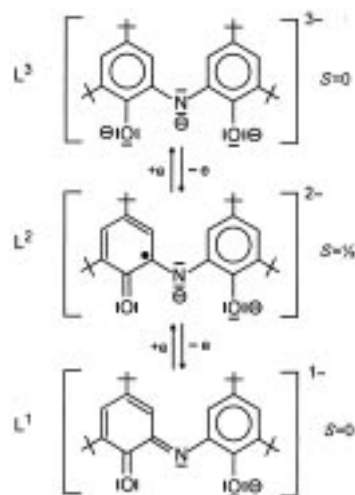
Phalguni Chaudhuri,* Martina Hess,
Thomas Weyhermüller, and Karl Wieghardt*

Recently we reported^[1] a dinuclear Cu^{II}-phenoxyl radical complex, which catalyzes efficiently the aerobic oxidation of primary and secondary alcohols to the corresponding aldehydes and ketones or to 1,2-glycols by oxidative C–C coupling with concomitant formation of H₂O₂. This complex together with that of Stack et al.^[2] are the first reported functional models for the enzyme galactose oxidase (GO).^[3] We report here a new mononuclear Cu^{II}-iminosemiquinone catalyst that selectively transforms primary alcohols (e.g. ethanol but not methanol) with O₂ to aldehydes and H₂O₂; secondary alcohols are not at all substrates for the catalyst.

The trifluoroacetate salt of the cation *N,N*-bis(2-hydroxy-3,5-di-*tert*-butylphenyl)ammonium [H₄(L³)](CF₃CO₂) was prepared through the condensation of 3,5-di-*tert*-butylcatechol with NH₃ in *n*-heptane and subsequent acidification with trifluoroacetic acid. It is known^[4] that the diamagnetic trianion (L³)^{3–} (Scheme 1), present as a tridentate ligand in complexes, can be easily oxidized to the radical dianion (L²)^{2–} and then to the diamagnetic monoanion (L¹)^{1–} in two successive one-electron oxidation steps. Speier and Pierpont et al. have, for example, described the complexes [Cu^{II}(L¹)₂]

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Scheme 1.

and $[\text{Cu}^{\text{II}}(\text{L}^2)(\text{pyridine})_2]$.^[5] In absence of air $[\text{H}_4(\text{L}^3)]-(\text{CF}_3\text{CO}_2)$ reacts with $[\text{Cu}^{\text{I}}(\text{NCCH}_3)_4]\text{ClO}_4$ in CH_3CN in the presence of excess NEt_3 to yield a yellow solution, which upon exposure to air at 20°C provides the neutral complex $[\text{Cu}^{\text{II}}(\text{L}^2)(\text{NEt}_3)]$ (**1**) as green-black crystals.

On the contrary, a solution of $\text{Cu}^{\text{I}}\text{Cl}$ and $[\text{H}_4(\text{L}^3)](\text{CF}_3\text{CO}_2)$ (1:1) neutralized by NEt_3 in CH_3OH upon treatment with pure dry O_2 at -50 to -70°C yields deep red crystals of $[\text{Cu}^{\text{II}}(\text{L}^3\text{H}_2)(\text{O}_2^{\cdot-})(\text{NEt}_3)]$ (**2**). Compound **2** is diamagnetic (2–300 K) due to strong intramolecular antiferromagnetic coupling between the Cu^{II} ion and the bonded superoxide ion. The $\text{Cu}^{\text{II}}(\text{O}_2^{\cdot-})$ moiety exhibits typical charge transfer absorptions at 524 and 650 nm and an infrared stretch $\tilde{\nu}({}^{16}\text{O}-{}^{16}\text{O})$ at 964 cm^{-1} , which on ${}^{18}\text{O}$ -labeling drops^[6] to 909 cm^{-1} . Complex **2** can be directly prepared by adding 100-fold excess of H_2O_2 to a green solution of **1** in dry methanol. On the other hand, a solution of **2** in tetrahydrofuran (THF) at 20°C forms **1** and H_2O_2 due to the equilibrium (1).



The X-ray structure^[7] of **1** shows that the Cu^{II} ion, coordinated to the tridentate $(\text{L}^2)^{2-}$ ligand along with an NEt_3 molecule, is in a slightly distorted square-planar N_2O_2 environment (Figure 1).^[8] The metrical parameters of $(\text{L}^2)^{2-}$ correspond to those for the reported $[\text{M}^{\text{IV}}(\text{L}^2)_2]$ complexes ($\text{M} = \text{V}, \text{Ti}, \text{Sn}$).^[4d, 9] That **1** possesses a diamagnetic ground state ($S=0$) arising from the intramolecular antiferromagnetic coupling between the spins of radical–ligand ($S_{\text{L}}^2 = 1/2$) and that of the Cu^{II} ion ($S_{\text{Cu}} = 1/2$) has been shown by variable-temperature susceptibility measurements (SQUID, 2–400 K)^[10] on solid **1**. This behavior is in accordance with that of the active form of GO, in which a Cu^{II} ion is coordinated to a tyrosyl radical.

The cyclic voltammogram of **1** in CH_2Cl_2 (0.1 M $[(n\text{Bu})_4\text{N}]\text{PF}_6$, glassy carbon electrode, 20°C) exhibits two reversible one-electron processes at $E_{1/2}^1 = -0.14\text{ V}$ and $E_{1/2}^2 = -1.06\text{ V}$ versus Fc^+/Fc in the potential range $+0.2$ to -1.3 V . Spectroelectrochemical measurements on the oxidized and reduced forms, generated by coulometry of **1**, show the redox processes to be ligand-centered, as shown in Equation (2). In

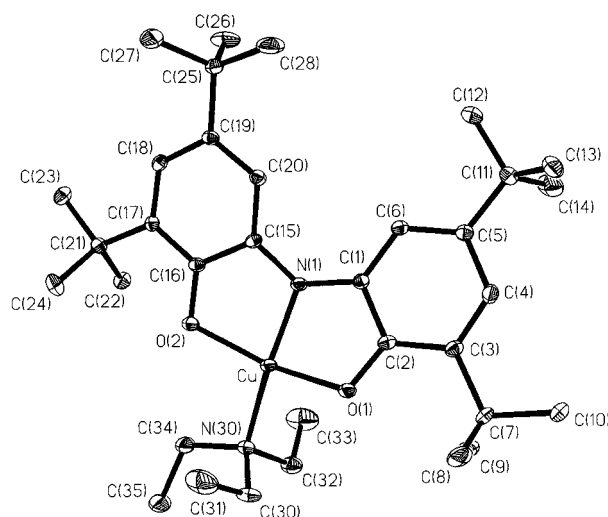
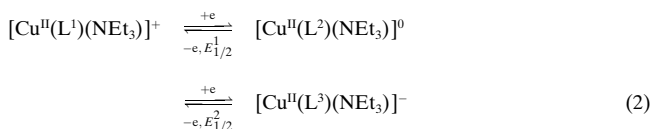
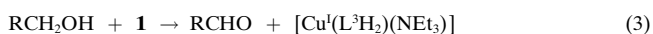


Figure 1. Crystal structure of the neutral complex **1**. Selected bond lengths [Å] and angles [°]: Cu–N(1) 1.906(2), Cu–O(1) 1.926(1), Cu–O(2) 1.959(1), Cu–N(30) 2.010(2), O(1)–C(2) 1.322(2), O(2)–C(16) 1.310(2), N(1)–C(1) 1.384(2), N(1)–C(15) 1.358(2); N(1)–Cu–O(1) 83.83(6), N(1)–Cu–O(2) 83.34(6), O(1)–Cu–O(2) 159.73(6), N(1)–Cu–N(30) 168.13(7), O(1)–Cu–N(30) 96.08(6), O(2)–Cu–N(30) 99.68(6).

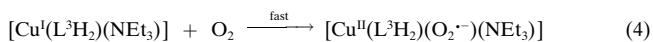
the absence of protons the Cu^{II} ion is reduced at even more negative potentials.



Under anaerobic conditions at 20°C , a solution of **1** in THF oxidizes stoichiometrically benzyl and ethyl alcohol—but not isopropyl alcohol or methanol—to the corresponding aldehydes [Eq. (3)], while **1** itself undergoes a two-electron reduction process forming most probably $[\text{Cu}^{\text{I}}(\text{L}^3\text{H}_2)(\text{NEt}_3)]$.



On the other hand, as $[\text{Cu}^{\text{I}}(\text{L}^3\text{H}_2)(\text{NEt}_3)]$ in THF or CH_2Cl_2 reacts with molecular O_2 to yield at first **2** and subsequently slowly **1** and H_2O_2 [Eq. (4) and (5)], the combination of Equations (3)–(5), in principle, leads to the homogeneously catalyzed oxidation of primary alcohols [Eq. (6)].



This is, indeed, the case. A THF solution of **1** (or $[\text{Cu}^{\text{I}}(\text{L}^3\text{H}_2)(\text{NEt}_3)]$) ($[\text{Cu}]_{\text{t}} = 2.65 \times 10^{-5}\text{ M}$) mixed with benzyl alcohol or ethanol (0.125 M) as substrate, provides after stirring in open air at 20°C for 20 h the corresponding aldehyde in an overall yield of about 55%. The catalysis can also be performed in neat alcohols without any additional solvent. The gas chromatographic product analysis established aldehydes as the sole oxidation product; no formation of carboxylic acid or oxidative C–C coupling^[1] products ($>1\%$)

was observed. The catalyst does not show any catalase activity: disproportionation of H_2O_2 does not occur.

From the measurements of the kinetics^[11] of these catalytic reactions the rate law [Eq. (7)] has been derived which establishes a mononuclear copper complex to be the active

$$v = k[\text{Cu}][\text{alcohol}] \quad [\text{NEt}_3] = 2 \times 10^{-3} \text{ M} = \text{const.} \quad (7)$$

form of the catalyst, as is found also in the compound described by Stack et al.^[2] and in GO.^[1] With the selectively deuterated substrates PhCD_2OH and $\text{CH}_3\text{CD}_2\text{OH}$ kinetic isotope effects $k_{\text{H}}/k_{\text{D}}$ of about 8 have been evaluated. As for other functional models^[1, 2] and GO,^[3] hydrogen abstraction from the α -carbon atom of the coordinatively bound alcoholate is apparently also in this case the rate-determining step. The turnover frequency for **1** in neat alcohol of 0.5 s^{-1} is somewhat bigger than that of the system described by Stack et al. for the oxidation of benzyl alcohol (ca. 0.005 s^{-1}); the catalysis proceeds overall relatively slowly.

Figure 2 shows that the rate of aldehyde formation increases with increasing $[\text{NEt}_3]$ concentration until a maximum is reached and then starts to decrease. An excess of NEt_3

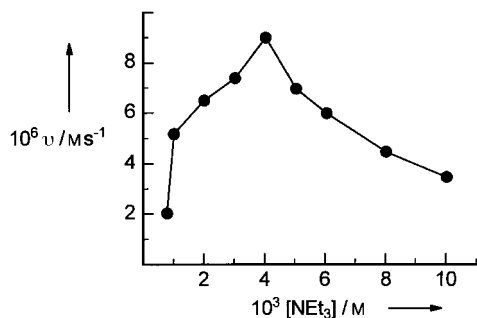
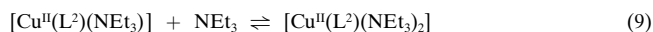
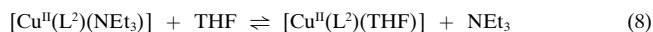


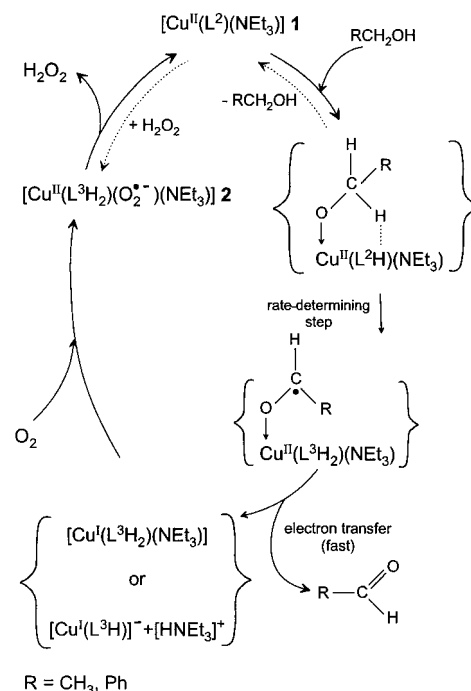
Figure 2. Effect of the concentration of base $[\text{NEt}_3]$ on the catalytic rate. Experimental conditions: $[\text{Cu}^{\text{I}}\text{Cl}] = 2.5 \times 10^{-4} \text{ M}$ (constant), $[\text{H}_4\text{L}^3] = 2.5 \times 10^{-4} \text{ M}$ (constant), $[\text{benzyl alcohol}] = 0.125 \text{ M}$ (constant), $[\text{NEt}_3] = (1.0 - 10.0) \times 10^{-3} \text{ M}$ in THF at 20°C and 1 atm air. Formation of benzaldehyde as a function of time was quantitatively determined.

inhibits the catalysis. Our qualitative (and speculative) interpretation of this behavior is as follows: In absence of excess NEt_3 , the monodentate ligand NEt_3 dissociates from **1** in THF [Eq. (8)], while in presence of excess NEt_3 the five-coordinate complex $[\text{Cu}^{\text{II}}(\text{L}^2)(\text{NEt}_3)_2]$ is formed [Eq. (9)].



As $[\text{Cu}^{\text{II}}(\text{L}^2)(\text{NEt}_3)_2]$ and $[\text{Cu}^{\text{II}}(\text{L}^2)(\text{THF})]$ (or its dimeric form, detected by ESI-MS) are catalytically inactive, there is a particular NEt_3 concentration, at which the catalytically active form **1** is present in the highest concentration. If the experimental concentration of NEt_3 is smaller than that at the maximum, the catalysis becomes slowed down by increasing formation of the monomeric dissociation product (or its dimer); a higher concentration of NEt_3 than that at the maximum results in more adduct formation also causing slow catalysis or even complete inhibition.

Our mechanistic proposal for the catalysis is displayed in Scheme 2. At the beginning **1** binds an alcohol; in the rate-determining step hydrogen-atom transfer from the α -C atom of the alcoholate ligand to the radical-ligand $(\text{L}^2\text{H})^\cdot$ occurs, in which it is reduced to $(\text{L}^3\text{H}_2)^-$. The resulting coordinated



Scheme 2. Proposed mechanism for the catalysis.

ketyl radical anion, a strong one-electron reducing agent, transfers very fast an electron to the Cu^{II} ion, during which the “bad” ligand aldehyde is formed and dissociates. The Cu^{I} form can now react with O_2 to form the Cu^{II} superoxide complex **2**, which then provides noncoordinated H_2O_2 and regenerates the active form **1**.

Experimental Section

N,N-Bis(2-hydroxy-3,5-di-*tert*-butylphenyl)ammonium trifluoroacetate (2.7 g, 48%) $[\text{H}_4\text{L}^3](\text{CF}_3\text{CO}_2)$ was obtained as a violet solid through the condensation of 3,5-di-*tert*-butylcatechol (5 g) with 25% aqueous ammonia (0.75 mL) in *n*-heptane (50 mL) under an argon atmosphere (48 h stirring at 20°C) and subsequent addition of trifluoroacetic acid (20 mL) and H_2O (50 mL). The crude product was washed several times thoroughly with *n*-pentane to yield a colorless product, which is slightly light-sensitive but strongly O_2 -sensitive. Remark: For the following syntheses and catalysis only the colorless pure product should be used. C, H, N analyses (%): $\text{C}_{30}\text{H}_{44}\text{F}_3\text{NO}_4$: calcd (found): C 66.64 (66.34), H 8.39 (8.30), N 2.59 (2.48); EI-MS: m/z : 425 $[M^+]$; ^1H NMR (CDCl_3): δ = 7.11 (s, 2H), 6.83 (s, 2H), 5.61 (s, br, 4H; OH, NH_2), 1.44 (s, 18H, $\text{C}(\text{CH}_3)_3$), 1.22 (s, 18H, $\text{C}(\text{CH}_3)_3$).

1: A solution of $[\text{Cu}^{\text{I}}(\text{NCCH}_3)_4](\text{ClO}_4)$ (0.16 g, 0.5 mmol), $[\text{H}_4\text{L}^3](\text{CF}_3\text{CO}_2)$ (0.272 g, 0.5 mmol), and triethylamine (0.5 mL) was refluxed in dry CH_3CN (50 mL) for 0.5 h under argon. Exposure of the resulting pale yellow solution to air at about 20°C initiated a color change to deep green, from which within 1 h green-black crystals of **1** precipitated (0.21 g, 71%). C, H, N, Cu analyses (%): $\text{C}_{34}\text{H}_{55}\text{N}_2\text{O}_2\text{Cu}$: calcd (found): C 69.52 (69.73), H 9.23 (9.22), N 4.77 (4.62), Cu 10.81 (10.63).

2: A solution of $\text{Cu}^{\text{I}}\text{Cl}$ (0.10 g, 1.0 mmol) $[\text{H}_4\text{L}^3](\text{CF}_3\text{CO}_2)$ (0.54 g, 1.0 mmol) and NEt_3 (0.5 mL, 3.5 mmol) in dry CH_3OH (50 mL) was heated to boiling for a short time under argon and then cooled to a

temperature in the range of -50 to -70°C and treated with pure O_2 to yield a deep red solution. Within 5–10 min a precipitation of a deep red microcrystalline substance **2** (0.12 g; 19%) occurred. An alternative method for crystallizing **2** was to add 30% H_2O_2 (0.26 mL) to a solution of **1** (160 mg, 2.23 mmol) in CH_3OH (25 mL) at -80°C . C, H, N, Cu analyses (%; $\text{C}_{34}\text{H}_{57}\text{N}_2\text{O}_4\text{Cu}$): calcd (found): C 65.72 (65.45), H 9.25 (9.23), N 4.51 (4.52), Cu 10.23 (10.20); UV/Vis (CH_2Cl_2): λ_{max} [nm] (ϵ [$\text{L mol}^{-1}\text{cm}^{-1}$]) = 391 (2.1×10^3), 423 (1.8×10^3), 524 (2.1×10^3), 649 (1.6×10^3); ^1H NMR (CD_2Cl_2 , 400 MHz): δ = 0.99 (t, 9H), 1.18 (s, 18H), 1.29 (s, 18H), 1.80 (br. s, 2H), 2.50 (dt, 6H), 7.20 (s, 2H), 7.33 (s, 2H).

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there is significantly different spin density at the two *para* carbon atoms with respect to the imino nitrogens. P. Chaudhuri, M. Hess, K. Hildenbrand, E. Bill, T. Weyhermüller, K. Wieghardt, *Inorg. Chem.* submitted.

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Synthesis, Structure, and Redox Properties of $[\{(\eta^5\text{-C}_5\text{H}_5)\text{Co}(\text{S}_2\text{C}_6\text{H}_4)\}_2\text{Mo}(\text{CO})_2]$, a Novel Metalladithiolene Cluster**

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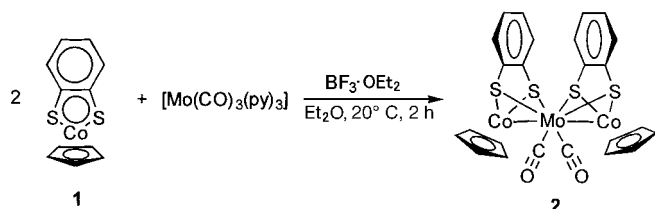
Dithiolato complexes of late transition metals exhibit interesting physical and chemical properties such as reversible redox activity, deep colors,^[1] and various substitution and addition reactions due to the quasiaromaticity and electronic unsaturation of the metalladithiolene ring.^[2] However, no reports on metal–metal bond formation by metalladithiolene complexes are available to our knowledge. Here we report the first formation of a cluster from a cobalt dithiolene complex, namely, $[\text{CoCp}(\text{S}_2\text{C}_6\text{H}_4)]$ (**1**, $\text{Cp} = \eta^5\text{-C}_5\text{H}_5$), by reaction with $[\text{Mo}(\text{CO})_3(\text{py})_3]$ and BF_3 to give $[\{\text{CpCo}(\text{S}_2\text{C}_6\text{H}_4)\}_2\text{Mo}(\text{CO})_2]$ (**2**), which has an almost linear Co–Mo–Co arrangement and four $\mu\text{-S}$ bridges. It can be regarded as two cobaltadithiolene rings bridged by a molybdenum dicarbonyl moiety. The redox

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properties of **2** could indicate how the Co–Co electronic interaction is transmitted by the Mo atom, since cobaltadithiolene undergoes a reversible one-electron reduction.^[3]

The mixed-metal cluster **2** was obtained by reaction of two equivalents of **1** with one equivalent of $[\text{Mo}(\text{CO})_3(\text{py})_3]$ in the presence of more than three equivalents of BF_3 in diethyl ether at room temperature (Scheme 1). The combination of



Scheme 1. Synthesis of **2**.

$[\text{Mo}(\text{CO})_3(\text{py})_3]$ and BF_3 is an effective way of generating a reactive $[\text{Mo}(\text{CO})_3]$ moiety that can bind to arenes.^[4] However, we could not detect any products in which the benzene ring ligates to Mo, even at different $1/[\text{Mo}(\text{CO})_3(\text{py})_3]$ molar ratios. This is presumably due to the higher reactivity of the electronically unsaturated cobaltadithiolene ring for addition reactions compared to the benzene ring. We assume that $[\text{Mo}(\text{CO})_3]$ adds to **1** to give a 1:1 complex with a Co–Mo and two Co–S–Mo bonds, but because this complex has an odd number of electrons at Mo, it reacts with a further molecule of **1** with loss of CO to give the 2:1 complex **2**.

The crystal structure of **2** is displayed in Figure 1.^[5] The Mo center is eight-coordinate with a square-antiprismatic (or dodecahedral) geometry, and the two S–Mo–S planes are twisted by 28.17° with respect to one another. The coordination

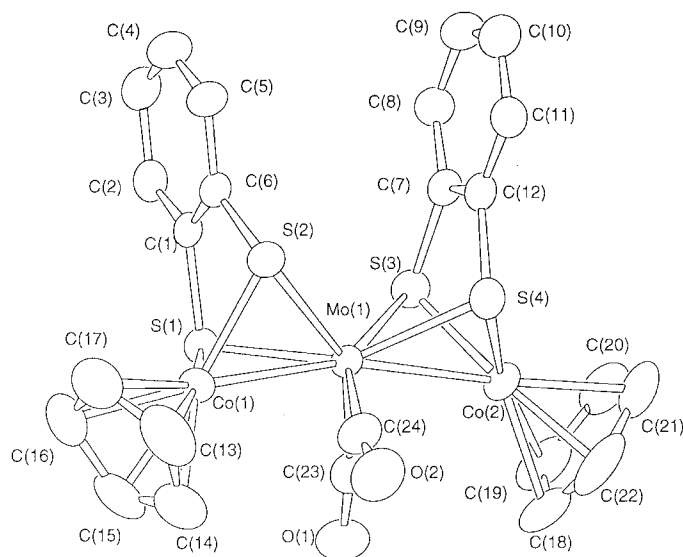


Figure 1. ORTEP plot of **2** with 50% probability ellipsoids. Selected bond lengths [Å] and angles [$^\circ$]: Mo1–Co1 2.6182(8), Mo1–Co2 2.6327(9), Mo1–S1 2.478(1), Mo1–S2 2.520(1), Mo1–S3 2.514(1), Mo1–S4 2.477(1), Co1–S1 2.199(2), Co1–S2 2.237(2), Co2–S3 2.227(2), Co2–S4 2.191(2); Co1–Mo1–Co2 165.03(3), Co1–Mo1–S1 51.04(4), Co1–Mo1–S2 51.58(4), Mo1–C23–O1 171.8(5).

environment of the Mo center with respect to the nonmetallic ligands (S and CO) is distorted trigonal-prismatic. Hence, the molecule belongs to the point group C_2 . The cobaltadithiolene rings are nonplanar owing to the coordination of the S atoms to Mo. The Co–S bond length of 2.218 Å is 0.107 Å longer than that of a free cobaltadithiolene ring.^[6] The Co–Mo bond length is 2.625 Å, which lies in the usual range for Co–Mo single bonds.^[7] The number of total valence electrons around the Co_2Mo core is 50; the four S atoms each contribute three electrons.

In the IR spectrum of **2**, two $\nu(\text{CO})$ bands are observed, and this is consistent with the C_2 symmetry at the Mo center. In the electronic spectrum of **2**, a strong absorption band is observed at $\lambda_{\text{max}} = 476 \text{ nm}$ ($\epsilon = 9800 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$). A preliminary molecular orbital calculation for **2** by the DV-X α method indicates that this band is characteristic of metal-to-metal charge transfer (MMCT) from Mo to Co rather than ligand-to-metal charge transfer (LMCT),^[8] because the energy levels of the d orbitals of Mo are close to those of Co. The ^1H NMR signals of the benzene rings of **2** appear at $\delta = 6.34$ and 6.79 and are shifted upfield by 0.79 and 1.30 ppm, respectively, relative to those of **1**. This effect can be attributed to the ring currents of the benzene rings.

A cyclic voltammogram of **2** in $[\text{Bu}_4\text{N}][\text{ClO}_4]/\text{MeCN}$ is shown in Figure 2. The first cyclic scan between -0.4 and -1.8 V versus Ag/Ag^+ shows two couples of reduction and

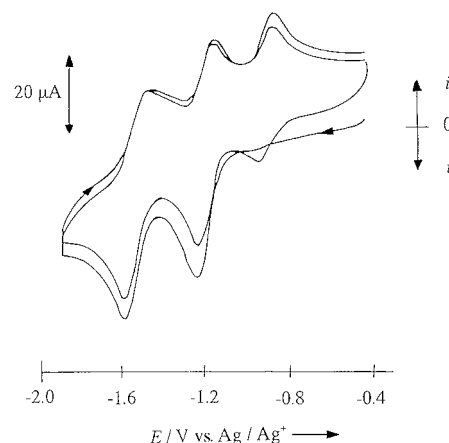


Figure 2. Cyclic voltammogram of **2** at a glassy carbon electrode in $0.1 \text{ mol dm}^{-3} [\text{Bu}_4\text{N}][\text{ClO}_4]/\text{MeCN}$ at a scan rate of 0.1 V s^{-1} .

reoxidation waves at $E^0 = -1.20$ and -1.53 V and an oxidation wave at $E_{\text{p,a}} = -0.90 \text{ V}$. A reduction wave corresponding to this oxidation wave is observed at $E_{\text{p,c}} = -0.96 \text{ V}$ in the second cyclic scan. This additional redox couple was assigned to $1/1^-$ by comparison of the E^0 values and UV/Vis absorption spectra of **1** and the electrolysis product of **2**. The redox wave due to $1/1^-$ does not appear when the negative limit of the potential scan is -1.4 V , which causes only the first reduction step of **2**. These results indicate that reduction of **2** gives successively the monoanion and dianion, after which the dianion decomposes to two monanions of **1** and a $[\text{Mo}(\text{CO})_2]$ fragment, the fate of which has not been determined. Thus, the redox process of **2** can be expressed as the EEC mechanism given in Equations (1)–(3).



The two redox potentials and the rate constant of the decomposition reaction of 2^{2-} were estimated by computer simulation to be $E_1^0 = -1.20$ V, $E_2^0 = -1.53$ V, and $k = 0.19$ s⁻¹. On the basis of these redox potentials, it is reasonable to assume that not the Mo atom but the two Co centers undergo reduction. Hence, the valence balance of 2^- can be roughly expressed as Co^{III}Mo⁰Co^{II}, which suggests that the Mo bridge assists electronic interaction between the Co sites to form a thermodynamically favorable mixed-valence state. This is supported by the electronic spectrum of 2^- , generated by the reduction of **2** with Na in THF, in which a broad band at 1160 nm ($\epsilon = 60$ mol⁻¹ dm³ cm⁻¹) is attributed to intervalence transfer.^[9] Detailed studies on the optical and magnetic properties of the reduced forms of **2** are in progress

Experimental Section

2: All manipulations were carried out under nitrogen or argon. BF₃ · OEt₂ (0.134 mL, 95.0%, 1.0 mmol) was added dropwise to a stirred solution of **1**^[10] (0.106 g, 0.40 mmol) and [Mo(CO)₃(py)₃]^[11] (0.084 g, 0.20 mmol) in diethyl ether (30 mL) at room temperature, and the mixture was stirred for 2 h. The solvent was evaporated under vacuum, and the components of the residue were separated by thin-layer chromatography on silica gel with toluene/hexane (2/1) as eluent. The component in the first band was eluted with toluene and recrystallized from hexane to give 50 mg (0.074 mmol, 37%) of fine brown crystals of **2**. Elemental analysis calcd for C₂₄H₁₈Co₂MoO₂S₄ (%): C 42.39, H 2.99, S 18.85; found: C 42.18, H 2.82, S 18.59; ¹H NMR (270 MHz, CDCl₃, 25 °C): δ = 5.15 (s, 10H, Cp), 6.34 (dd, J(H,H) = 5.5, 3.3 Hz, 4H, Ph), 6.79 (dd, 4H, Ph); IR (KBr disk): $\tilde{\nu}$ = 1860, 1923 cm⁻¹ (CO).

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coefficient μ for MoK α radiation is 22.3 cm⁻¹. An empirical absorption correction based on azimuthal scans of several reflections was applied and resulted in transmission factors ranging from 0.88 to 1.00. The data were corrected for Lorentzian and polarization effects. The structure was solved by direct methods and expanded by Fourier techniques. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 4707 observed reflections ($I > 3\sigma(I)$) with unweighted and weighted agreement factors of $R = 0.041$ and $R_w = 0.040$. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corporation. 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-108002. 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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The Synthesis and Molecular Structure of the First Two-Coordinate, Dinuclear σ -Bonded Mercury(II) RHgHgR Compound**

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In memory of Nicolai S. Vyazankin

Most of the known mercury compounds have a formal +2 oxidation state.^[1–5] σ -Bonded organomercury(II) compounds with a +1 oxidation state for example RHgHgR, R = organic substituent, have been frequently cited in the literature as

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- [5] X-ray structure analysis of **2**: A crystal (0.20 × 0.20 × 0.60 mm) was mounted in a glass capillary, and data were collected at 296 K on a Rigaku Afc7R diffractometer with graphite-monochromated MoK α radiation ($\lambda = 0.7107$ Å) and rotating-anode generator. Crystal data: C₂₄H₁₈O₂MoCoS₄, $M_r = 680.45$, triclinic, space group $P\bar{1}$, $a = 12.040(3)$, $b = 15.858(4)$, $c = 6.587(2)$ Å, $\alpha = 101.89(2)$, $\beta = 98.86(2)$, $\gamma = 92.78(2)^\circ$, $V = 1211.8(6)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.865$ g cm⁻³; of 7381 reflections ($6 < 2\theta < 60^\circ$), 7068 were unique. The linear absorption

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of a solution of **1** in hexane at $\lambda > 330$ nm leads selectively to the corresponding coupling product $[(\text{Me}_3\text{SiMe}_2\text{Si})_3\text{Si}]_2$ (**6**). We are currently attempting to prepare other compounds that possess the novel $\text{Hg}^{\text{I}}-\text{Hg}^{\text{I}}$ σ bond and are studying the chemistry of **1** further.

Experimental Section

Standard Schlenk techniques were used for all synthesis and all sample manipulations. NMR spectra were recorded in solution at room temperature in CDCl_3 or C_6D_6 using Bruker EM-200 or Bruker-400 instruments. Mass spectra (MS) were obtained with a Finnigan MAT TSQ 45 triple-stage quadrupole mass spectrometer. Elemental analyses were performed by the Microanalytical Laboratory of the University of Essen.

$(\text{Me}_3\text{SiMe}_2\text{Si})_3\text{SiH}$ (**4**): A solution of Cl_3SiH (1.35 mmol) in toluene (10 mL) was slowly added under vacuum using Schlenk techniques to a solution of $\text{Me}_3\text{SiMe}_2\text{SiLi}^{[14]}$ (4.1 mmol) in toluene (10 mL). The product was isolated by filtration of the reaction mixture, evaporation of the solvent, and column chromatography.

^1H NMR (C_6D_6): $\delta = 0.18$ (27H, s, SiMe_3), 0.34 (18H, s, SiMe_2), 2.81 (1H, s, SiH); ^{13}C NMR (C_6D_6): $\delta = -1.61$ (Me_3Si), -1.27 (Me_2Si); ^{29}Si NMR (C_6D_6): $\delta = -116.21$ (HSi), -39.93 (SiMe_2), -15.69 (SiMe_3); MS(EI): m/z : 349 [$M^+ - \text{SiMe}_3$].

1 (**5**): $t\text{Bu}_2\text{Hg}$ (3.15 mmol) and **4** (6.50 mmol) (for the preparation of **5** 3.40 mmol of **4** were used) were heated under an argon atmosphere up to 120°C . The reaction mixture was stirred for 4 h, during which time

isobutane (5.25 mmol) (2.83 mmol for the preparation of **5**) was formed. Evaporation of volatile compounds from the reaction mixture yielded **1** (67%) (or **5** (85%)). Compound **1** could be purified by crystallization from pentane. $[(\text{Me}_3\text{SiMe}_2)_3\text{Si}]_2\text{Hg}_2$ (**1**): ^1H NMR (C_6D_6): $\delta = 0.28$ (27H, s, SiMe_3), 0.48 (18, s, SiMe_2); ^{13}C NMR (C_6D_6): $\delta = -0.68$ (SiMe_3), 1.37 (SiMe_2); ^{29}Si NMR (C_6D_6 , 25°C): $\delta = -49.64$ and -46.02 (SiHg), -30.72 and -30.35 (SiMe_2), -14.67 and -13.02 (SiMe_3); ^{29}Si NMR (C_6D_6 , 60°C): $\delta = -48.67$ (SiHg), -30.54 (SiMe_2), -14.82 (SiMe_3). ^{199}Hg NMR (C_6D_6): $\delta = 1142.3$; elemental analysis calcd for $\text{C}_{30}\text{H}_{90}\text{Si}_{14}\text{Hg}_2$ (%): C 28.93, H 7.28; found: C 29.34, H 7.47; MS(CI): m/z : 1043 [$M^+ - \text{Hg}$]. $(\text{Me}_3\text{SiMe}_2)_3\text{Si}-\text{SiHg}t\text{Bu}$ (**5**): ^1H NMR (C_6D_6): $\delta = 0.23$ (27H, s, SiMe_3), 0.39 (18H, s, SiMe_2), 1.65 (9H, s, CMe_3); ^{13}C NMR (C_6D_6): $\delta = -0.83$ (SiMe_3), 0.73 (SiMe_2), 30.78 (CMe_3), 79.85 (CMe_3); ^{29}Si NMR (C_6D_6): $\delta = -91.4$ (SiHg), -30.5 (SiMe_2), -16.3 (SiMe_3); ^{199}Hg (C_6D_6): $\delta = -104.5$.

6: A solution of **1** (or of **5**) in hexane was irradiated with a 80-W Hanau mercury vapor lamp (Q-81) equipped with an uranium glass filter ($\lambda > 330$ nm). After separation of the hexane solution from metallic mercury, **6** was isolated by crystallization from hexane (yield 72–78%). ^1H NMR (C_6D_6): $\delta = 0.28$ (54H, s, SiMe_3), 0.48 (36H, s, SiMe_2); ^{13}C NMR (C_6D_6): $\delta = 1.02$ (SiMe_3), 3.40 (SiMe_2); ^{29}Si NMR (C_6D_6): $\delta = -87.38$ (Si); -34.83 (SiMe_2), -10.97 (SiMe_3); MS(CI): m/z : 842 [M^+].

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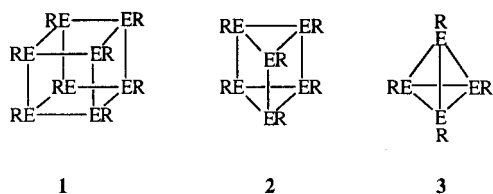
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- [15] A single crystal of dimensions $0.4 \times 0.4 \times 0.3$ mm was immersed in Nujol, transferred into a capillary and mounted on a Siemens P4 diffractometer. Data were collected at 173 K using MoK_α radiation ($0.71069 \text{ \AA}$, ω scan): 8588 collected, 2421 unique, and 2418 observed reflections; 81 parameters. $\text{C}_{30}\text{H}_{90}\text{Hg}_2\text{N}_2\text{Si}_{14}$, $M_r = 1245.46 \text{ g mol}^{-1}$, rhombohedral, space group $R\bar{3}$ (no. 148), $a = 18.247(3)$, $c = 15.574(4) \text{ \AA}$, $V = 4491(2) \text{ \AA}^3$, $Z = 3$, $\rho_{\text{calcd}} = 1.382 \text{ g cm}^{-3}$, $\mu = 5.421 \text{ mm}^{-1}$, $wR2 = 0.0837$ ($R1 = 0.0355$ based on 1838 reflections with $F_o > 4\sigma(F_o)$). Largest difference peak/hole: $+3.014/-1.940 \text{ e \AA}^{-3}$ (near Hg). The structure was solved by Patterson methods using the SHELXTL-PLUS package (G. M. Sheldrick, SHELXTL-PLUS An Integrated System for Solving, Refining and Displaying Structures from Diffraction Data, Rel.5.03, Siemens Analytical X-Ray Instruments Inc., **1989**). Refinement was achieved with the program SHELXL93 employing full-matrix least-squares methods on F_o^2 . All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were introduced at calculated positions and refined by using a riding model. 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-103313. 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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Hexasupersilyl-*triprismo*-hexastannane (*t*Bu₃Si)₆Sn₆—The First Molecular Tin Compound Containing a Sn₆ Prism**

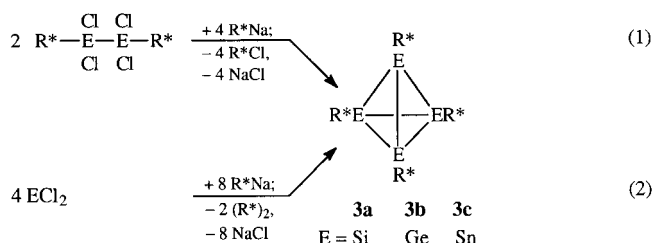
Nils Wiberg,* Hans-Wolfram Lerner, Heinrich Nöth,
and Werner Ponikwar

Compounds R_nE_n with cubic (1), trigonal-prismatic (2), or tetrahedral polyhedra E_n (3) of carbon homologues E have only recently (since 1988) become accessible through the use of sterically demanding groups R.^[1, 2] Evidently, less sterically demanding substituents do not provide sufficient protection to deter the polyhedral compounds from transforming into less strained compounds.^[3, 4] In certain cases, compounds with polyhedral frameworks of type 1 and also types 2 and 3 can be synthesized for E = Si and Ge,^[1, 2] whereas for E = Sn only those of type 1, but not of type 2 and 3 are known. However,



for E = Sn, compounds with a pentagonal Sn₁₀ prismane and those with a Sn₅ propellane framework also exist,^[5] whereas for E = Pb polyhedral compounds R_nE_n are still unknown.

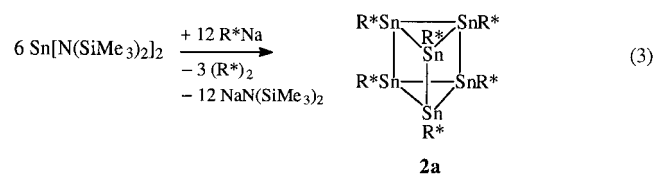
After our successful synthesis of a tetrasilatetrahedrane **3a**^[6] along path (1) and then of a tetragermatetrahedrane **3b**^[7] (in each case R in 3 is tri-*tert*-butylsilyl R* = Si*t*Bu₃ (supersilyl)) along paths (1) and (2) in the last few years, we decided



to study the formation of a tetrastannatetrahedrane **3c** along path (2) as a continuation of our work. Accordingly, a solution of tin dichloride SnCl₂ in THF was added dropwise to a solution containing two molar equivalents of supersilylsodium *t*Bu₃SiNa in THF cooled to –78 °C. On allowing the reaction mixture to slowly warm to room temperature it becomes dark brown, and a brown-black, insoluble tin compound as well as—according to NMR spectra of the reaction solution—

superdisilane *t*Bu₃SiSi*t*Bu₃^[8] and a soluble tin compound, tetrasupersilylristannacyclopropene (*t*Bu₃Si)₄Sn₃^[9, 10] are formed. The desired tetrahedrane **3c**, which was expected to be soluble in THF, is thus not obtained by this route.^[10] The above-mentioned tristannacyclopropene also forms in reactions of tin di-*tert*-butoxide Sn(OrBu)₂ as well as those of tin bis[bis(trimethylsilyl)amide] Sn[N(SiMe₃)₂]₂ with *t*Bu₃SiNa in pentane—along the path via isomeric tetrasupersilylristannaallene (*t*Bu₃Si)₄Sn₃^[9]—or by dehalogenation of R₂²SnCl₂ with sodium in benzene.^[11]

The reaction of Sn[N(SiMe₃)₂]₂ with *t*Bu₃SiNa (R*Na), proceeds differently when the pentane is replaced by *tert*-butyl methyl ether at –78 °C. After 4 h the dark blue reaction mixture was allowed to warm from –78 °C to –25 °C, and in the course of two days dark violet crystals precipitated from the mixture. According to the X-ray structure analysis^[12] the crystals contain hexasupersilyl-*triprismo*-hexastannane (**2a**) in addition to superdisilane^[8] and *tert*-butyl methyl ether. Thus again no tetrahedrane **3c** is formed, but instead along path (3) a polyhedral compound of tin with lower strain energy.^[3] The crystals can be stored at room temperature in the absence of



air, water, and solvents, and decompose in the presence of organic solvents above about –5 °C (formation of dark brown solutions). Remarkably, solutions of the crystals in toluene or THF at –25 °C only give a very weak ¹H and ²⁹Si NMR signal, respectively, and no ¹¹⁹Sn NMR signal, which is consistent with the very low solubility of the product at the measurement temperature.

Figure 1 shows the molecular structure of **2a** in the crystal. Compound **2a** comprises a near equilateral Sn₆ prismane framework with almost parallel Sn₃ faces that are only slightly twisted away from each other (see Figure 2; angle between the angle bisector of the upper and lower Sn₃ faces is 5.1°). Thus, both with respect to the Sn₆ structure as well as with respect to the 6 × 3(Sn) = 18 framework electrons (two electrons per bond), compound **2a** is classified as electron-precise; in contrast, the [Sn₆{Cr(CO)₃]₆]^{2–} ion, which has been known for some time, is classified as a *closo* compound^[13] both with respect to the Sn₆ structure (octahedron) as well as with respect to the 6 × 2(Sn) + 2(e[–]) = 14 = 2n + 2 framework electrons present (n = number of Sn atoms). The three Sn–Sn distances between the Sn₃ faces of the Sn₆ prismane are each 2.91 Å, and within the Sn₃ faces two of the Sn–Sn distances are also 2.91 Å and one is 2.94 Å; they are thus—for steric reasons—slightly longer than the sum of the radii of two Sn atoms (2.80 Å^[14]). Through the twisting of the Sn–Si bonds from the two Sn₃ units in opposite directions, the supersilyl groups achieve staggered (octahedral) packing (Figure 2). The Si–Sn distances are on average 2.71 Å and are thus longer than the sum r_{Sn} + r_{Si} = 2.57 Å,^[14] but similar to the Si–Sn distances in (*t*Bu₃Si)₂SnCl₂ (2.70 Å^[15]).

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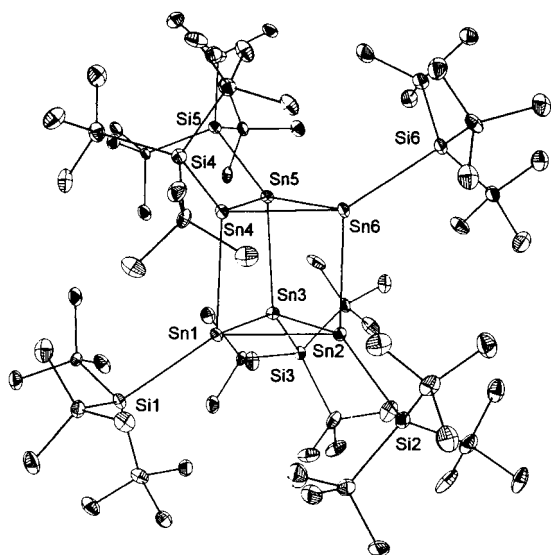


Figure 1. Side view of the molecular structure of $R_6^*Sn_6$ in the crystal (ORTEP plot; 25% thermal probability ellipsoids; for clarity hydrogen atoms are not shown) and atom numbering scheme. Selected bond lengths [Å] and angles [°]: Sn1–Sn2 2.910(1), Sn1–Sn3 2.939(1), Sn1–Sn4 2.907(2), Sn2–Sn3 2.907(1), Sn2–Sn5 2.907(1), Sn3–Sn6 2.903(1), Sn4–Sn5 2.907(1), Sn4–Sn6 2.916(1), Sn5–Sn6 2.941(1), Si1–Sn1 2.743(3), Si2–Sn2 2.687(3), Si3–Sn3 2.700(3), Si4–Sn4 2.683(3), Si5–Sn5 2.752(3), Si6–Sn6 2.724(3), Si–C 1.940 (av); Sn_3 ring angles at Sn1/Sn2/Sn3 and Sn4/Sn5/Sn6 59.59(3)/60.70(3)/59.71(3) and 60.67(3)/59.82(3)/59.51(3), respectively, Sn_4 ring angles at Sn1/Sn2/Sn5/Sn4, Sn2/Sn3/Sn6/Sn5, and Sn1/Sn3/Sn6/Sn4 89.39(5)/90.22(5)/89.44(5)/90.29(5), 92.42(5)/88.01(5)/91.80(5)/87.28(5), and 86.69(5)/92.64(5)/87.18(5)/93.05(5), respectively, Si1–Sn1–Sn2 114.74(6), Si1–Sn1–Sn3 145.25(6), Si1–Sn1–Sn4 128.05(6), analogous Si–Sn–Sn angles (see Figure 2) at Sn2 115.56(6)/146.29(6)/123.32(7), at Sn3 115.74(6)/147.11(7)/124.70(7), at Sn4 119.31(7)/144.81(6)/123.79(6), at Sn5 117.77(6)/146.21(7)/126.23(7), and at Sn6 117.74(6)/144.28(7)/127.91(6), C–Si–C 111.1 (av).



Figure 2. View of the structure of $R_6^*Sn_6$ in the crystal (SCHAKAL plot; atoms with arbitrary radii, for clarity *t*Bu groups are not shown).

Dark violet $(R^*Sn)_6$ is after orange-colored $(RSn)_{10}$ with a pentagonal Sn_{10} framework and ruby $(RSn)_8$ with a square Sn_8 prisms framework ($R = 2,6-Et_2C_6H_3$ in each case)^[5] a further molecular Sn_n prisms framework of tin. It is based for the first time on a trigonal Sn_6 prisms framework. Thus, whereas the 2,6-diethylphenyl substituent leads to the formation of less strained^[3] Sn_n prisms, the sterically more demanding supersilyl substituent results in the formation of the more strained Sn_6 prisms. Evidently the faces in the Sn_{10}

and in the Sn_8 prisms—unlike those in the Sn_6 prisms—do not offer sufficient space for one SiR_3 group per Sn atom.^[16] However, the SiR_3 substituents cover the faces of the Sn_4 tetrahedra of **3c**—unlike those of the Ge_4 or Si_4 tetrahedra **3b** and **3a**,^[6, 7] respectively—although not completely. Consequently, this polyhedral framework is not formed when tBu_3Si substituents are used owing to its particularly high strain:^[3] substituents more sterically demanding than supersilyl groups are necessary for the stabilization of a Sn_4 tetrahedra.

To date very little is known about the mechanism of the formation of $R_6^*Sn_6$ (or of $R_4^*Sn_3$ ^[9]) from $Sn[N(SiMe_3)_2]_2$ and R^*Na in $tBuOMe$ (or pentane). It proceeds possibly by an initial substitution of the amide groups by supersilyl groups with formation of the stannylene R_2^*Sn , the dimerization of which to give the distannene $R_2^*Sn=SnR_2^*$, however, does not take place for steric reasons. On the other hand, the composition of the reaction products obtained— $R_6^*Sn_6$ as well as $R_4^*Sn_3$ in addition to R^*R^* —indicate that the oligomerization of R_2^*Sn proceeds by elimination of supersilyl radicals R^* , which subsequently dimerize; the path of this oligomerization is determined by the nature of the reaction medium used.

Experimental Section

$Sn[N(SiMe_3)_2]_2$ ^[17] (0.710 g, 1.45 mmol) in pentane (5 mL) was added dropwise to a solution of tBu_3SiNa (0.660 g, 2.97 mmol) in pentane (10 mL) at $-78^\circ C$. The reaction mixture was maintained at $-78^\circ C$ for one day and then at $-90^\circ C$ for three days. Then the solution cooled to $-90^\circ C$ was decanted off from insoluble material ($Na[N(SiMe_3)_2]$). After the pentane had been condensed off at $-78^\circ C$, the remaining residue was dissolved in $tBuOMe$ at $-78^\circ C$ and the dark blue solution was allowed to warm to $-25^\circ C$. Dark violet crystals (0.107 g, 0.045 mmol; 18%) of the composition $(tBu_3Si)_6Sn_6(\mathbf{2a}) \cdot 0.4(tBu_3Si)_2 \cdot 2tBuOMe$ (confirmed by the X-ray structure analysis) crystallized from this solution in the course of two days. The crystals decomposed in the absence of solvent at about $80^\circ C$ and in the presence of a solvent at about $-5^\circ C$. In $[D_8]toluene$ cooled to $-25^\circ C$ the crystals display ^1H NMR signals at $\delta = 1.373$ (s; 18 *t*Bu from **2a**?), 1.383 (s; 6 *t*Bu from $(tBu_3Si)_2$), and 1.057/3.017 (s/s; *t*Bu/Me from $tBuOMe$). The determined ratio of areas of the *t*Bu signals is 3.56:1.00:5.79. In THF or $[D_8]toluene$ cooled to $-25^\circ C$ the crystals display ^{29}Si NMR signals at $\delta = 35.4$ (tBu_3SiSiR_3) and 61.0 (**2a**?). Since for the latter ^{29}Si NMR signal no $^{119/117}Sn$ satellites were observed owing to its low intensity, this cannot be assigned unambiguously to the prisms $(tBu_3Si)_6Sn_6$. At room temperature the above-mentioned ^1H and ^{29}Si NMR signals assigned to compound **2a** disappear. In THF or $[D_8]toluene$ cooled to $-20^\circ C$, compound **2a** displays an ESR signal without fine structure, which could not be assigned to date.

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- ($n = \text{constant}$) decreases in the order $(\text{SiH})_n$, $(\text{GeH})_n$, $(\text{SnH})_n$, $(\text{PbH})_n$; it is, however, about 250 kJ mol^{-1} even for $(\text{PbH})_8$.
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 - [12] Crystal structure analysis of $(t\text{Bu}_3\text{Si})_6\text{Sn}_6 \cdot 0.4(t\text{Bu}_3\text{Si})_2 \cdot 2t\text{BuOMe}$: Siemens P4 diffractometer, $\text{MoK}\alpha$ radiation, graphite monochromator, CCD area detector, crystal dimensions $0.03 \times 0.15 \times 0.14 \text{ mm}^3$. The crystal was mounted in perfluoroether oil, $T = 163(2) \text{ K}$, $\text{C}_{90.6}\text{H}_{204.6}\text{O}_2\text{-Si}_{6.8}\text{Sn}_6$, $M_r = 2229.54$, black-red prisms, triclinic, $a = 16.86(1)$, $b = 17.229(9)$, $c = 21.64(1) \text{ \AA}$, $\alpha = 89.17(1)$, $\beta = 67.15(2)$, $\gamma = 84.25(2)^\circ$, $V = 5764(6) \text{ \AA}^3$, $Z = 2$; space group $P\bar{1}$, $\rho_{\text{calc}} = 1.287 \text{ Mg m}^{-3}$, $\mu = 1.391 \text{ mm}^{-1}$, $F(000) = 2280$. Data collection: 2θ from 2.38 to 58.26° in $-20 \leq h \leq 20$, $-22 \leq k \leq 22$, $-24 \leq l \leq 26$, 32 656 measured reflections, 18 048 of which are independent and 11 502 observed ($F > 4\sigma(F)$), semiempirical absorption correction with SADABS (max/min transmission $1.000/0.928$), $R_1 = 0.0618$, $wR_2 = 0.1185$ ($F > 4\sigma(F)$), $\text{GOF}(F^2) = 1.042$; maximum residual electron density $1.287 \text{ e \AA}^{-3}$. The weighting factor is $w^{-1} = \sigma^2 F_o^2 + (P)^2 + P$ with $P = (F_o^2 + 2F_c^2)/3$. The structure was solved by direct methods (SHELXL; G. M. Sheldrick **1997**). All non-hydrogen atoms of the prismane were refined anisotropically. The hydrogen atoms were introduced in calculated positions and included in the refinement with a riding model and fixed isotropic U_i values. The unit cell contains in addition to the nondisordered molecules $(t\text{Bu}_3\text{Si})_6\text{Sn}_6$ strongly disordered molecules $(t\text{Bu}_3\text{Si})_2$ with an occupancy factor of 0.8 and four molecules of *tert*-butyl methyl ether. The disorder could only be approximately resolved. The *t*Bu groups of the disilane and the atoms of the ether molecules were refined with isotropic thermal parameters. 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-113897. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Syntheses and Ligating Properties of Molybdocene Alkoxides—The First Heterodimetallic Alkoxide Containing Molybdenum and Bismuth**

Marc Hunger, Christian Limberg,* and Peter Kircher

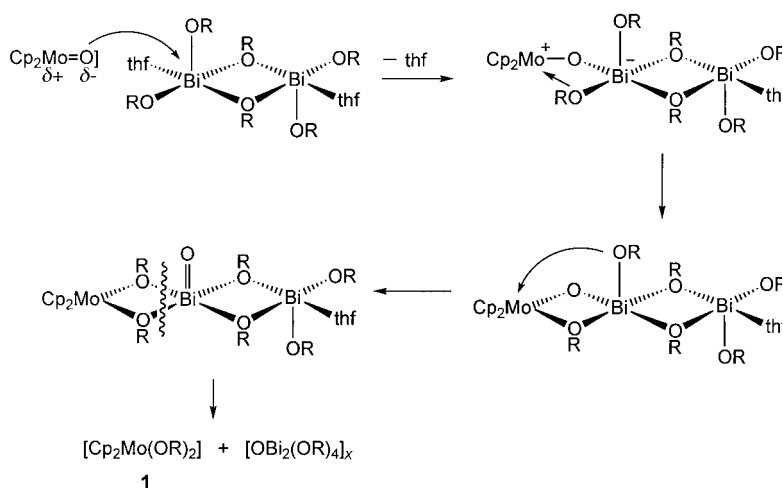
The unique property of $[\text{Bi}_2\text{MoO}_6]$ to act as a heterogeneous catalyst for the technical propene oxidation^[1a] remains a subject of intense discussion.^[1b] According to recent investigations, the oxygenation occurs at Bi- μ -O-Mo moieties,^[1c] which encourages research aimed at preparing molecular species containing both Mo and Bi in oxo environments. In this context bismuth–molybdenum heterometallic alkoxides are of particular interest, as studies on metal alkoxides have clearly shown their potential to simulate the properties and structures of metal oxides.^[2] There is, however, a dearth of information about even the homometallic alkoxides of bismuth.^[3] While several groups have claimed the existence of a bismuth/transition metal precursor in their sol–gel processes,^[4] only two heterometallic alkoxide complexes containing Bi and a transition metal ($\text{V}^{[3]}$ and $\text{Ti}^{[5]}$) have been isolated and structurally characterized. This is particularly surprising as an increasing number of advanced oxide materials based on bismuth appear to display attractive properties,^[5] and mixed-metal alkoxides could act as single-source precursors for such oxides. In an extension of our studies on the synthesis and formation of novel molybdenum alkoxides^[6] we present here the first mixed Bi/Mo alkoxide, which to our knowledge is also the first structurally characterized example of a molecular compound with a discrete oxo linkage of any type between Bi and Mo.

All initial attempts to prepare the aforementioned type of species by methods established for the synthesis of other heterometallic alkoxides^[7] such as Lewis acid–base reactions of certain alkoxides, or by applying the principles of the nonhydrolytic gel formation process,^[8] yielded—in agreement with observations described in the literature^[4]—insoluble, intractable solids. Since this is almost certainly a result of high aggregation levels, we decided to employ organomolybdenum compounds in which a part of the coordination sphere at the Mo center is protected by tightly bound organic ligands. For several reasons $[\text{Cp}_2\text{Mo}(\text{OR})_2]$ compounds seemed to be ideally suited for our purpose, but—despite the vast literature on species bearing the Cp_2Mo fragment^[9]—these alkoxides have not been described yet. This is probably due to the electron richness of the Cp_2Mo unit, which accordingly does not accept any strong π -donor ligands (note that the corresponding thiolates^[9] are readily made!). Consequently $[\text{Cp}_2\text{Mo}=\text{O}]$ was chosen as an alternative reagent to treat bismuth alkoxides, since its oxo functionality possesses

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nucleophilic character^[10] due to substantial negative charge accumulation and may therefore be capable of displacing weakly bound ligands at Bi centers. As already pointed out, the choice of employable bismuth alkoxides is narrow. $[\text{Bi}(\text{OCH}(\text{CF}_3)_2)_3(\text{thf})_2]$ is one of the few showing a relatively low degree of aggregation and possessing labile ligands in form of coordinated THF molecules (according to the solid-state structure).^[11] The reaction with $[\text{Cp}_2\text{Mo}=\text{O}]$ in THF yields after appropriate workup an insoluble amorphous material and a red-brown solid, which can be recrystallized from toluene. Spectroscopic and single-crystal X-ray analyses^[12] of the red-brown crystals (Figure 1) revealed that not a Bi-containing Mo alkoxide but $[\text{Cp}_2\text{Mo}\{\text{OCH}(\text{CF}_3)_2\}_2]$ **1** had been ob-



Scheme 1. Possible mechanism for the formation of **1**, $\text{R} = \text{CH}(\text{CF}_3)_2$.

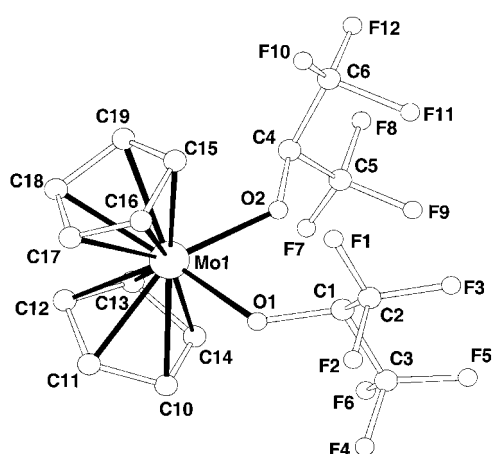


Figure 1. Molecular structure of **1**; all hydrogen atoms were omitted for clarity. Selected bond lengths [Å] and angles [°]: Mo1–O2 2.065(5), Mo1–O2 2.101(5), O1–C1 1.384(8), O2–C4 1.376(9), O2–Mo1–O1 78.84(18), C1–O1–Mo1 126.5(4), C4–O2–Mo1 129.4(5).

tained—the first representative of the elusive $[\text{Cp}_2\text{Mo}(\text{OR})_2]$ class of complexes.^[13] This is probably a consequence of the comparatively poor π -donor properties of the $\text{OCH}(\text{CF}_3)_2$ group (vide supra). As anticipated the Mo–O bonds are rather long (2.065(5) and 2.101(5) Å, cf. 1.880(1) Å in $[\text{Mo}_2(\text{OCMe}(\text{CF}_3)_2)_6]$ ^[14]), while the O–Mo–O angle (78.8(2)°) lies within the usual range found for Cp_2MoX_2 (X = singly bonded ligand) compounds.

How can the formation of **1** be rationalized? Under the assumption that $[\text{Cp}_2\text{Mo}=\text{O}]$ attacks one Bi center under expulsion of THF (Scheme 1), the resulting intermediate Mo–O–Bi unit would surely be stabilized by alkoxide bridging, which in turn could initiate its decomposition to yield **1** and an oxo-containing Bi compound. The latter is expected to aggregate and display a solubility as observed. The mechanism proposed in Scheme 1 implies that—in contrast to the corresponding thiolates $[\text{Cp}_2\text{Mo}(\text{SR})_2]$, which act as ligands for transition metal halides or organometallic fragments—**1** cannot serve as a kind of “tweezer” for Lewis acidic BiL_x fragments. This apprehension proved true in many experiments in which bismuth alkoxides and chlorides were allowed

to react with **1**: The donor capacity of the $(\text{CF}_3)_2\text{CHO}$ ligands in **1** is not sufficient to stabilize a second metal fragment between them. Recourse had to be made to “classical” alkoxides but apart from $[\text{Bi}(\text{OtBu})_3]_\infty$, which is too bulky to be employed, these are poorly characterized and display low solubilities.^[15] The in situ preparation of $[\text{Bi}(\text{OEt})_3]$ by ethanolysis of $[\text{Bi}(\text{OtBu})_3]_\infty$ was reported to proceed quantitatively^[16] but a reaction with $[\text{Cp}_2\text{Mo}=\text{O}]$ is not reasonable as the latter decomposes in the presence of EtOH, so that a different Cp_2Mo -containing starting material had to be employed. Reactions of metal chlorides with metal alkoxides have proved to be applicable for the synthesis of a large number of heterometallic alkoxides, which form after rapid ligand site exchanges as the thermodynamically most stable products, and consequently $[\text{Cp}_2\text{MoCl}_2]$ was treated with in situ prepared $[\text{Bi}(\text{OEt})_3]$ at 70 °C. After appropriate workup brown crystals were obtained, which were very sensitive to moisture. An X-ray crystal structure analysis^[12] (Figure 2)

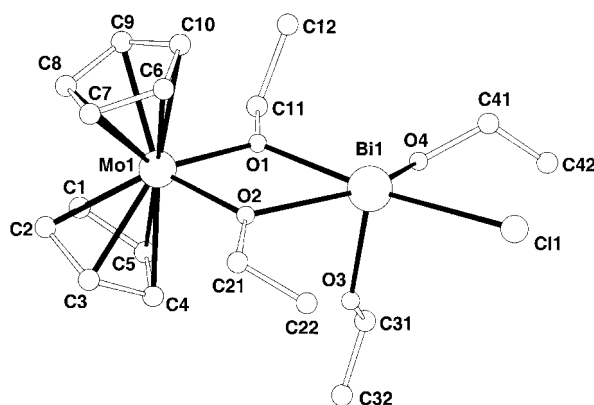


Figure 2. Molecular structure of **2**; all hydrogen atoms were omitted for clarity. Selected bond lengths [Å] and angles [°]: Bi1–O3 2.093(10), Bi1–O4 2.095(10), Bi1–O2 2.323(8), Bi1–O1 2.537(8), Bi1–C11 2.918(4), Mo1–O2 2.041(9), Mo1–O1 2.116(8), O3–Bi1–O4 88.9(5), O4–Bi1–O1 85.1(4), O1–Bi1–O2 61.6(3), O2–Bi1–C11 119.3(2), O1–Mo1–O2 73.7(3).

identified the corresponding compound as $[\text{Cp}_2\text{Mo}(\mu\text{-OEt})_2\text{-Bi}(\text{OEt})_2\text{Cl}]$ (**2**), where indeed a Mo and a Bi center are bridged by two ethanolate ligands. The coordination sphere of

the Bi center, which is completed by two terminal ethanolate ligands and a chloro ligand, can be described as square pyramidal with the stereoactive lone pair of electrons occupying the ligand site missing for an octahedron. The terminal Bi–OEt distances (2.09(1) and 2.09(1) Å) are quite normal for such ligands in bismuth alkoxides. Due to the different *trans* ligands of each μ -OEt group, the Bi– μ -OEt bond lengths differ quite significantly (2.537(8) (O2) and 2.323(8) Å (O1)) but are comparable with the Bi–O_μ distances in the two other known heterometallic bismuth alkoxides,^[3,5] while the Bi–Cl bond of 2.918(4) Å is rather long (in comparison with the average value of 2.532 Å in a Cl₃Bi(μ -OR)₃ moiety^[3]). The Mo–O distances (2.041(9) (O2) and 2.116(8) Å (O1)) can be described as normal for Mo(μ -OEt)₂ moieties,^[6a] and the fact that the distances of the terminal ligands in **1** are comparable yet again stresses that the Cp₂Mo centers reject the donor capacity the terminal alkoxide ligands have on offer.

Apparently, in agreement with above arguments the electron-donating character of the ethyl group allows μ -OR bridging between the Cp₂Mo fragment and the Bi center, while synergetically the electron-withdrawal by the Bi center ensures that the π -electron density at the Cp₂Mo fragment is not too high (this role is played by the F atoms in **1**). According to the solid-state structure the diamagnetic compound **2** should show signal sets for four different CH₃CH₂ groups in the ¹H NMR spectrum. Only two sets are found for solutions of the compound in CD₂Cl₂, but lowering of the temperature leads to a broadening of all signals,^[17] which further split at about –40 °C to give the complex spectrum expected for **2**. Consequently, at room temperature the compound shows dynamic behavior in solution involving site exchanges of the terminal ligands at Bi, which make the two bridging and the two terminal EtO ligands equivalent.

The formation of **2** can be assumed to proceed by μ -Cl bridging of [Cp₂MoCl₂] to in situ generated, coordinatively unsaturated [Bi(OEt)₃] molecules, which initiates ligand exchange reactions. The bridging positions are finally occupied by EtO ligands, which possess the more favorable properties for this purpose.^[6a] However, only one of the two original Cl ligands binds at the Bi center in **2**, the second one is replaced by external EtO[–].

In conclusion, the results presented suggest that alkoxides of Cp₂Mo can only be isolated if the π -donor strength of the alkoxide ligands is decreased by electron-withdrawing groups or by their complexation to a Lewis acidic center. This principle was used to prepare the first Bi/Mo alkoxide, which represents the first step towards molecular Bi/Mo alkoxides or even oxides suitable to serve as structural or functional models for fragments of the [Bi₂MoO₆] surface. Moreover, the first representative of the [Cp₂Mo(OR)₂] class of compounds was isolated by employing a fluorinated alkoxide ligand. In view of the antitumor activity of [Cp₂MoCl₂], the origin of which is under current investigation,^[18] compounds like **1** may be suitable alternatives.

Experimental Section

1: A solution of [Bi(OCH(CF₃)₂)₃(thf)]^[11] (0.64 g (0.4 mmol) in THF (20 mL) was added dropwise to a solution of [Cp₂MoO]^[10] (0.20 g,

0.82 mmol) in THF (40 mL). After the mixture had been stirred for 1 h, all volatiles were removed from the resulting brown solution and the solid brown residue was extracted with toluene. The filtrate was reduced to about 20 mL and stored at –40 °C to afford analytically pure **1** (0.32 g, 0.58 mmol; 70 % yield). ¹H NMR ([D₈]THF): δ = 5.69 (s, 10H, H_{Cp}), 4.54 (sept, 1H, ³J(H,F) = 6.83 Hz, CH); ¹³C NMR: δ = 124.1 (q, 2 C, ¹J(C,F) = 287.7 Hz, CF₃), 103.0 (s, 10C, Cp), 73.6 (sept, ³J(C,F) = 28.5 Hz, CH); ¹⁹F NMR: δ = 75.56 (d, 6 F, CF₃); MS (EI): *m/z* (%): 563 (100) [(1+H)⁺], 498 (8) [(1+H–Cp)⁺], 396 (51) [(1+H–OCH(CF₃)₂)⁺], 366 (50) [(Cp₂Mo(CF₃)₂)⁺], 228 (44) [Cp₂Mo⁺], 198 (38) [(MoOCH(CF₃)₂)⁺].

2: [Cp₂MoCl₂]^[10] (0.74 g, 2.5 mmol) was suspended in EtOH (30 mL) and the mixture was added to [Bi(OrBu)₃]_∞ (2.15 g, 5.0 mmol) dissolved in EtOH (30 mL). All [Cp₂MoCl₂] was taken up into the brown solution after stirring for 2 h, and the mixture was subsequently heated for 40 min to 70 °C. All volatiles were removed and the residue was extracted with THF (15 mL). Cooling of the solution to –80 °C afforded analytically pure **2** (0.60 g, 0.9 mmol; 37 % yield). ¹H NMR (CD₂Cl₂): see ref. [17]; ¹³C NMR (CD₂Cl₂): δ = 101.6 (Cp), 73.8 (t-OCH₂), 59.9 (μ -OCH₂), 26.0 (t-OCH₂CH₃), 19.9 (μ -OCH₂CH₃).

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with $I > 2\sigma(I)$, Lp correction ($\mu = 7.120 \text{ mm}^{-1}$), 247 refined parameters with $R = 0.074$, residual electron density (max/min): $2.747/-1.984 \text{ e } \text{\AA}^{-3}$ (caused by the data collection at room temperature and the stereoactive lone pair of electrons at Bi); $\text{MoK}\alpha$ radiation, $\lambda = 0.71073 \text{ \AA}$, was used in both cases and the structures were solved by direct methods (program: SHELXS-97), refined versus F^2 (program: SHELXL-97) with anisotropic temperature factors for all non-hydrogen atoms. 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-112043 and CCDC-112044. 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 Spreader-Bar Approach to Molecular Architecture: Formation of Stable Artificial Chemoreceptors**

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A struggle between chaos and order is especially hardened in the nano-world, where local concentration gradients are very high and diffusion processes extremely fast. Moreover, as is typical for many systems ordered on the nanometer scale, even small structural changes can lead to a total loss of function of the whole system. Major progress in these systems led to the concept of molecular architecture and chemical nanotechnology.^[1, 2] Introduction of self-assembly, which allowed the formation of molecularly organized multilayer systems with relatively slow diffusion between different molecular layers. We describe herein a spreader-bar approach that allows for the first time noncross-linked monolayers to be obtained, whose structure can not be distorted by lateral diffusion.

The most effective technique to form artificial receptors for small molecules is based on molecular imprinting,^[3–8] which

comprises the formation of a three-dimensional porous polymer whose cavities match the molecular structure of the analyte of interest. Only a few attempts to apply this principle to form two-dimensional chemical receptors have been reported (Figure 1a)^[9–11], but lateral diffusion of molecules in the noncross-linked monolayers distorts the receptor structure (Figure 1b). We describe herein a novel approach for the preparation of stable artificial interfaces with pre-defined affinity in which the stability against lateral diffusion was achieved by means of a molecular spreader-bar (Figure 1c).

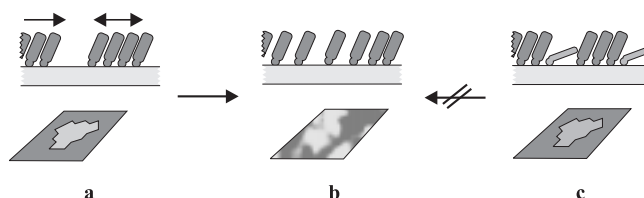


Figure 1. Schematic representation of the possibilities of building artificial receptors on a surface as viewed from above (below) and in cross section (above). By two-dimensional molecular imprinting the binding sites (a) can be distorted by lateral diffusion (b). The binding sites can be stabilized—as shown here for the first time—by using a template (c) in the monolayer.

An artificial interface with a high affinity for barbituric acid (a starting material for many pharmaceuticals) was created by co-adsorption of thiobarbituric acid **1** (the template) and dodecanethiol **2** (the matrix) onto a gold substrate (Figure 2). This process leads to the formation of binding sites with a structure complementary to that of thiobarbituric acid. Binding of barbituric acid **3** and of other species to the respective surface was detected by capacitance measurements: an increase in the dielectric thickness decreases the electrode capacitance.^[12] A high selectivity of this artificial chemoreceptor for barbituric acid was observed (Figure 3): while there is a considerable response to the addition of barbituric acid, the addition of diethylbarbituric acid caused no effect. Moreover, the effects that arise from additions of barbituric acid in the presence of a high concentration of diethylbarbituric acid were exactly the same as in its absence. The capacitance response to pyridine was also much lower than to barbituric acid. The response of such a receptor is shown in Figure 4.

To study the structure of the binding sites the affinity properties of several other types of interface were compared (Table 1). No binding of barbituric acid to the surface formed by a pure monolayer of dodecanethiol or by a pure monolayer of thiobarbituric acid was observed.^[13] Therefore, the barbituric acid binding sites on the interface formed by the co-adsorption of dodecanethiol and thiobarbituric acid must include both types of molecules and one can exclude binding mechanisms based on the formation of hydrogen bonds or other interactions between barbituric acid and thiobarbituric acid only. Also, no binding of barbituric acid on gold electrodes covered by a mixed monolayer of thiobarbituric acid and short-chain alkylthiols (butanethiol and 2-methyl-2-propanethiol) was observed. Therefore, for a strong specific interaction with the template, the matrix is required to form a sufficiently thick monolayer.

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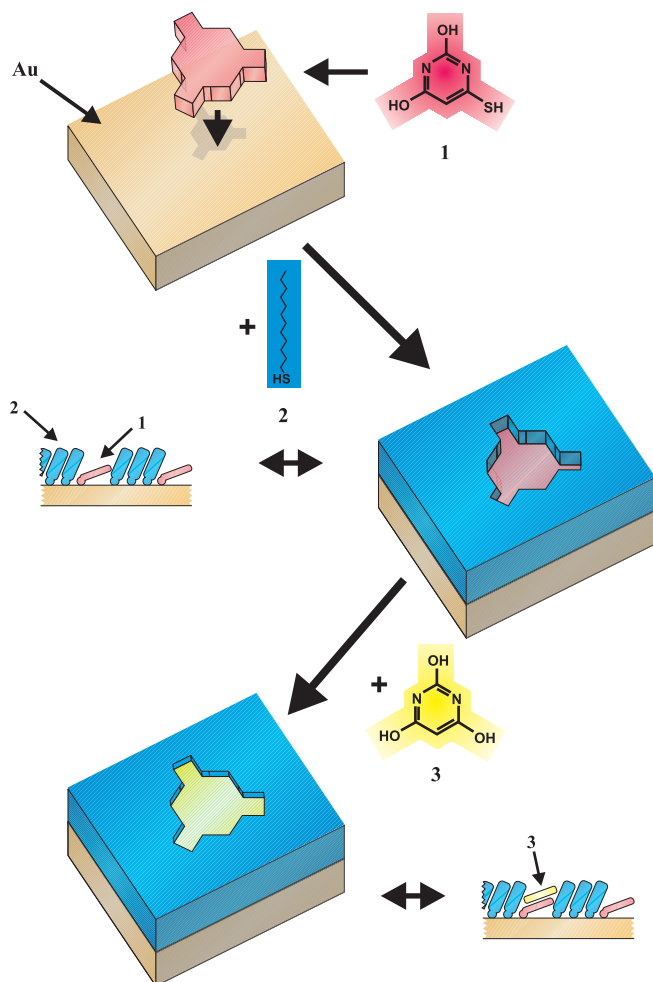


Figure 2. Preparation and operation of artificial chemoreceptors. The binding sites are formed by co-adsorption of the template and matrix molecules. The template should have a shape similar to that of the analyte, and both the template and the matrix molecules must be able to bind strongly to the surface. As an example, co-adsorption of thiobarbituric acid **1** (template) and dodecanethiol **2** (matrix) on the gold surface results in the formation of binding sites for barbituric acid **3**.

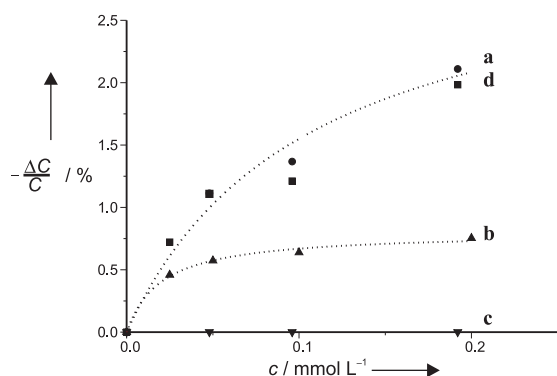


Figure 3. Detection of binding by measuring the capacitance changes of the artificial receptors: a: barbituric acid, b: pyridine, c: diethylbarbituric acid, d: barbituric acid in the presence of 1 mmol L⁻¹ diethylbarbituric acid. Template: thiobarbituric acid, matrix: dodecanethiol. Electrolyte: 5 mmol L⁻¹ phosphate buffer, 100 mmol L⁻¹ KCl, pH 5.5.

The electrodes covered by the dodecanethiol/thiobarbituric acid mixture have a specific capacitance of $4.4 \pm 0.4 \mu\text{F cm}^{-2}$. From comparison with specific capacitances of the electrodes

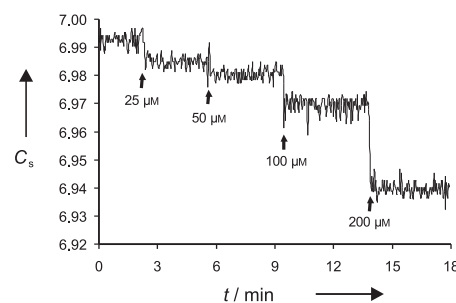


Figure 4. Response time of the artificial receptors as measured by the changes in capacitance C_s [$\mu\text{F cm}^{-2}$] on binding barbituric acid to an electrolyte coated with thiobarbituric acid and hexanethiol.

covered by matrix or template molecules only and assuming independent contributions of both species to the capacitance of the electrode covered by mixed monolayers, one can estimate that about 48% of the electrode area is covered by thiobarbituric acids. A modification of the alkanethiol/thiobarbituric acid ratio at the interface, achieved by varying this ratio in the solution used for electrode coating or by varying the adsorption time, resulted in a decrease or even total loss of the sensor response to barbituric acid.

The requirement that both types of molecules should be present at the interface at a definite ratio implies that the binding sites have a definite structure. This structure can be assumed to consist of cavities in the monolayer of the matrix with a cross-section exactly defined by the shape of the template molecules. These binding sites cannot be distorted by the lateral diffusion of molecules in the monolayer: like a boat in the ocean, they can conserve their definite shape anywhere at the interface and are independent of any movements of the surrounding molecules.

A comparative study of the temporal stability of the binding sites to barbituric acid that were formed by the two-dimensional molecular imprinting^[9] and by the novel spreader-bar technique was performed. The two-dimensional molecular imprinting technique also resulted in a formation of selective binding sites, however, a total loss of these receptor properties was observed already after the first adsorption/desorption cycle. On the contrary, the binding sites formed by the molecular spreader-bar technique display receptor properties during many adsorption/desorption cycles with only a slight loss of the affinity for every next cycle.

Table 1. Formation of artificial binding sites.^[13]

matrix	Monolayer composition template	Capacitance increase after addition of barbituric acid	5,5-diethyl- barbituric acid	pyridine
1-dodecanthiol	—	none	—	none
—	2-thiobarbituric acid	none	none	none
1-dodecanthiol	2-thiobarbituric acid	high	none	low
1-hexanethiol	2-thiobarbituric acid	high	none	low
1-butanethiol	2-thiobarbituric acid	none	none	—
2-methyl-2-propanthiol	2-thiobarbituric acid	none	none	—

Two mechanisms for the selective capacitive response can be considered: a) different binding constants for different species and b) a sievelike effect, namely, there are steric

limitations for large molecules. In this case, the molecules smaller than the cavities provide lower capacitive effects than the molecules whose shape allows them to fill the cavities.

The new spreader-bar technique provides a simple way of forming stable nanostructures without chemical polymerization. One can suggest possible applications of this principle in the preparation of artificial receptors (for chemosensors, active phases for chromatography, stereoselective catalysis, and membrane filtration), molecular electronic devices, or other nanostructures.

Experimental Section

The interface with artificial binding sites for barbituric acid was prepared on the gold surface by firstly cleaning it with a hot solution of concentrated H_2SO_4 (3 mL) and 30% H_2O_2 (1 mL), then rinsing with water, and drying.

Caution: this solution reacts violently with most organic materials and must be handled with extreme care. For the preparation of the artificial receptors by the spreader-bar technique, the electrodes were placed into a solution of dodecanethiol ($10 \mu\text{mol L}^{-1}$) and thiobarbituric acid (10 mmol L^{-1}) in methanol/water (1/9) for 70 h at 22°C , then rinsed with chloroform, and dried under nitrogen. The electrodes were further investigated during the following few days; no systematic effect on the surface properties was observed with storage. A coating of the electrodes by other species were performed using the same conditions and with the same concentrations of template and matrix compounds. A mixture of dodecanethiol ($10 \mu\text{mol L}^{-1}$) and barbituric acid (14 mmol L^{-1}) in the same solvent and coating conditions was used for the preparation of the artificial receptors according to two-dimensional molecular imprinting.

All adsorption measurements were performed at room temperature in the electrolyte consisting of phosphate buffer (5 mmol L^{-1}) and KCl (100 mmol L^{-1}) at pH 5.5. The capacitive method used to study adsorption, as well as essential experimental details, are described in reference [12].

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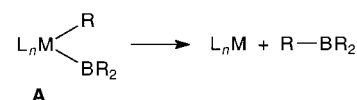
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Synthesis and Structures of *cis*- and *trans*-[Os(Bcat)(aryl)(CO)₂(PPh₃)₂]: Compounds of Relevance to the Metal-Catalyzed Hydroboration Reaction and the Metal-Mediated Borylation of Arenes**

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Alex Williamson, and L. James Wright*

Dedicated to Professor Helmut Werner
on the occasion of his 65th birthday

In some discussions of the mechanism of the metal-catalyzed hydroboration of unsaturated hydrocarbons^[1, 2] the final step is the reductive elimination of an organoborane from a postulated intermediate **A**, which has both a boryl ligand and a σ -bound carbon ligand (Scheme 1). Theoretical



Scheme 1. Reductive elimination of RBR_2 from postulated alkyl, boryl intermediate **A**.

studies of this reaction support the intermediacy of such a metal complex.^[3] In related studies arenes and alkenes have been borylated with the metal complexes $[\text{CpFe}(\text{Bcat})(\text{CO})_2]$, $[\text{Mn}(\text{Bcat})(\text{CO})_5]$, and $[\text{Re}(\text{Bcat})(\text{CO})_5]$ (H_2cat = catechol, $1,2\text{-(HO)}_2\text{C}_6\text{H}_4$) under photolytic conditions.^[4] Even alkanes can be functionalized in a similar way with $[(\text{C}_5\text{Me}_5)\text{M}(\text{Bcat}')(\text{CO})_n]$ ($\text{M} = \text{Fe}, \text{Ru}, n = 2$; $\text{M} = \text{W}, n = 3$; $\text{H}_2\text{cat}' = 1,2\text{-(HO)}_2\text{C}_6\text{H}_2\text{-3,5-Me}_2$).^[5] Although the mechanism of these borylation reactions is not known with certainty, an intermediate of type **A** has been suggested as a reasonable possibility.^[5] Despite the obvious significance of characterizing stable examples of metal boryl complexes that also contain a σ -bound carbon ligand, only two rather special examples of such complexes have been described more recently. The first of these is an iridium(IV) complex in which both the boryl and aryl donor atoms are part of a complex chelating ligand system^[6] and the second is $[\text{Ir}(\text{Bcat})(\text{C}\{\text{CO}_2\text{CH}_3\}=\text{CH}\{\text{CO}_2\text{CH}_3\})\text{Cl}(\text{PMe}_3)_3]$ in which the vinyl function arises from insertion of dimethyl acetylenedicarboxylate into an iridium–hydrogen bond.^[7]

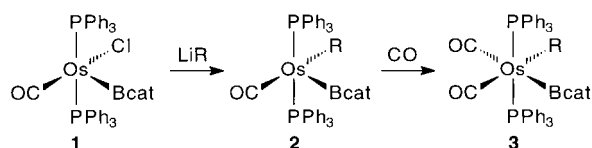
Herein we describe 1) the first stable examples of both coordinatively unsaturated and saturated metal complexes containing boryl and simple σ -bound aryl ligands, 2) structure determinations of both *cis* and *trans* isomers of the coordinatively saturated octahedral examples with implications for the bonding characteristics of the Bcat ligand, and 3) the facile

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reductive elimination of aryl borane from the *cis* isomer with subsequent oxidative addition of HBcat.

Reaction of the coordinatively unsaturated complex **1**^[8] with *o*-tolyllithium in benzene at 5 °C gave the coordinatively unsaturated *o*-tolyl complex **2** as an air-stable solid in high yield (Scheme 2). Remarkably the boryl ligand was not



Scheme 2. Synthesis of *o*-tolyl, boryl complexes **2** and **3**. R = *o*-tolyl.

attacked in this reaction. Attempts to synthesize the phenyl and *p*-tolyl analogues were not successful, and the steric protection afforded by the bulkier *o*-tolyl ligand could be important in stabilizing the coordinatively unsaturated **2**. No tractable products from the reaction of the ruthenium complex [Ru(Bcat)Cl(CO)(PPh₃)₂]^[8] with *o*-tolyllithium could be isolated.

The ¹H and ¹³C NMR spectra of **2**, both at room temperature and at –40 °C, show two sets of signals for the *o*-tolyl, the Bcat, and the triphenylphosphane ligands in a ratio of approximately 7:2. We interpret this as indicating the presence of two isomers in solution arising from hindered rotation about the osmium–*o*-tolyl bond. Complex **2** reacted rapidly with carbon monoxide to give the corresponding six-coordinate complex **3** in high yield. Solutions of **3** were unstable at room temperature (see following discussion), but a crystal structure determination was carried out on a single crystal grown from toluene/*n*-hexane at –20 °C.^[9] The structure (Figure 1) reveals a distorted octahedral arrangement

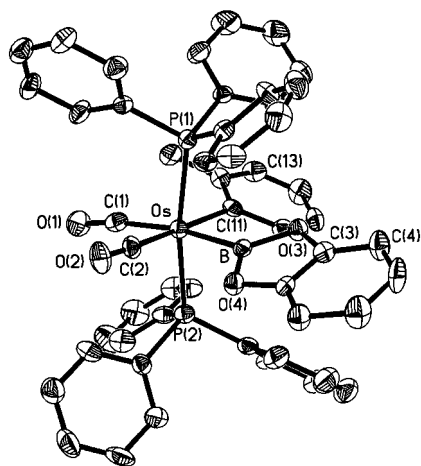
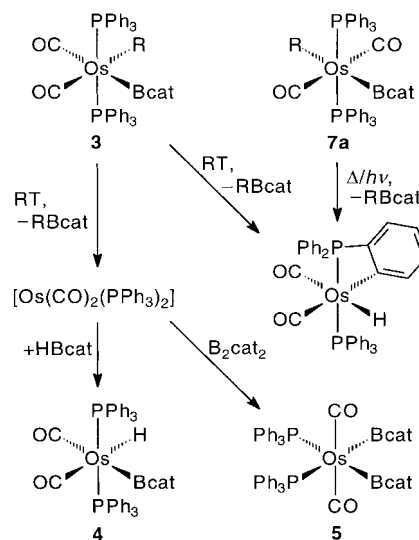


Figure 1. Molecular structure of **3** (ORTEP plot) in the crystal. Selected bond lengths [Å] and angles [°]: Os–B 2.154(7), Os–C(1) 1.964(7), Os–C(2) 1.903(6), Os–C(11) 2.202(6), Os–P(1) 2.4060(17), Os–P(2) 2.4050(17); C(1)–Os–B 169.6(3), P(1)–Os–P(2) 171.50(6), C(1)–Os–C(2) 88.1(2), C(1)–Os–C(11) 95.1(2), C(2)–Os–B 82.9(2), C(11)–Os–B 94.0(2), C(2)–Os–C(11) 176.5(2).

about osmium in which the *o*-tolyl and boryl ligands are mutually *cis* and the phosphane ligands mutually *trans*. The very strong *trans* influence of the boryl ligand is illustrated by the fact that the Os–CO distance *trans* to the boryl ligand

(1.964(7) Å) is greater than the corresponding distance for the carbonyl ligand *trans* to the *o*-tolyl ligand (1.904(6) Å). Since *o*-tolyl is recognized as a strong σ -bonding ligand, the observed greater *trans* influence for the Bcat ligand indicates that in this molecule Bcat is not only a good σ -bonding ligand but also a good π acceptor. A consequence of this bonding situation is that a carbonyl ligand *trans* to Bcat can be activated towards nucleophilic attack (see later discussion). Both the boryl ligand and the *o*-tolyl ligand are tilted out of the plane of best fit through the Os, C(1), C(2), C(11), and B atoms by 22.87(13) and 24.96(10)°, respectively. The Os–B distance is 2.155(7) Å, which is longer than those reported for other six-coordinate osmium–Bcat complexes.^[8b] The Os–C(11) distance of 2.202(6) Å is at the long end of the range of Os–aryl bond lengths.

Complex **3** eliminated *o*-tolylBcat slowly at room temperature in benzene to give [Os(CO)₂(PPh₃)₂], which could not be isolated, and in the absence of a further reactant orthometalation occurred to give [Os(C₆H₄PPh₂)H(CO)₂(PPh₃)] (Scheme 3).^[10] Complex **3** is therefore a convenient

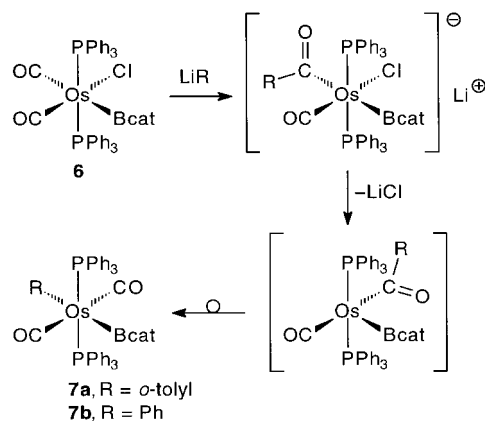


Scheme 3. Reductive elimination of *o*-tolylBcat from *o*-tolyl, boryl complexes. R = *o*-tolyl.

precursor for [Os(CO)₂(PPh₃)₂], which can react with suitable compounds in situ before orthometalation can occur. Examples are the oxidative addition with HBcat to give **4**,^[11] and with B₂cat₂ to give the bis(Bcat) complex **5**.

These two reactions, reductive elimination of arylborane and the oxidative addition of HBcat, model two consecutive steps in the catalytic cycle often proposed for transition metal catalyzed hydroboration.^[2] The elimination of *o*-tolylBcat from **3** in CDCl₃ at 24 °C was monitored by ¹H NMR spectroscopy. After approximately 100 min half of complex **3** had undergone elimination, and this was accompanied by formation of [OsCl₂(CO)₂(PPh₃)₂] from the reaction of [Os(CO)₂(PPh₃)₂] and the solvent. The reductive elimination of *o*-tolylBcat from **3** was suppressed at temperatures at or below –20 °C.

A geometrical isomer of complex **3** with *o*-tolyl and Bcat ligands located mutually *trans* can be prepared as shown in Scheme 4. Carbonylation of complex **1** gave the *cis* complex



Scheme 4. Postulated mechanism for formation of the *trans* isomer **7**.

6.^[11] Reaction of **6** with *o*-tolyllithium yielded **7a**. We propose that the reaction proceeds initially by nucleophilic attack of *o*-tolyl anion at the activated carbonyl ligand *trans* to Bcat. Subsequent loss of the chloro ligand forms a neutral complex in which the acyl ligand may adopt either an η^1 or η^2 bonding mode. An alternative possibility is that the acyl oxygen atom may interact weakly with the boron atom, thereby controlling the geometry of the intermediate and so influencing the specificity of the observed product. Reverse migratory insertion occurs such that the *o*-tolyl group assumes the position *trans* to the Bcat ligand giving the observed isomer **7a** (Scheme 4). Complex **6** reacted in the same way with PhLi to give **7b**.

The X-ray crystal structure of **7a** is shown in Figure 2. The geometry about osmium is a distorted octahedron in which the two carbonyl ligands are mutually *trans* and the Bcat

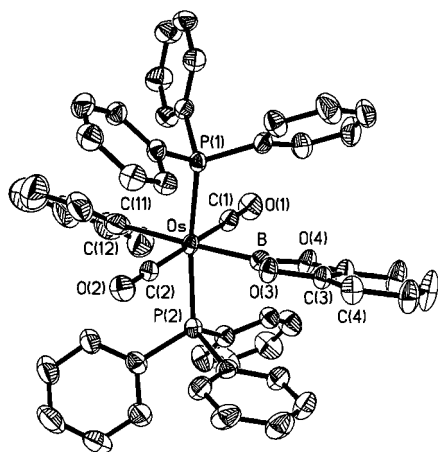


Figure 2. Molecular structure of **7a** (ORTEF plot) in the crystal. Selected bond lengths [Å] and angles [°]: Os–B 2.132(7), Os–P(1) 2.3791(17), Os–P(2) 2.3874(17), Os–C(1) 1.952(7), Os–C(2) 1.930(7), Os–C(11) 2.277(5); C(1)–Os–B 88.5(3), P(1)–Os–P(2) 169.76(5), C(1)–Os–C(2) 178.6(3), C(1)–Os–C(11) 94.2(3), C(2)–Os–B 91.8(3), C(11)–Os–B 175.5(3), C(2)–Os–C(11) 85.6(3).

ligand is *trans* to the *o*-tolyl ligand. Both the Bcat and the *o*-tolyl ligands are tilted out of the plane of best fit through the Os, C(1), C(2), C(11), and B atoms by 16.72(41) and 36.82(40)°, respectively. The Os–B distance of 2.132(7) Å is

close to that of complex **3**. However, the Os–C(11) distance of 2.277(5) Å is significantly greater than that of complex **3** (2.202(6) Å), illustrating again the very strong *trans* influence of the Bcat ligand relative to the carbonyl ligand.

Complex **7a** is considerably more robust with respect to reductive elimination of *o*-tolylBcat than complex **3**. Unchanged **7a** was recovered after heating under reflux in benzene for 2 h. However, formation of [Os(C₆H₄PPh₂)H(CO)₂(PPh₃)] occurred when the boiling solution was irradiated with a 1000-W tungsten–halogen lamp (see Scheme 3). Presumably, under these conditions the molecule rearranges to form the *cis* isomer **3**, which immediately reductively eliminates *o*-tolylBcat and undergoes orthometallation. These observations indicate that the requirement for facile reductive elimination is that the aryl and Bcat ligands be adjacent to one another. Complex **3** is therefore a useful model for a key intermediate proposed in both metal-catalyzed hydroboration and aryl borylation.

Experimental Section

2: To a rapidly stirred suspension of **1** (166 mg, 0.185 mmol) in benzene at 5 °C was slowly added a solution of *o*-tolyllithium in diethyl ether (0.75 M, 0.32 mL, 0.24 mmol). The mixture was allowed to warm to room temperature and was then stirred for 30 min. During this time the starting material dissolved to form a cloudy orange solution. The benzene was then reduced to a volume of 1 mL in vacuo, and *n*-hexane was added to precipitate **2** as a yellow solid. This was collected and washed with ethanol and *n*-hexane. Yield 162 mg (92 %); ¹H NMR (400 MHz, CDCl₃, –40 °C, TMS): major isomer: δ = 0.31 (s, 3H, C₆H₄CH₃), 5.23 (d, *J* = 7.2 Hz, 1H, C₆H₄CH₃), 6.10 (t, *J* = 7.1 Hz, 1H, C₆H₄CH₃), 6.45 (d, *J* = 7.3 Hz, 1H, C₆H₄CH₃), 6.58 (t, *J* = 7.1 Hz, 1H, C₆H₄CH₃), 6.88 (m, 4H, Bcat), 7.25–7.41 (m, 30H, PPh₃); minor isomer: δ = 0.15 (s, 3H, C₆H₄CH₃), 5.72 (d, *J* = 7.1 Hz, 1H, C₆H₄CH₃), 6.53 (t, *J* = 7.1 Hz, 1H, C₆H₄CH₃), 6.66 (t, *J* = 7.0 Hz, 1H, C₆H₄CH₃), 6.79 (m, 2H, Bcat), 6.97 (m, 3H, Bcat with C₆H₄CH₃), 7.16–7.23 (m, PPh₃); ¹³C{¹H} NMR (100 MHz, CDCl₃, –40 °C, TMS): major isomer: δ = 24.65 (s, C₆H₄CH₃), 110.17 (Bcat), 118.99 (s, C₆H₄CH₃), 120.28 (s, Bcat), 121.80 (s, C₆H₄CH₃), 127.54 (t', ¹²J(C,P) = 9 Hz, *o*-PPh₃), 129.59 (s, *p*-PPh₃), 131.13 (s, C₆H₄CH₃), 132.06 (s, C₆H₄CH₃), 132.66 (t', ¹³J(C,P) = 49 Hz, *i*-PPh₃), 134.43 (t', ³⁵J(CP) = 10 Hz, *m*-PPh₃), 148.89 (s, C₆H₄CH₃), 150.20 (s, Bcat), 180.03 (t, ²J(C,P) = 13 Hz, *i*-C₆H₄CH₃), 196.66 (t, ²J(C,P) = 8 Hz, CO); minor isomer: δ = 18.07 (s, C₆H₄CH₃), 119.91 (s, Bcat), 121.11 (s, C₆H₄CH₃), 127.42 (*o*-PPh₃), 128.81 (s, C₆H₄CH₃), 129.19 (s, *p*-PPh₃), 133.68 (t', ¹³J(C,P) = 50 Hz, *i*-PPh₃), 133.91 (t', ³⁵J(C,P) = 10 Hz, *m*-PPh₃), 140.56 (s, C₆H₄CH₃), 149.88 (s, Bcat), 171.07 (t, ²J(C,P) = 12 Hz, *i*-C₆H₄CH₃), other signals not observed; IR (Nujol): $\tilde{\nu}$ = 1903 cm^{–1} (vs, CO); elemental analysis calcd for C₅₀H₄₁BO₃OsP₂ (%): C 63.03, H 4.34; found: C 62.88, H 4.54.

3: Complex **2** (150 mg, 0.157 mmol) was dissolved in benzene (10 mL) to give a yellow solution through which a stream of CO gas was passed for 5 s, turning the solution colorless. The volume of the benzene was reduced to about 1 mL in vacuo, and *n*-hexane was added to precipitate **3** as a white solid, which was collected and washed with ethanol and *n*-hexane. Yield 137 mg (89 %); ¹H NMR: δ = 1.96 (s, 3H, C₆H₄CH₃), 6.17 (t, *J* = 7.2 Hz, 1H, C₆H₄CH₃), 6.46 (d, *J* = 7.4 Hz, 1H, C₆H₄CH₃), 6.63 (t, *J* = 7.1 Hz, 1H, C₆H₄CH₃), 6.79 (m, 4H, Bcat), 7.05–7.23 (m, 31H, PPh₃ and C₆H₄CH₃); ¹³C{¹H} NMR: δ = 33.13 (s, C₆H₄CH₃), 110.74 (s, Bcat), 119.87 (s, Bcat), 122.35 (s, C₆H₄CH₃), 122.60 (s, C₆H₄CH₃), 127.22 (t', ²⁴J(C,P) = 9 Hz, *o*-PPh₃), 127.35 (s, C₆H₄CH₃), 129.41 (s, *p*-PPh₃), 133.47 (t', ¹³J(C,P) = 54 Hz, *i*-PPh₃), 133.69 (t', ³⁵J(C,P) = 10 Hz, *m*-PPh₃), 145.37 (t, ²J(C,P) = 11 Hz, *i*-C₆H₄CH₃), 147.84 (s, C₆H₄CH₃), 148.98 (s, C₆H₄CH₃), 149.64 (s, Bcat), 183.37 (t, ²J(C,P) = 7 Hz, CO), 186.09 (t, ²J(C,P) = 7 Hz, CO); IR (Nujol): $\tilde{\nu}$ 1997 (vs), 1937 cm^{–1} (vs, CO); elemental analysis calcd for C₅₁H₄₁BO₄OsP₂ (%): C 62.45, H 4.21; found: C 62.15, H 4.23.

7a: A colorless solution of **6** (0.415 g, 0.449 mmol) in benzene (25 mL) was cooled to 5 °C. A solution of *o*-tolyllithium in diethyl ether (0.75 M, 0.78 mL, 0.58 mmol) was added slowly to the rapidly stirred solution, and the resulting mixture was pale yellow and cloudy. The mixture was allowed to

warm to room temperature and then stirred for 30 min. The volatiles were removed in vacuo and the resultant solid dissolved in dichloromethane (15 mL). Ethanol (7 mL) was then added and the dichloromethane removed in vacuo to precipitate a white solid, which was collected and washed with ethanol and *n*-hexane. The solid was recrystallized from dichloromethane/ethanol to give pure **7a**. Yield 0.324 g (74 %); ^1H NMR (24 °C): δ = 1.74 (s, 3H, $\text{C}_6\text{H}_4\text{CH}_3$), 6.33 (t, J = 6.8 Hz, 1H, $\text{C}_6\text{H}_4\text{CH}_3$), 6.72 (m, 2H, Bcat), 6.80 (m, 3H, Bcat and $\text{C}_6\text{H}_4\text{CH}_3$), 6.85 (d, J = 6.8 Hz, 1H, $\text{C}_6\text{H}_4\text{CH}_3$), 7.08–7.25 (m, 30H, PPh₃); $^{13}\text{C}\{^1\text{H}\}$ NMR (24 °C): δ = 31.17 (s, $\text{C}_6\text{H}_4\text{CH}_3$), 110.27 (s, Bcat), 119.85 (s, Bcat), 122.55 (s, $\text{C}_6\text{H}_4\text{CH}_3$), 124.13 (s, $\text{C}_6\text{H}_4\text{CH}_3$), 127.47 (t', $^2, ^4J(\text{C}, \text{P})$ = 9 Hz, *o*-PPh₃), 127.98 (s, $\text{C}_6\text{H}_4\text{CH}_3$), 129.48 (s, *p*-PPh₃), 133.60 (t', $^3, ^5J(\text{C}, \text{P})$ = 9 Hz, *m*-PPh₃), 134.70 (t', $^1, ^3J(\text{C}, \text{P})$ = 51 Hz, *i*-PPh₃), 146.47 (t, $^2J(\text{C}, \text{P})$ = 9 Hz, *i*- $\text{C}_6\text{H}_4\text{Me}$), 149.41 (s, $\text{C}_6\text{H}_4\text{CH}_3$), 149.84 (s, $\text{C}_6\text{H}_4\text{CH}_3$), 150.35 (s, Bcat), 189.75 (t, $^2J(\text{C}, \text{P})$ = 7 Hz, CO), 192.71 (t, $^2J(\text{C}, \text{P})$ = 7 Hz, CO); IR (Nujol): $\tilde{\nu}$ = 1941 (vs), 2038 cm^{-1} (w, CO); elemental analysis calcd for $\text{C}_{51}\text{H}_{41}\text{BO}_4\text{OsP}_2 \cdot 1.2\text{CHCl}_3$ (%): C 55.77, H 3.78; found: C 55.74, H 3.82.

5: A mixture of **3** (89 mg, 0.091 mmol) and B_2cat_2 (60 mg, 0.25 mmol) was dissolved in benzene (10 mL) and stirred at room temperature for 16 h. The benzene was removed from the resulting orange solution, and then dichloromethane (10 mL) was added. The resultant suspension was filtered through Celite, and ethanol (5 mL) was added to the filtrate. The dichloromethane was removed in vacuo to give **5** as a white precipitate which was collected and washed with ethanol. Yield 46 mg (50 %); ^1H NMR: δ = 6.69 (m, 4H, Bcat), 6.82 (m, 4H, Bcat), 7.08–7.18 (m, 18H, PPh₃), 7.30 (m, 12H, PPh₃); $^{13}\text{C}\{^1\text{H}\}$ NMR: δ = 110.71 (s, Bcat), 120.04 (s, Bcat), 127.86 (t', $^2, ^4J(\text{C}, \text{P})$ = 10 Hz, *o*-PPh₃), 129.49 (s, *p*-PPh₃), 133.20 (t', $^3, ^5J(\text{C}, \text{P})$ = 12 Hz, *m*-PPh₃), 136.40 (m, *i*-PPh₃), 150.17 (s, Bcat), 189.76 (t, $^2J(\text{C}, \text{P})$ = 8.5 Hz, CO); IR (Nujol): $\tilde{\nu}$ = 1963 cm^{-1} (vs, CO); elemental analysis calcd for $\text{C}_{50}\text{H}_{38}\text{B}_2\text{O}_6\text{OsP}_2$ (%): C 59.54, H 3.80; found: C 59.30, H 3.51.

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68.9500(10), β = 76.4950(10), γ = 73.2160(10)°, Z = 2, V = 2655.5(2) Å³, ρ_{calcd} = 1.376 g cm^{-3} ; T = 203 K; $2\theta_{\text{max}}$ = 55.02°; total reflections 22438 of which 11362 were unique (R_{int} = 0.0366); Siemens SMART CCD diffractometer, $\text{MoK}\alpha$ radiation (λ = 0.71073 Å); empirical absorption correction (μ = 2.652 mm^{-1} , $T_{\text{min}}/T_{\text{max}}$ = 0.4021/0.7963). The structure was solved by Patterson and Fourier methods and refined by full-matrix least squares on F^2 on all data. The asymmetric unit cell contains one molecule of **7a** and one molecule of chloroform split between two sites; R_1 = 0.0557 for the 10128 observed data ($I > 2\sigma(I)$) and wR_2 = 0.1640 for all data. Programs used: SHELXS-97 (structure solution) and SHELXL-97 (structure 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 nos. CCDC-104125 (**3**) and CCDC-104126 (**7a**). 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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Thermally Stable Heterobinuclear Bivalent Group 14 Metal Complexes $\text{Ar}_2\text{M}-\text{Sn}[\text{1,8-(NR)}_2\text{C}_{10}\text{H}_6]$ ($\text{M} = \text{Ge}, \text{Sn}$; $\text{Ar} = \text{2,6-(Me}_2\text{N)}_2\text{C}_6\text{H}_3$; $\text{R} = \text{CH}_2\text{iBu}$)**

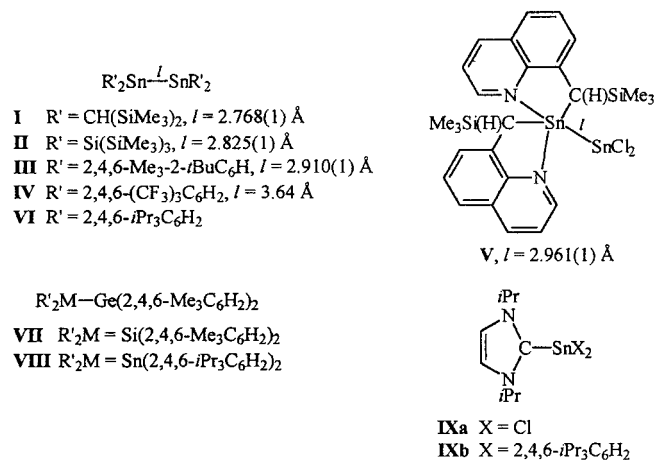
Christian Drost, Peter B. Hitchcock, and Michael F. Lappert*

Homobinuclear tin(II) compounds $\text{Sn}_2\text{R}'_4$, free of bridging ligands, and their germanium(II) analogues have attracted much attention.^[1] The first such crystallographically characterized compound **I** was reported 25 years ago,^[2] followed more recently by **II**,^[3] **III**,^[4] and **IV**.^[5] Only one example of a crystalline, bivalent bis[tin(II)] compound with different ligands attached to each tin atom, namely, the thermally labile **V**, had previously been described.^[6] In **I–III** and **V**, the Sn–Sn distance l was in the range 2.77–2.91 Å (cf. 2.81 Å in tetrahedral tin), while in **IV** (which has close Sn...F contacts) the tin–tin distance of 3.64 Å is too large for significant Sn...Sn bonding. Each of **I–IV**, unlike **V**, was either partially (**III** > **I**)^[1] or completely dissociated in toluene or methylcyclohexane. There was some evidence for formation of the transient

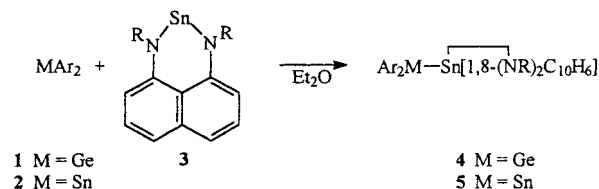
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low-temperature dimer **VI** from the crystalline cyclotrimer $(\text{SnR}'_2)_3$ ($\text{R}' = 2,4,6\text{-}i\text{Pr}_3\text{C}_6\text{H}_2$) by dissolution in $\text{MeC}_6\text{H}_{11}$ and photolysis at 205 K.^[7] Two transient heterobinuclear complexes **VII**^[8] and **VIII**^[9] were identified by spectroscopy or by trapping experiments.



We now report the synthesis and characterization of two novel, thermally stable, crystalline compounds: the first thermally robust heterobimetallic $\text{Ge}^{\text{II}}\text{-Sn}^{\text{II}}$ compound **4** and a second (cf. **V**) $\text{Sn}^{\text{II}}\text{-Sn}^{\text{II}}$ compound **5**, which maintain the integrity of their metal–metal bonds in solution. Treatment of the crystalline, monomeric, base-stabilized yellow diarylgermylene GeAr_2 (**1**) or -stannylene SnAr_2 (**2**)^[10] with a suspension of the new [(1,8-dineopentylamino)naphthalene]-based stannylene **3**^[11] in diethyl ether afforded after workup orange-red crystals of **4** or **5** in good yields (Scheme 1).



Scheme 1. Synthesis of the diarylmethylene–stannylene adducts **4** and **5** ($\text{Ar} = 2,6\text{-(Me}_2\text{N)}_2\text{C}_6\text{H}_3$, $\text{R} = \text{CH}_2t\text{Bu}$).

Compounds **3–5** gave satisfactory microanalytical as well as EI-MS and multinuclear NMR spectroscopic data (Table 1). In addition, single-crystal X-ray diffraction studies revealed the molecular structure of crystalline **5** (Figure 1). Complex **3** is sparingly soluble in diethyl ether and aliphatic hydrocarbons, whereas not only **1** and **2**^[10] but also **4** and **5** are readily soluble in these solvents.

The asymmetric unit cell of crystalline **5** contains two independent molecules of essentially identical geometry (structural parameters of only one are discussed) and a molecule of diethyl ether. The molecular structure of **5** is best described as a heteroleptic distannene comprising two independent stannylene moieties **2** and **3** linked solely by their tin–tin “bond” (Sn1-Sn2 3.087(2) Å), which is only slightly longer than those of **I–III**, **V**,^[2–4, 6] and tetrahedral tin. The tin atoms of **5** differ in their coordination number, Sn1 being five- and Sn2 three-coordinate. The Sn1–C and Sn1–N distances in

Table 1. Selected data for **3–5**. NMR spectra at 298 K in C_6D_6 (^1H , ^{13}C) or $\text{PhMe}/\text{C}_6\text{D}_6$. ^1H NMR: 250.0 MHz; $^{13}\text{C}\{^1\text{H}\}$ NMR: 62.86 MHz; $^{119}\text{Sn}\{^1\text{H}\}$ NMR: 186.36 MHz.

3 : Orange-red; 81 % yield; m.p. ca. 154 °C; $^{119}\text{Sn}\{^1\text{H}\}$ NMR: $\delta = 183$; EI-MS (70 eV): m/z (%): 416 (40) [M^+].
4 : Orange; 72 % yield; m.p. ca. 115 °C; ^1H NMR: $\delta = 1.12$ (s, 18H, CMe_3), 2.45 (s, 24H, NMe_2), 3.49 (br, 4H, CH_2CMe_3), 6.39 (d, 4H, H-3/5, $^3J(^1\text{H},^1\text{H}) = 7.9$ Hz), 7.04 (t, 2H, H-4, $^3J(^1\text{H},^1\text{H}) = 7.9$ Hz), 6.74–7.15 (m, 6H, H_{Naph}); $^{13}\text{C}\{^1\text{H}\}$ NMR: $\delta = 29.6$ (CC_3), 35.5 (CC_3), 45.6 (NC_2), 62.0 (CH_2), 104.3, 111.8, 115.3, 125.7, 130.3, 140.8, 157.7 (C_{Ar}); $^{119}\text{Sn}\{^1\text{H}\}$ NMR: $\delta = -58$; EI-MS (70 eV): m/z (%): 812 (1) [M^+].
5 : Orange; 87 % yield; m.p. (decomp) > 120 °C; ^1H NMR $\delta = 1.13$ (s, 18H, CMe_3), 2.49 (s, 24H, NMe_2), 3.54 (br, 4H, CH_2CMe_3), 6.39 (d, 4H, H-3/5, $^3J(^1\text{H},^1\text{H}) = 7.9$ Hz), 7.04 (t, 2H, H-4, $^3J(^1\text{H},^1\text{H}) = 7.8$ Hz), 6.75–7.15 (m, 6H, H_{Naph}); $^{13}\text{C}\{^1\text{H}\}$ NMR: $\delta = 29.5$ (CC_3), 35.5 (CC_3), 45.5 (NC_2), 61.8 (CH_2), 103.7, 111.1, 115.5, 125.6, 129.8, 140.9, 158.6 (C_{Ar}); $^{119}\text{Sn}\{^1\text{H}\}$ NMR: $\delta = -30$ (Sn_{Naph}), 275 (Sn_{Ar}); EI-MS (70 eV): m/z (%): 861 (3) [M^+].

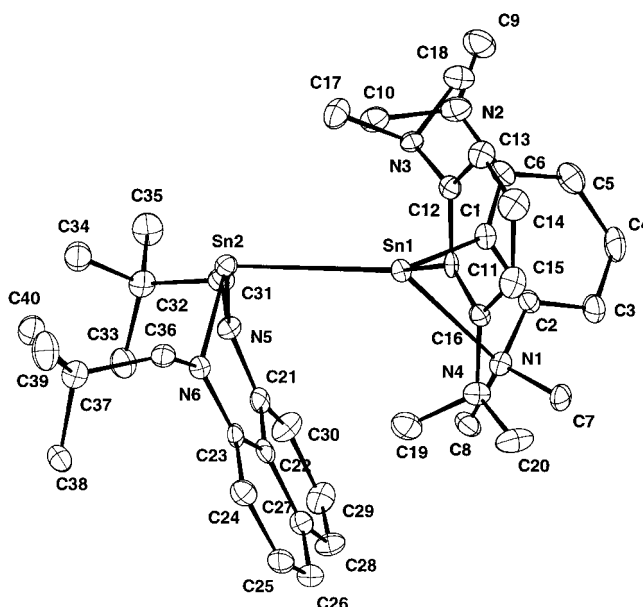


Figure 1. Molecular structure of **5**. Selected bond lengths [Å] and angles [°]: Sn1–Sn2 3.087(2), Sn1–C1 2.196(7), Sn1–C11 2.186(6), Sn1–N1 2.517(5), Sn1–N3 2.580(5), Sn2–N5 2.083(6), Sn2–N6 2.086(6); C11–Sn1–C1 110.7(2), C1–Sn1–N1 59.9(2), C11–Sn1–N1 92.7(2), C11–Sn1–N3 59.2(2), C1–Sn1–N3 92.6(2), N1–Sn1–N3 132.2(2), C11–Sn1–Sn2 122.8(2), C1–Sn1–Sn2 122.6(2), N1–Sn1–Sn2 130.20(13), N3–Sn1–Sn2 97.32(13), N5–Sn2–N6 86.9(2), N5–Sn2–Sn1 95.8(2), N6–Sn2–Sn1 96.7(2).

the Sn1Ar_2 fragment of **5** are significantly shorter than those of free **2**,^[10] a feature previously observed, but in a much more pronounced fashion, in $\text{Ar}_2\text{Sn} \cdot \text{BH}_3$ ^[13] (Table 2). The sum of the three angles subtended at Sn1 by the *ipso*-C atoms C1 and C11 and Sn2 is 356.1°, and this indicates that C1, C11, and Sn2 are equatorially disposed about a distorted trigonal Sn1 centroid; N1 and N3 occupy quasi-axial positions; the N1–Sn1–N3 bond angle is 132.2(2)°. To ensure optimal electron saturation at Sn1, the Sn1–C1 and Sn1–C11 bonds are strongly tilted towards the coordinating nitrogen atoms N1 and N3, leaving the nonbonding nitrogen atoms N2 and N4 remote from Sn1.

The sum (Σ) of the bond angles subtended at Sn2 in **5** is 279.4°, with Sn2 in a pyramidal (PX_3 -like) environment (bite angle N5–Sn2–N6 86.9(2), N5–Sn2–Sn1 95.8(2), N6–Sn2–Sn1

72 %) or **5** (1.30 g, 87 %). Recrystallization from hexane yielded very fine needles of both compounds.

Crystal data for **5**: $C_{84}H_{126}N_{12}OSn_4$, $M_r = 1794.73$, monoclinic, space group $P2_1/c$ (no. 14), $a = 22.911(4)$, $b = 18.441(6)$, $c = 22.250(13)$ Å, $\beta = 115.30(3)^\circ$, $V = 8499(6)$ Å³, $F(000) = 3688$; $Z = 4$, $\rho_{\text{calc}} = 1.40$ g cm⁻³, $\mu(\text{MoK}\alpha) = 14.2$ cm⁻¹, crystal dimensions $0.3 \times 0.2 \times 0.1$ mm, 12 145 reflections collected for $2 < \theta < 23^\circ$, 11 805 independent reflections, $R1 = 0.047$ for 8396 reflections with $I > 2\sigma(I)$, $wR2 = 0.09$ (for all data), $S = 0.989$. Data collected at $T = 173(2)$ K, Enraf Nonius CAD-4 diffractometer, absorption correction, structural solution by direct methods, full-matrix least-squares refinement on F^2 with SHELXL-93 with non-hydrogen atoms anisotropic. The asymmetric unit contains two independent molecules of the Sn_2 complex and one molecule of diethyl ether solvent. 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-107 662. 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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Kinetic Resolution of Diiron Acyl Complexes—An Approach to Asymmetric Bicyclic β -Lactams**

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In the last few years we have been interested in the dipolar cycloaddition of nitrones with diiron acyl complexes.^[1, 2] This chemistry has been shown to be effective in the diastereo- and enantioselective addition of a variety of nitrones to mono- and disubstituted α, β -unsaturated acyl complexes. It was found that, after cycloaddition, oxidation of the resultant complex gives a thioester isoxazolidine product.^[1–3] It has been shown that isoxazolidines can be converted to amino alcohols through reduction of the N–O bond.^[4–6] In the case of the cycloadducts discussed below, this results in the formation of thioester derivatives of β -amino acids. Here in we show that reaction of a cyclic nitrone derived from proline proceeds with one enantiomer of the diiron complex significantly faster than with the other enantiomer. Additionally the utility of this approach is demonstrated through the synthesis of a simple carbapenem.

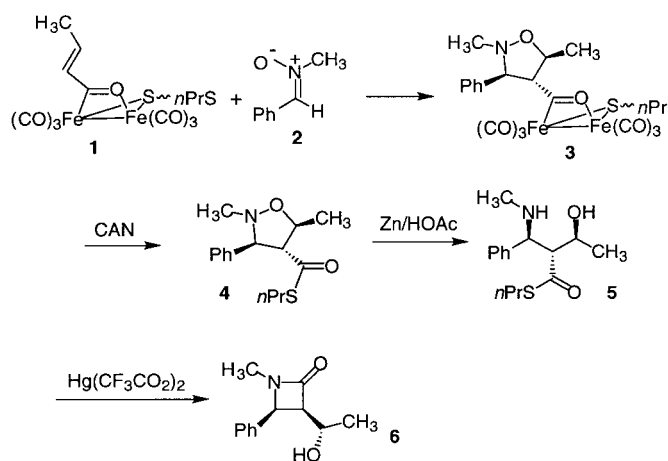
Reaction of complex **1** with the *Z*-nitrone **2** gave the expected isoxazolidine product, which could be oxidized to the corresponding thioester **4**. Reaction with zinc and acetic acid then yielded the β -amino β -hydroxy thioester **5**. The hope was that treatment of the thioester with mercury trifluoroacetate would result in removal of the sulfur with commensurate trapping by the amine group to give the β -lactam.^[7–9] Since optically active diiron acyl complexes are accessible, this approach would potentially provide a mild route to optically active β -lactams.^[2] Unfortunately the initial attempt to form the β -lactam from amino alcohol **5** resulted in less than a 15 % yield of the desired product **6**. It has been reported that the cyclization to give β -lactams with groups *cis* on the adjacent carbon atoms of the four-membered ring is difficult (Scheme 1).^[10]

Because of this observation, and the fact that a large number of the known β -lactam antibiotics possess the opposite stereochemistry, the dipolar cycloaddition was run on an *E*-nitrone. The stereochemistry of acyclic nitrones is typically *Z*. Consequently, it was necessary to attempt the cyclization with a cyclic nitrone. *Endo* addition of such a nitrone would give a β -amino acid with the correct stereochemistry to readily form a β -lactam. Cyclization of nitrone **7** with diiron complex **1** followed by oxidative removal of the

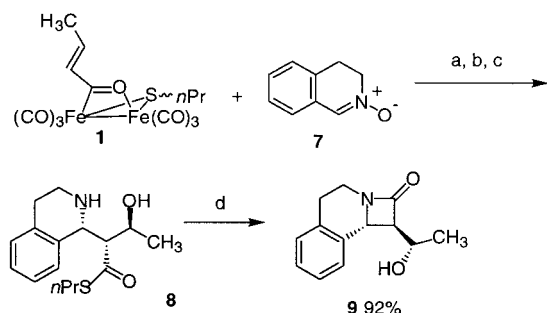
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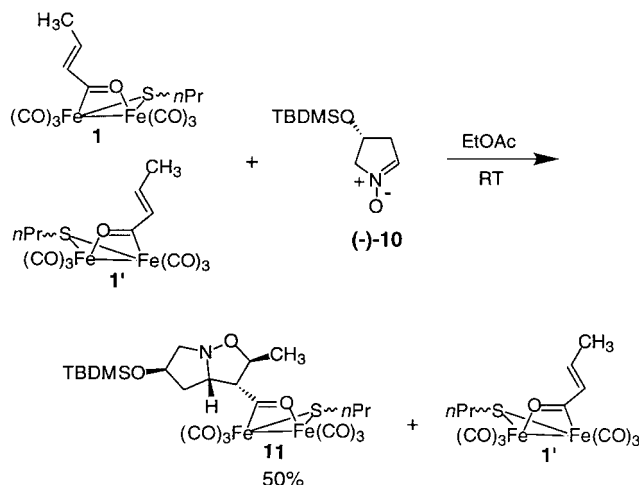
 Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.


 Scheme 1. Synthesis of β -lactam **6** from **1** and **2**.

metal gives the thio-ester product. Reduction of the N–O bond provides an amino thioester (**8**) that, upon treatment with mercury trifluoroacetate, readily cyclizes to the β -lactam **9**.^[11] The stereochemistry obtained in this reaction not only facilitates cyclization to the β -lactam but corresponds to the stereochemistry of important carbapenems, such as thienamycin (Scheme 2).^[12–17]


 Scheme 2. Synthesis of **9** from **1** and **7**. a) Cycloaddition, b) CAN, CH_3CN , c) Zn, HOAc, d) $\text{Hg}(\text{CF}_3\text{CO}_2)_2$.

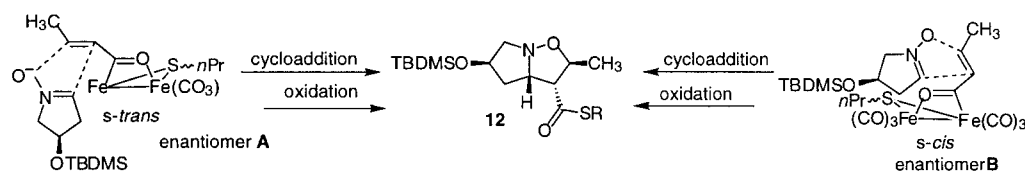
Once it had been established that this approach was a viable route to β -lactams, an attempt to use this reaction sequence in the synthesis of carbapenem structures was undertaken. Therefore, the racemic complex **1** was treated with enantiomerically pure nitron **10**. This reaction resulted in a 50% yield of the expected bicyclic isoxazolidine but, surprisingly, as a single diastereomer. If both enantiomers of the racemic complex reacted with the enantiomerically pure nitron (–)-**10**, two diastereomeric products (*S,S* and *S,R*) should have been obtained. Isolation of the unreacted starting material and measurement of its optical rotation revealed that the unreacted starting material was optically active, which confirmed that reaction with the nitron (–)-**10** effected a kinetic resolution of the racemic complex. It was subsequently observed that, if allowed sufficient time, the other enantiomer of the iron complex also reacts with nitron (–)-**10** (Scheme 3).


 Scheme 3. Compound **11** is predominately one diastereomer, at least 20:1. The recovered starting material is highly enriched in favor of one enantiomer. TBDMS = *tert*-butyldimethylsilyl.

The selection of one enantiomer of the diiron complex by the enantiomerically pure cyclic nitron requires consideration of a number of factors. First, the nitron must approach the olefin from one side, most likely the side opposite the sulfur (Scheme 4). Second, given the product formed, the nitron must react selectively through an *endo* transition state. Third, only one face of the nitron can add to the complex; this is likely to be the face opposite the large *tert*-butyldimethylsilyl group. The last issue to consider is what conformation the complex reacts in, *s-cis* or *s-trans*. From the high selectivity, we know that either enantiomer **A** reacts in the *s-trans* conformation or enantiomer **B** reacts in the *s-cis* conformation (Scheme 4). Since we do not yet know the absolute sense of chirality of the complex at the metal center, we do not know which conformation the molecule reacts in. Given the high diastereoselectivity, we know it must be predominately one. All these issues combine to cause the enantiomerically pure nitron to react with one of the two enantiomers of the racemic mixture with a significantly faster rate.

It is important to note that, while the stereochemistry at the metal center has not been determined, the absolute stereochemistry of the three stereogenic carbon atoms in the product is known. They have been assigned relative to the known chirality of the carbon atom which bears the hydroxy group of the nitron. When the nitron from *cis*-hydroxy proline ((+)-**10**), the enantiomer of (–)-**10**, is used the other enantiomer of the β -lactam is obtained.

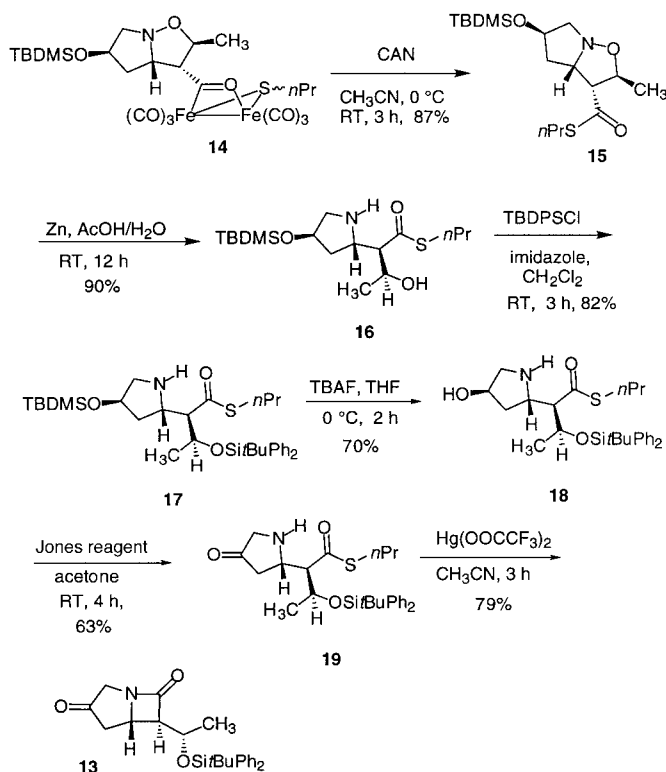
The relative rates of the reaction between a matched and mis-matched nitron complex pair has been determined. This was done by resolution of the complex through reaction with the enantiomerically pure nitron (–)-**10**. After resolution of


 Scheme 4. The two possible *endo* transition states for product formation.

the racemic complex with nitron (–)-**10**, the recovered, optically active starting material was then allowed to react with its matched and mismatched nitrones, (–)-**10** and its enantiomer (+)-**10**. It was observed that the complex resolved through reaction with (–)-**10** (i.e. the recovered starting material) reacted eleven times faster with nitron (+)-**10**.

To demonstrate the potential of this approach for the synthesis of β -lactams, carbapenem **13** was synthesized (Scheme 5).^[18] Cycloadduct **14** was obtained from the cyclo-

be used in a variety of asymmetric cycloadditions. The reaction of nitron **10** with diiron complexes provides β -amino acids that can be converted to β -lactams with absolute stereochemistry that corresponds to the carbapenems, such as thienamycin. We are currently developing chemistry for the conversion of intermediates such as **13** into a series of biologically active β -lactams. Additionally we are attempting to determine the absolute sense of chirality of these complexes at the metal centers.



Scheme 5. Synthesis of carbapenem **13** from **14**. TBDPS = *tert*-butyldiphenylsilyl.

addition of nitron (–)-**10** and a racemic mixture of complex **1**. The resulting complex **14** was then treated with cerium ammonium nitrate (CAN) to liberate thioester **15**. To complete the synthesis of **13**, the hydroxy group that was used to set the stereochemistry must be oxidized to a ketone. It was found that the best time for this transformation was before the β -lactam was formed. This was accomplished through reduction of the N–O bond with zinc and acetic acid, to give β -amino thioester **16**, followed by protection of the free hydroxy group as the *tert*-butyldiphenylsilyl ether (**17**). Selective removal of the *tert*-butyldiphenylsilyl group with tetrabutylammonium fluoride (TBAF) then gave the desired alcohol (**18**) ready for oxidation. Jones oxidation of the free alcohol followed by cyclization with mercuric trifluoroacetate afforded optically active bicyclic β -lactam **13**. When the nitron from *cis*-hydroxy proline ((+)-**10**) was used, the stereochemistry of the three stereogenic centers of β -lactam **13** corresponded to the stereochemistry of many therapeutically useful carbapenems, including thienamycin.

Through the reaction of nitron (–)-**10**, optically active diiron acyl complexes can be obtained. These complexes can

Experimental Section

General synthesis of the α,β -unsaturated acyl complexes:

A 100 mL, round-bottomed flask was charged with $[\text{Fe}_3(\text{CO})_{12}]$ (5.00 g, 9.93 mmol) and flushed with nitrogen. THF (120 mL) was added, followed by the addition of *n*-propanethiol (0.87 g, 11.40 mmol) and triethylamine (1.17 g, 11.53 mmol). The solution was stirred for 20 min, during which time a color change of green to yellow-brown occurred. Then *trans*-crotonyl chloride (2.10 g, 20.25 mmol) was added. During this addition, gas evolution was observed. The mixture was stirred for 15 h, during which time the reaction mixture turned dark red and a white precipitate formed. The solution was filtered and the solvent removed to leave a red oil. The product was purified by silica gel chromatography (hexane) to give 3.40 g (8.02 mmol, 81 %) of a slightly air-sensitive, dark red oil. A product which corresponded to the CO deinsertion product, as described by Seyferth et al., eluted before the desired product.^[3, 19–21]

Sample dipolar cycloaddition: Crotonyl acyl complex **1** (1.6 g, 3.78 mmol) was dissolved in ethyl acetate (20 mL) and transferred to a 50 mL Schlenk tube. Nitron (–)-**10** (974.0 mg, 4.53 mmol) was added and the mixture was degassed by three freeze-pump-thaw cycles. Once degassed the reaction mixture was stirred for 3 h. The product was purified by column chromatography (silica gel, ethyl acetate/hexane 5/95) and isolated as a red oil; 1.89 mmol, 50 %. ^1H NMR (300 MHz, CDCl_3): δ = 4.33 (dd, J = 7.8, 7.2 Hz, 1H), 4.25 (dq, J = 6.0, 4.5 Hz, 1H), 3.85 (dd, J = 9.0, 8.1 Hz, 1H), 3.72 (m, 1H), 3.04 (dd, J = 10.0, 4.8 Hz, 2H), 2.81 (m, 2H), 1.86 (m, 2H), 1.53 (m, 1H), 1.38 (m, 2H), 1.13 (t, J = 7.5 Hz, 3H), 1.01 (d, J = 6 Hz, 3H), 0.87 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); minor isomer: 4.40 (m, 1H), 3.77 (m, 1H), 3.11 (t, J = 5.9 Hz, 1H), 2.97 (d, J = 5.74 Hz, 1H), 1.14 (t, J = 7.3 Hz, 3H), 1.00 (d, J = 5.6 Hz, 3H), 0.86 (s, 9H); 0.03 (s, 3H); 0.02 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3): δ = 300.3, 211.5, 211.2, 208.9, 208.7, 208.6, 81.4, 72.3, 72.0, 65.4, 65.0, 40.6, 37.3, 26.8, 25.8, 18.0, 17.4, 13.4, –4.7, –4.8; minor isomer: 82.4, 72.9, 72.7, 65.9, 65.3, 40.8, 36.7, 25.7, 18.1, 13.5, –4.9; IR (thin film): $\tilde{\nu}$ = 2932, 2898, 2858, 2074, 2031, 1997, 1970, 1462, 1114, 1071 cm^{-1} ; FAB-MS: m/z (%): 641 ($[M+H]$, 10), 133 (100); HRFAB-MS: calcd for $\text{C}_{23}\text{H}_{34}\text{Fe}_2\text{NO}_3\text{Si}$: m/z : $[M+H]$ 640.0422, found 640.0413; TLC: R_f : 0.52 (ethyl acetate/hexane 15/85).

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Surface Coordination Chemistry: Corrosion Inhibition by Tetranuclear Cluster Formation of Iron with Salicylaldehyde**

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The corrosion of metals represents a waste of both natural resources and money. Corrosion can cause catastrophic accidents because of premature failure of metallic equipment. The world's supply of metals is limited, and wastage due to corrosion leads to consumption of energy and water reserves

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for producing and fabricating metallic structures. Acorga P5000 (referred to as P50) is a modern corrosion inhibitor for iron and comprises 5-nonylsalicylaldehyde as a mixture of carbon-chain isomers.^[1] P50 was developed as an extraction agent for copper,^[2] and bis-salicylaldehyde complexes of many divalent metal ions are known.^[3] However, there is no information on the mode of action of P50 on iron surfaces, and data available on the mode of action of P50 on iron surfaces, and complexes are scant and contradictory.^[4–7] We have already identified a variety of bonding modes for salicylaldehyde oligomers with vanadium and zinc.^[8] When steel is treated with P50, a purple coating is formed on the surface and constitutes the protective film. Here we describe the iron chemistry of salicylaldehyde and alkyl-substituted analogues and compare the products of these reactions with corresponding materials produced by treating mild steel with these reagents.

Reaction^[9] of iron(III) chloride with salicylaldehyde (H_2SalH)^[8] led to crystals formulated as $[\{\text{Fe}(\text{SalH})(\text{H-SalH})\}_4] \cdot \text{H}_2\text{SalH} \cdot 2\text{C}_8\text{H}_{10}$ (**1**).^[10] Compound **1** comprises two molecules of xylene, a molecule of H_2SalH , and a cluster containing four Fe^{III} centers, each of which has a distorted octahedral coordination environment with four O and two *cis*-N donor atoms (Figure 1). Each Fe^{III} center is ligated by a terminal, bidentate HSalH *N,O*-donor ligand (those containing N1, N3, N5, and N7 function in this manner) and four atoms (1 N and 3 O) of three of the four bridging SalH ligands (those containing N2, N4, N6, and N8). Each of these bridging ligands joins two Fe^{III} centers through its oximate oxygen atom ($\mu\text{-O}$); the attached nitrogen atom links this Fe-O-Fe moiety to a third Fe^{III} center ($\mu\text{-ON}$), and the phenolate oxygen atom is bound to this Fe^{III} ion to form a six-membered FeNCCCO chelate ring. The structure of the cluster is further stabilized by four intramolecular hydrogen bonds between a terminal oxime NOH group of the bidentate HSalH ligand and the adjacent phenolate oxygen atom of another such ligand. A similar diversity in the bonding modes of this type of ligand occurs in $[\{\text{VO}(\text{SalEt})(\text{HSalEt})\}_2]$.^[8]

The four Fe^{III} centers of the tetranuclear cluster of **1** have a distorted tetrahedral arrangement with $\text{Fe} \cdots \text{Fe}$ distances of about 3.6 Å for linkage by one $\mu\text{-O}$ and one $\mu\text{-ON}$, and about 4.1 Å for linkage by two $\mu\text{-ON}$ groups. Tetranuclear Fe^{III} clusters, albeit with different structures, were identified in $[\text{Fe}^{\text{III}}\{\text{Fe}^{\text{III}}(\text{salicylhydroximate})(\text{MeOH})(\text{acetate})\}_3] \cdot 3\text{MeOH}$, in which three Fe^{III} centers are arranged about a central Fe^{III} ion,^[12a] and $[\text{L}_2\text{Fe}_2^{\text{III}}(\mu_3\text{-O}_2)(\mu_2\text{-CH}_3\text{COO})_3(\text{SalH})_2\text{Fe}_2^{\text{III}}\text{L}_2]\text{X}$ ($\text{L} = 1,4,7\text{-trimethyltriazacyclononane}$; $\text{X} = \text{ClO}_4$, PF_6), the cation of which contains a butterfly arrangement of the four Fe^{III} centers formed by two edge-sharing $\text{Fe}_3^{\text{III}}(\mu_3\text{-O})$ triangular units in which deprotonated NO groups bridge the wing and body pairs of iron atoms.^[12b]

Elemental analyses, spectroscopic data, and other physical measurements^[13] have demonstrated that the predominant constituent of the red-brown product obtained when an Fe salt reacts with salicylaldehyde is the tetranuclear cluster characterized in **1**. The ^{57}Fe Mössbauer isomer shift (0.53 mm s^{-1} at 77 K, 0.54 mm s^{-1} at 4.2 K) and quadrupole splitting (1.02 mm s^{-1} at 77 K, 1.05 mm s^{-1} at 4.2 K) are consistent^[16–18] with a high-spin Fe^{III} system. The linewidth

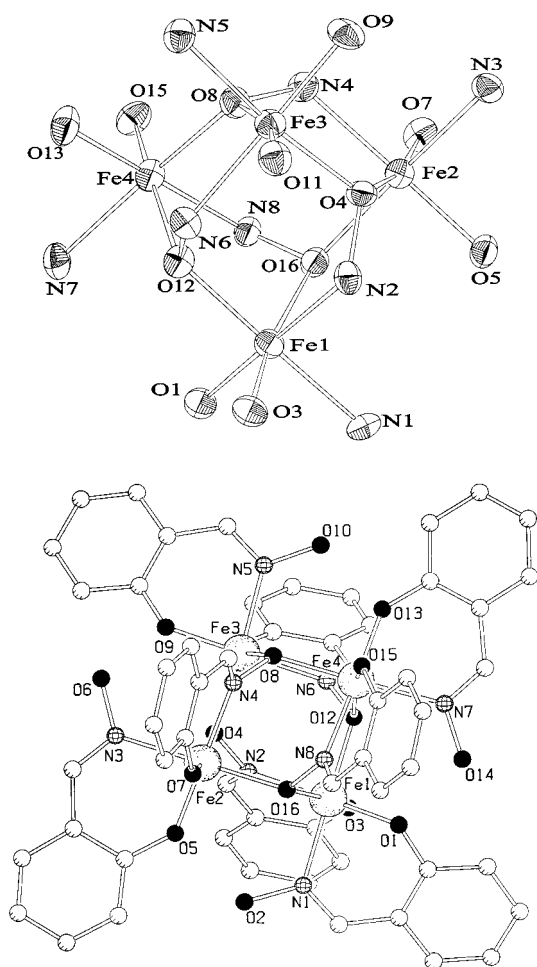


Figure 1. The molecular structure of $[\text{Fe}(\text{C}_7\text{H}_6\text{O}_2\text{N})(\text{C}_7\text{H}_5\text{O}_2\text{N})_3]_4$. Top: The tetranuclear Fe^{III} cluster showing the coordination sphere of each metal center. Bottom: The overall structure of the cluster viewed down the approximate molecular C_2 (S_4) axis with the H atoms omitted for clarity. Selected bond lengths [Å] and angles [°]: Fe1–O1 1.929(7), Fe1–O3 1.890(8), Fe1–O12 2.041(7), Fe1–O16 2.069(7), Fe1–N1 2.156(9), Fe1–N2 2.113(9), Fe2–O4 2.087(7), Fe2–O5 1.898(7), Fe2–O7 1.900(8), Fe2–O16 2.052(7), Fe2–N3 2.161(9), Fe2–N4 2.120(9), Fe3–O4 2.041(7), Fe3–O8 2.056(7), Fe3–O9 1.925(7), Fe3–O11 1.889(8), Fe3–N5 2.159(9), Fe3–N6 2.137(9), Fe4–O8 2.062(7), Fe4–O12 2.065(7), Fe4–O13 1.924(7), Fe4–O15 1.887(8), Fe4–N7 2.155(9), Fe4–N8 2.135(9); N1–Fe1–O1 84.7(3), N2–Fe1–O3 84.7(4), O1–Fe1–O12 91.7(3), O1–Fe1–O16 95.8(3), N3–Fe2–O5 85.6(3), N4–Fe2–O7 84.4(3), O5–Fe2–O7 92.7(3), O5–Fe2–O16 91.6(3), N5–Fe3–O9 84.5(4), O8–Fe3–N6 81.4(3), O11–Fe3–N6 84.4(4), N6–Fe3–O4 90.5(3), N7–Fe4–O13 85.8(4), O12–Fe4–O8 85.2(3), N8–Fe4–O15 83.6(3), N7–Fe4–O12 85.5(3); Fe...Fe distances [Å]: Fe1–Fe4 3.547(3), Fe1–Fe2 3.607(3), Fe1–Fe3 4.050(2), Fe2–Fe3 3.568(3), Fe2–Fe4 4.068(3), Fe3–Fe4 3.603(3).

(0.38 mm s^{−1} at 77 K, 0.66 mm s^{−1} at 4.2 K) and the appearance of a magnetic pattern at 1.5 K with an overall splitting of 4.7 T are consistent with the presence of a small cluster.^[14–16] In aqueous, aerobic media, the same product is obtained irrespective of whether an iron(II) or an iron(III) salt is used as starting material. 5-*tert*-Butyl- and 5-(1,1,3,3-tetramethylbutyl)salicylaldoxime (which are close analogues of P50) react with Fe^{II} or Fe^{III} salts to produce dark purple precipitates that were shown by spectroscopy to contain a tetranuclear Fe^{III} core analogous to that in **1**. Whilst freshly precipitated Fe(OH)₂ or Fe(OH)₃ yielded such products, aged samples of

these hydroxides, as well as $\alpha\text{-FeOOH}$,^[17] $\alpha\text{-Fe}_2\text{O}_3$,^[17] $\gamma\text{-FeOOH}$,^[18] $\beta\text{-FeOOH}$,^[19] and rust (produced by the exposure of a Q panel^[20] to the Manchester environment for one month) showed no reaction with salicylaldoxime or its alkyl-substituted derivatives.

Degreased Q panels were treated with 1M H₂SO₄ for 30 s, washed with distilled water, and treated, by spinning, with an alcoholic solution of salicylaldoxime. Scanning electron micrographs showed the formation of a thick surface layer. The Fe(2p), Fe(3p), O(1s), and N(1s) X-ray photoelectron spectra (XPS) of the treated panels were identical to those of the isolated tetranuclear cluster. Therefore, the differences between the O(1s) and N(1s) XPS chemical shifts of the treated panels and the free ligands are particularly significant. Similarly, the dominant features in the attenuated total reflection FT-IR spectrum of the treated panels closely resembled those of the isolated tetranuclear cluster. Extraction of the surface coating allowed analytical and further spectroscopic investigations to be accomplished. Elemental analyses, mass spectrometric, EPR, FT-IR, XPS, UV/Vis absorption, and magnetic susceptibility measurements were all consistent with the presence in the surface coating of a high proportion of the tetranuclear cluster identified in **1**. The Fe K-edge X-ray absorption spectra of the isolated tetranuclear cluster and the solid extracted from the surface coating were essentially identical. The energy of each of the K absorption edges is consistent with the presence of Fe^{III}.^[21] The Fe K-edge EXAFS spectra of the surface coating and **1** are each well described by backscattering contributions from shells of light atoms at 1.93, 2.10, 2.91, and 3.06 Å (assigned as 4O, 2N, 4C, and 4C, respectively), consistent with the crystal structure of **1**. Although Fe K-edge EXAFS provided no clear evidence for the proximity of Fe atoms, this is not unexpected, given the spread in the crystallographically determined Fe...Fe distances (3.547(3)–4.068(3) Å). When 5-*tert*-butyl or 5-(1,1,3,3-tetramethylbutyl)salicylaldoxime was used, the spectroscopic properties of the surface product were also consistent with the formation of compounds structurally analogous to the tetranuclear cluster of **1**.

Therefore, we postulate that the corrosion inhibition by P50 on an iron surface is due to the generation of a tetranuclear cluster complex.^[22] Iron ions must be available for the formation of such species, and the efficiency of P50 is therefore enhanced by treatment of an oxidized iron surface with a mild acid. The results reported here provide evidence, in addition to the information obtained on the nature of the interaction of oxidized Cu surfaces with benzotriazole,^[23] that corrosion inhibition at a metal surface can involve the production of metal clusters. The low ligand/metal ratio which arises for a ligand in immediate contact with an oxidized metal surface means that polyfunctional ligands have advantages over monofunctional ligands as corrosion inhibitors. Polyfunctional ligands tend to produce compact poly-metallic assemblies and, when formed at the surface of a metal, such species can form a protective barrier which inhibits corrosion.

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- [10] The crystal and molecular structure of a crystal of dimensions $0.03 \times 0.2 \times 0.45$ mm was determined on a Rigaku AFC5R diffractometer with graphite-monochromated $\text{Cu}_{K\alpha}$ radiation ($\lambda = 1.54178$ Å, $\mu = 67.04$ cm⁻¹) and a 12-kW rotating anode generator at room temperature. $\text{C}_{79}\text{H}_{71}\text{N}_9\text{O}_{18}\text{Fe}_4$, triclinic, space group $P\bar{1}$ (no. 2), $a = 14.136(2)$, $b = 21.815(6)$, $c = 12.828(2)$ Å, $\alpha = 97.64(2)^\circ$, $\beta = 105.65(1)^\circ$, $\gamma = 84.95(2)^\circ$, $V = 3770(1)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.460$ g cm⁻³, $F(000) = 1712$. The scan was of the $\omega/2\theta$ type with a scan rate of 8.0° min⁻¹. Of the total of 11 210 unique reflections collected with $2\theta_{\text{max}} = 120.3^\circ$, 4851 with $I > 3\sigma(I)$ were used for structure determination by direct methods.^[11] An absorption correction using the program DIFABS was applied to the data (N. P. C. Walker, D. I. Stuart, *Acta Crystallogr. Sect. A* **1983**, 39, 158–166). Full-matrix least-squares refinement with anomalous dispersion for all non-hydrogen atoms gave residuals of $R = 0.068$ and $R_w = 0.069$, where $R = \sum |\Delta| / \sum |F_o|$ and $R_w = (\sum w^2 F_o^2 / \sum w^2 F_o^2)^{1/2}$, $w = 1/[\sigma^2(F_o) + 0.00022 |F_o|^2]$. 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-103013. 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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Stereoselective Solid-Phase Synthesis of β -Lactams—A Novel Cyclization/Cleavage Step towards 1-Oxacephams**

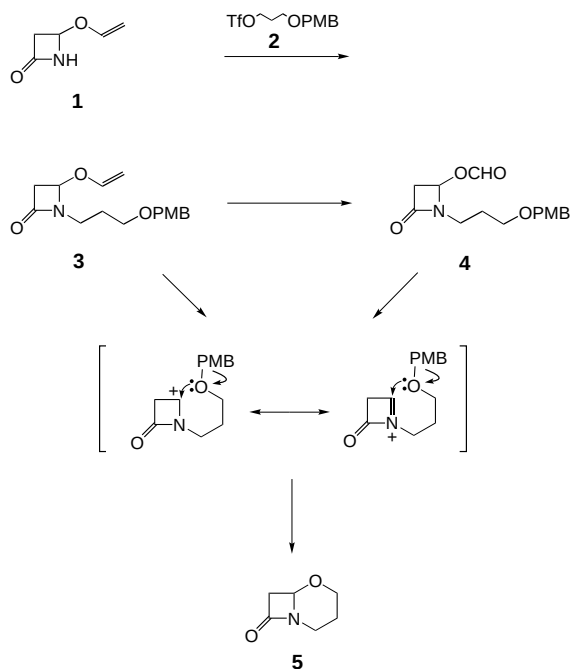
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The solid-phase method is an extremely efficient approach for the synthesis of small molecules.^[1] A current trend is to extend the principles of organic reactions that take place in homogenous phase to analogous reactions that take place on solid supports. To date, the syntheses of a variety of classes of heterocycles by solid-phase synthesis methodology have been reported.^[1] However the solid-phase synthesis of β -lactams, though attractive structures because of their antibiotic activity, have been barely reported.^[2] Herein we describe new resin-based chemistry for the straightforward construction of 1-oxacephams, which represent a class of compounds with potential biological activity.^[3] The development of new

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antibiotics is of the highest interest because of the increasing bacterial resistance against commonly used drugs.

Recently Kałuza et al.^[4] reported a new approach to 1-oxacephams from 4-vinyloxyazetidin-2-one **1**. The strategy consists of N-alkylation of **1** by the 1,3-propanediol derivative **2** that bears sulfonyl and 4-methoxybenzyl substituents, oxidation of the vinyloxy group in **3** to the formyloxy (or acetoxy group), and finally cyclization of compound **4** by the nucleophilic displacement at C-4 of the azetidin-2-one ring (Scheme 1). The 4-methoxybenzyl protection of the hydroxyl



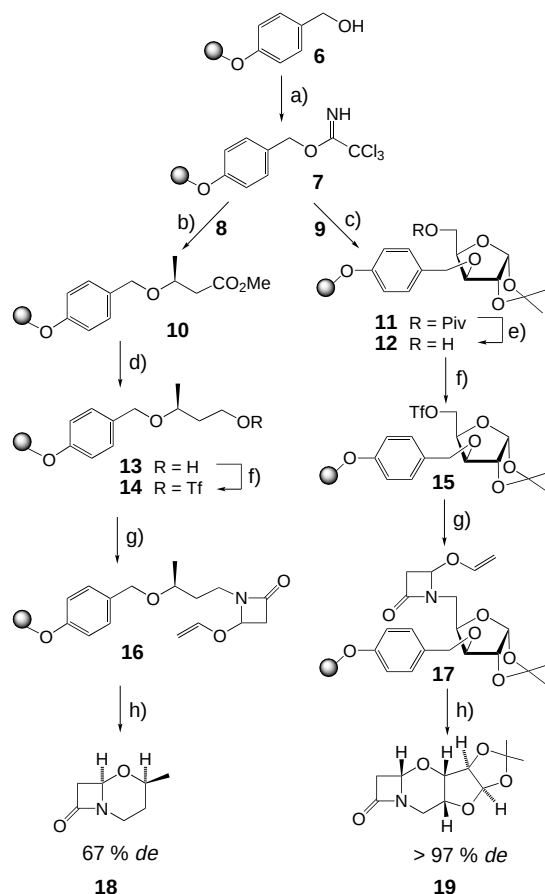
Scheme 1. Synthesis of 1-oxacepham **5**.^[4,5] TfO = trifluoromethylsulfonyl, PMB = 4-methoxybenzyl.

group increases the nucleophilicity of the oxygen atom and allows efficient cyclization with the liberation of the 4-methoxybenzyl cation. Further studies on the cyclization step have shown that conversion of the vinyloxy into the acyloxy group is not essential to perform the cyclization.^[5] The 4-vinyloxy-*N*-substituted- β -lactam **3** gave the 1-oxacepham **5**, in 51% yield in the presence of Lewis acids. It should be pointed out that the strategy based on N-alkylation prior to the cyclization step when applied to chiral compounds **2** and **1** as a starting material has offered better asymmetric induction at C-4 of the azetidin-2-one ring than that found for the commonly used condensation of 4-acetoxyazetidin-2-one with chiral alcohols.^[6]

A new concept in solid-phase synthesis is the so-called cyclization/cleavage approach.^[7–10] The last step of the reaction sequence on the solid support involves formation of the oxazine ring and proceeds with simultaneous cleavage of the molecule from the resin. The first solid-phase synthesis in which cyclization was performed on a solid support was reported by Hobbs DeWitt et al.^[7] in the synthesis of hydantoin libraries. Most of the reports that take advantage of cyclization/cleavage strategy result in the formation of an amide function.^[8] The metathesis for the synthesis of cyclo-

olefins^[9] and formation of macrocyclic lactones by a intramolecular ketophosphonate–aldehyde condensation reaction have been reported recently.^[10] The high purity of the detached final products is an important advantage of the well designed cyclization/cleavage strategy because only the required precursor of the target molecule undergoes cyclization.

Our synthetic strategy is outlined in Scheme 2. Commercially available Wang resin **6** (Rapp polymer, 1.13 mmol of OH per gram of resin) was treated with trichloroacetonitrile



Scheme 2. Solid-phase synthesis of 1-oxacephams **18** and **19**. a) CCl_3CN , DBU, CH_2Cl_2 , 20°C, 3 h; b) methyl (*S*)-3-hydroxybutyrate (**8**), $\text{BF}_3 \cdot \text{Et}_2\text{O}$, $\text{CH}_2\text{Cl}_2/\text{cyclohexane}$ 1/1, 20°C, 5 min; c) 1,2-*O*-isopropylidene-5-*O*-pivaloyl- α -D-xylofuranose (**9**), $\text{BF}_3 \cdot \text{Et}_2\text{O}$, $\text{CH}_2\text{Cl}_2/\text{cyclohexane}$ 1/1, 20°C, 5 min; d) DIBAL-H, THF, 20°C, 4 h; e) MeONa, MeOH, 20°C, 2 h; f) Tf_2O , 2,6-lutidine, CH_2Cl_2 , 0°C, 6 h; g) **1**, *n*BuLi, $\text{Bu}_4\text{NH}_2\text{SO}_4$, THF, $-70 \rightarrow -20^\circ\text{C}$, 12 h; h) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , 20°C, 3 h. Piv = pivaloyl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIBAL-H = diisobutylaluminum hydride.

and DBU and transformed into the Wang trichloroacetimidate resin **7**, which was recently shown to be a polymer-bound benzylating reagent and capable of immobilizing alcohols under mild conditions.^[11] The resin **7** was coupled with the chiral methyl (*S*)-3-hydroxybutyrate (**8**) or with the 1,2-*O*-isopropylidene-5-*O*-pivaloyl- α -D-xylofuranose (**9**) in the presence of catalytic amounts of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in $\text{CH}_2\text{Cl}_2/\text{cyclohexane}$ (1/1) for 5 min at room temperature according to the procedure of Hanessian and Xie^[11] to afford **10** and **11**, respectively. The IR spectra of **10** and **11** showed the absence of the strong C=N stretching band at 1664 cm^{-1} , present in the

starting polymer **7**, whereas the carbonyl stretching band at 1740 cm^{-1} and 1734 cm^{-1} , respectively, appeared. Reduction of the ester group in the resin-bound methyl (*S*)-3-hydroxybutyrate (**10**) with DIBAL-H (3 equiv) at 20°C in THF yielded the expected alcohol **13**. On the other hand the pivaloyl group of the resin-bound sugar derivative **11** was removed with sodium methoxide (10 equiv) in methanol to afford the alcohol **12**, after 2 h the infrared spectrum showed no carbonyl stretching band of the pivaloyl group.

Treatment of the resin-bound alcohols **12** and **13** with TiF_2O (5 equiv) and 2,6-lutidine (7 equiv) in CH_2Cl_2 at 0°C for 6 h, yielded the corresponding triflates **14** and **15**. The N-alkylation of 4-vinylazetidin-2-one (**1**) with triflates **14** and **15** was achieved with a mixture of tetrabutylammonium hydrogen sulphate (5.25 mmol), *n*BuLi (10.5 mmol) and **1** (5 mmol) in dry THF.^[4b] The mixture was stirred at -70°C for 20 min to generate the deprotonated azetidin-2-one. Subsequently, the resin-bound triflates **14** or **15** (2 mmol) were added and the respective solutions were allowed to warm up to room temperature and stirred overnight. The characteristic absorption bands of the carbonyl functions in the β -lactam rings at 1765 cm^{-1} and 1774 cm^{-1} of the resins **16** and **17**, respectively, proved the successful N-alkylation reaction had occurred in both cases. This N-alkylation method when applied in solution to compounds related to **14** and **15** with the 4-methoxybenzyl protection in place of the resin afforded respective *N*-substituted azetidinones in 55 % and 65 %, respectively.^[4b, 5] It should be stressed that cleavage of intermediates **16** and **17** from the resin to analyze any resin-bound by-products can not be performed because of the sensitivity of both intermediates to acid conditions.

The 1-oxacephams **18** and **19** were obtained in good overall yield (26–30 %, over six steps) and high diastereomeric purity (67 and 97 % *de*, respectively) by the cyclization/cleavage step from the corresponding resins **16** or **17** with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (1 equiv) in CH_2Cl_2 . The release of the products from the resins was completed after 3 h, and no carbonyl bands in the IR spectra of either remaining resins were observed. Also after additional treatment of the remaining resins with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ no release of the oxacephams was detectable.

In summary we have demonstrated that our synthetic strategy for the formation of 1-oxacephams can be performed on a solid phase with excellent results. The good yield and purity of the products, and simple procedure for both reaction sequences make this a very attractive method for the synthesis of 1-oxacephams.

Experimental Section

Typical procedure for a cyclization/cleavage reaction: The resin **16** or **17** (0.3 mmol) was swelled in CH_2Cl_2 (4 mL), and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.3 mmol) added. The reaction was stirred at room temperature for 3 h, then Et_3N (0.1 mL) added and stirring, continued for 10 min. The resin was separated and the solution collected. The resin was washed with CH_2Cl_2 several times, and all solutions were combined, and evaporated. The oily residue was dried in vacuo and purified by chromatography on silica gel to afford products **18** (67 % *de*) or **19** (>97 % *de*), respectively. The spectral and analytical data of **18** and **19** were identical to those published earlier.^[12]

Keywords: antibiotics • cyclizations • lactams • solid-phase synthesis

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A Method for the Selection of Catalytic Activity Using Phage Display and Proximity Coupling**

Jean-Luc Jestin,* Peter Kristensen, and Greg Winter*

Attempts have been made to endow proteins with catalytic activities by use of either design or selection strategies.^[1] Among the selection strategies employed, the use of phage display^[2–4] has facilitated the selection of proteins with binding activities.^[5] Repertoires of variant polypeptides are displayed on the surface of filamentous bacteriophages by fusion of the gene that encodes the polypeptide to that of a phage coat protein; binding of the phage to ligands coated on a solid phase thereby allows the isolation of those genes that encode polypeptides with ligand binding activities. However it has proved more difficult to apply this technology to the isolation of catalysts.

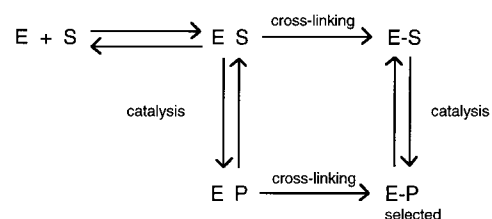
A first strategy, analogous to the methods used for the isolation of catalytic antibodies by immunization of animals with transition state analogues,^[6] involves the selection of the phage by binding of the displayed enzyme to transition state analogues and then the screening of the selected phage for catalytic activities.^[7] A second strategy involves the use of the catalytic mechanism to generate a covalent cross-link from the enzyme to a tagged mechanism-based enzyme-inhibitor,^[8] to a tagged and highly reactive moiety generated by the catalytic reaction,^[9] or to a tagged reaction intermediate.^[10] The displayed enzymes are then selected by capture of the phage by using the tag, for example with a biotin tag on a streptavidin-coated surface.

Both strategies have limitations. For example, they require a detailed knowledge of the reaction mechanism and may impose considerable challenges in the design and chemical synthesis of the substrate or transition state analogue. Furthermore such strategies may direct the selection process to a single feature of the catalytic cycle, for example to a single transition state (whereas enzyme catalysis is likely to involve multiple transition states) or to a reaction intermediate (whereas enzyme catalysis also involves product formation and release).^[11]

A third strategy has been developed recently, and was reported during the preparation of this paper, which may overcome some of these limitations.^[12] It is based on the selection of the product and the use of proximity effects. Thus a tagged substrate was cross-linked to the phage in the proximity of the displayed enzyme; the phage was thereby

attached to a solid phase and released by an intramolecular cleavage reaction catalyzed by the displayed enzyme.^[12]

We had independently devised a similar approach, as applied here to a synthetic reaction (Scheme 1). It involves two chemically independent reactions, the catalytic reaction



Scheme 1. Our strategy for the selection of catalytically active enzymes (see text for further information). E = enzyme, S = substrate, P = product.

that leads to a product (in this case distinguished by incorporation of a biotin tag) and a chemical cross-linking reaction in which the substrate (and product) is linked to the phage. Selection of the phage by streptavidin beads therefore selects for phages that are chemically attached to the tagged product; these reactions are more likely to be coupled on the same phage since reactions in a *cis* position should be favored by proximity effects over those in *trans* positions.

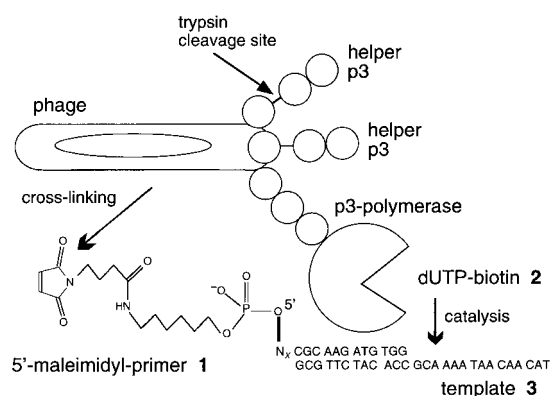
The sites for cross-linking could be engineered within the enzyme^[13] or phage,^[12] or more randomly as shown here. Indeed the use of a range of different sites for cross-linking may pose fewer steric constraints for the catalytic reaction, and provide multiple opportunities for a cross-linked substrate to be tagged. We used maleimides in a chemical cross-linking reaction, which are known to react with thiols and, in alkaline solutions, with amino groups,^[14] and are therefore capable of reacting with a wide range of sites on the phage and on the displayed enzyme. A covalently bound product between the major coat protein (p8) and *N*-biotinoyl-*N'*-(6-maleimido-hexanoyl)hydrazide, was detected by SELDI-MS^[*]. We believe that either of the two amino groups (the N-terminal Ala-1 and the residue Lys-8) are involved (data not shown in the table).^[15]

We tested the strategy using DNA polymerases in view of their central role in molecular evolution. We therefore introduced a maleimidyl group at the 5' end of a DNA primer (Scheme 2); the product was tagged by addition of biotinylated dUTP to the 3' end of the primer by the catalytic action of the polymerase. We cloned the Klenow and Stoffel fragments of DNA polymerase I from *Escherichia coli* and *Thermus aquaticus* for display by fusion to the pIII coat protein of filamentous bacteriophage (see experimental section). Both fragments lack the 5' → 3' exonuclease domains; the Stoffel fragment also lacks a 3' → 5' exonuclease activity.^[16]

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[*] Abbreviations: SELDI = surface-enhanced laser desorption and ionization; MALDI = matrix-assisted laser-desorption and ionization; PBS = aqueous solution of 0.1 M NaCl and 25 mM Na₂HPO₄ at pH 7.5, unless otherwise stated; TEN = aqueous solution of 0.3 M Tris-HCl, 33 mM EDTA, and 1.3 M NaCl at pH 9.0; dNTP = deoxynucleotide triphosphate; BSA = bovine serum albumin; EDTA = ethylenediaminetetraacetic acid; scFv = single-chain variable antibody fragment; ELISA = heterogeneous enzyme assay.



Scheme 2. Selection of catalytically active DNA polymerases obtained by phage display and proximity coupling. If the polymerase is active, then 5'-maleimidyl-derivatized primer **1** is extended with biotinylated dUTP **2** and dNTPs in a template **3** dependent manner. The maleimidyl group can react with the phage coat, and thereby allow the capture of catalytically active phage polymerases onto streptavidin-coated beads. N_{13} = TAA CAC GAC AAA G; N_{50} = T₅₀

We decided to use “monovalent” display,^[4] in which the fusion protein is cloned on a phagemid (pHEN1)^[17] and then rescued by a helper phage. The polymerase fragments were shown to be displayed on the phage (after rescue with the helper phage) by binding of the phage to wells coated with anti-polymerase antibodies (as detected by ELISA). The phages were also analyzed by Western blot with anti-p3 or anti-polymerase antibodies. This confirmed the presence of the fusion protein, but also indicated contamination by free polymerase. Presumably this arises by secretion from the bacterial host through incomplete suppression of the amber stop codon or by cleavage from the phage surface. This was removed by a further step of ultracentrifugation or by size-exclusion chromatography. The purified phages were assayed for DNA polymerase activity in a primer/template extension assay with radioactively labelled $\alpha^{32}\text{P}$ -dCTP (see experimental section) and found to be active (data not shown in the table).

However, the polymerase-p3 fusion protein was poorly incorporated into the phage compared to the p3 protein as indicated by Western blots (data not shown in the table). This appeared to be a consequence of extensive incorporation of p3 from the helper phage, as shown by our use of a helper phage (KM13) in which the p3 protein of the helper phage (but not that of fusion protein) can be cleaved with trypsin so as to render it incapable of mediating infection.^[18] Thus after proteolysis only those phages that had incorporated the fusion protein are infective; from the loss in titre after proteolysis we estimated that only one phage particle in a thousand had incorporated the fusion protein. We anticipated that the selection process, which relied on tagging by polymerase *cis*, would be compromised by such a great excess of phages that lacked the polymerase but were available for tagging *trans*. We therefore treated selected phages with trypsin to destroy the infectivity of those lacking the displayed polymerase.

We incubated the phage that displayed the Stoffel fragment with primer **13** (Table 1, which comprised a 5' maleimidyl group and a 3' biotinylated nucleotide. After incubation the

Table 1. Deoxyoligonucleotide sequences.^[a]

3: AAA TAC AAC AAT AAA ACG CCA CAT CTT GCG
4: TGG TCC CGG CGC AAG ATG TGG CGT
5: CGC AAG ATG TGG CGT
6: GTG GAC GCC ACA TCT TGC G
7: TTC AGA TAC CCA TGG CCT CTG GTG GCG GTG GCT GTG
GTG GCG GTG GCA GCC CCA AGG CCC TGG AGG AGG CC
8: GAG TCA TTC TGC GGC CGC CTC CTT GGC GGA GAG CCA
GTC C
9: TTC AGA TAC CCA TGG CCC AGG TGA TTT CTT ATG ACA ACT
ACG TC
10: GAG TCA TTC TGC GGC CGC GTG CGC CTG ATC CCA GTT
TTC GCC
11: CTC TCC CAG GAG CTA GCC ATC C
12: ACA CCC CCT CCA TGA CCT CCT TGG CCA GCC GGG CCA
CGG CCT CCG CCC TCT CTT TTG GGG CCT CGA GGA CCA GTG
CGG CGT GGA CCT GAA GGA GCA TCC
13: TTT CGC AAG ATG TGG CGT
14: GTG CCA GAC CTA GAG GCC
15: CTA TGC GGC CCC ATT CA
16: ACA GCT ATG ACC ATG ATT ACG CC
17: AAT AGG TGT ATC ACC GTA CTC AGG.

[a] Restriction sites are underlined. The TGT codon in primer **7** noted in bold encodes a cysteine at the N-terminus of the Stoffel-p3 fusion protein. Deoxyoligonucleotide **4** contains a 5'-biotinyl group and deoxyoligonucleotide **13** a 5'-maleimidyl group and a 3'-biotinyl group.

phage could be captured on streptavidin-coated beads, with a yield of about 1–5 % of infectious phage. This shows that the primer can be chemically cross-linked to the phage, presumably through the p8 protein as shown for the *N*-biotinoyl-*N'*-(6-maleimidohexanoyl)hydrazide. We then incubated phage with primer **1b**, which comprised a 5' maleimidyl group in the presence of biotin–dUTP **2** and template **3**. Capture of the phage was dependent on the presence of **1b**, **2**, and **3** (Table 2).

Table 2. Selection of catalytically active Stoffel-phage particles.

φ_i ^[a] [tu]	φ_f ^[b] [tu]	Yield [%]	Conditions ^[c]
8.4×10^5	2.0×10^4	2.4	
3.6×10^5	1.0×10^2	0.028	– primer 1b
4.4×10^5	3.0×10^2	0.068	– biotinylated dUTP 2
4.8×10^5	3.0×10^2	0.062	– template 3
4.4×10^9	4.0×10^6	0.091	– trypsin
1.5×10^9	5.5×10^5	0.037	– trypsin, – primer 1b

[a] φ_i and φ_f denote the number of transformation units (tu) prior and after the selection. [b] Yield = φ_f/φ_i . [c] with primer **1b**, biotinylated dUTP **2**, template **3**, and trypsin.

This observation shows that the primer tagged by the enzyme can be cross-linked to the phage. The addition of trypsin greatly reduced the infectivity of phages before and after capture. This suggests that many of the phages that are captured nevertheless lack the phage polymerase. The effect of trypsin, in eliminating these phages, is to greatly improve the capture yield as defined in Table 2.

We then used the selection process to select active from denatured polymerase (Table 3). We mixed Klenow-phage and Stoffel-phage particles, heated the mixture at 60 °C to favor the denaturation of the Klenow fragment, and then undertook a round of selection. Without heating we enriched the Klenow phage twofold; with heating we enriched the Stoffel phage 14-fold. The enrichment of the Stoffel phage

Table 3. Selection of Stoffel phage from Klenow phage.

Phage particles ^[a] (Stoffel/Klenow)	Enrichment in Stoffel	Conditions
(27/5) _i (21/10) _f	0.39	pH 7.5, 25 °C
(3/56) _i (23/30) _f	14	pH 7.5, 60 °C/25 °C ^[b]
(18/17) _i (110/4) _f	26	pH 8.5, 60 °C/25 °C ^[b]
(18/17) _i (22/6) _f	3	pH 8.5, 60 °C/25 °C ^[b] ; Taq 1U

[a] Ratios (Stoffel/Klenow) indicate the relative proportion of Stoffel phage and Klenow phage for the mixture before (i) and after selection (f). [b] The phage particles were treated at 60 °C for 3 min, then under catalytic and cross-linking conditions at 60 °C for 3 min followed by 1 h at 25 °C.

was improved (26-fold) at higher pH values. As expected, the addition of free polymerase resulted in a loss of enrichment (to threefold).

We also used the selection process to select between phages that displayed Stoffel fragments that bind to DNA but have different catalytic activities (Table 4). Two highly conserved

Table 4. Selection of Stoffel phage with different catalytic activities.

Phage particles ^[a] (WT/M)	Enrichment	Catalytic conditions
(4/44) _i (35/10) _f	35	1b 5 μ M, 60 °C/25 °C ^[b]
(4/44) _i (30/15) _f	20	1b 0.5 μ M, 60 °C/25 °C ^[b]
(4/44) _i (16/32) _f	5	1b 50 nM, 60 °C/25 °C ^[b]
(5/43) _i (27/21) _f	11	1b 0.5 μ M, 25 °C
(5/43) _i (40/8) _f	43	1b 0.5 μ M, 35 °C
(5/43) _i (34/14) _f	21	1b 0.5 μ M, 45 °C
(5/41) _i (45/3) _f	123	1a 5 μ M, 35 °C
(24/23) _i (20/22) _f	1	13 1.3 μ M, 60 °C/25 °C ^[b]
(38/10) _i (9/33) _f	0.071	no catalysis, DNA binding only ^[c]

[a] The ratios of wild-type Stoffel (WT) or mutant Stoffel (M) as in Table 3. [b] Polymerization and cross-linking at 60 °C for 3 min followed by 1 h at 25 °C. [c] See experimental section.

carboxylate residues are located at the active site of family I DNA polymerases such as the Klenow and Stoffel fragments.^[19] One of them (D882 in the Klenow and D785 in the Stoffel fragment) has been shown to bind a metal ion and to be directly involved in catalysis in homologous polymerases.^[20] Mutation of either residue in the Klenow polymerase leads to a major loss in catalytic activity (k_{cat}).^[21] We engineered the homologous mutations in the Stoffel fragment (D785A, D786A). As expected, mutant Stoffel phage proved much less active than the wild-type in a primer-extension assay (not shown). When the mutant Stoffel phage was mixed with the wild-type Stoffel phage, we obtained a 35-fold enrichment for the latter after the process of catalytic selection (Table 4). Using slightly different conditions ($T = 35$ °C instead of 25 °C and primer **1a** instead of **1b**), the enrichment of the Stoffel fragment could be improved to 123-fold over the less active mutant one.

The selection in favor of the wild-type Stoffel polymerase appears to reflect the catalytic rather than the binding step since the concentration of the DNA primer/template (9 μ M) is greatly in excess of its dissociation constant from Taq DNA polymerase (1.5 nM).^[22] The mutant Stoffel fragment appears to have an even better binding affinity than the wild-type Stoffel fragment. Thus when we mixed the mutant and wild-type Stoffel phage and selected for binding to duplex DNA we

obtained a 14-fold enrichment for the mutant in contrast to the selection in favor of wild-type under catalytic conditions.

Our results show that it is possible to base a selection process for enzymes that catalyze a synthetic reaction on the proximity of chemical cross-linking and catalytic tagging reactions on the same phage particle. The sites need not be adjacent; indeed the sites for catalysis and cross-linking may be separated by as much as 5.1 nm in primer **1a**. Here we let both reactions run together; the order of reactions (tagging then cross-linking, or cross-linking then tagging; Scheme 1) will depend on the relative rates of the cross-linking and tagging reactions and in turn on such factors as the concentrations of tagged substrate, the binding affinity of the enzyme for tagged substrate, and the chemical reactivity of the cross-linking moiety. In principle it should be possible to force the order of reactions, for example by first adding the reactive substrate, and later adding the tag. It should also be possible to adjust the selection procedure according to the kinetic parameters.

The strategy of proximity coupling seems likely to be general, as demonstrated now for both a cleavage^[12] and here for a synthetic reaction. It is illuminating to compare key technical features of the approach used here with that recently described.^[12] Each made use of helper phages engineered within the p3 protein but for very different purposes. We introduced a protease cleavage site to focus the selection process on those phages that displayed polymerase, whereas Pedersen and colleagues introduced a site of specific attachment for the substrate of the nuclease. We used random cross-linking of the maleimidyl group (probably to lysine residues in the major coat protein p8), whereas Pedersen and colleagues used disulfide bond formation with an engineered cysteine of the helper p3. It may be possible to combine these different features to advantage, or indeed to use the principle with other display^[23] or compartmentation strategies.^[24] This may facilitate their use for the evolution of enzymes in vitro.

Experimental Section

Deoxyoligonucleotides (Table 1) were prepared by solid-phase synthesis on a DNA synthesizer (394, Applied Biosystems) except **4**, which was provided by Eurogentec. The 5'-biotinyl group was introduced from 1-(dimethoxytriphenylmethyl)oxy-3-O-(*N*-biotinyl-3-aminopropyl)-triethyleneglycolylglyceryl-2-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite and the 3'-biotinyl group from 1-(dimethoxytriphenylmethyl)oxy-3-O-(*N*-biotinyl-3-aminopropyl)-triethyleneglycolylglyceryl-2-O-succinoyl modified pore glass. The 5'-aminohexyl modification was introduced with a 6-((4-monomethoxytriphenylmethyl)amino)hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite (Glen Research).

The deoxyoligonucleotide containing a 6-((4-monomethoxytriphenylmethyl)amino)hexyl group was purified on a C₁₈ reversed-phase HPLC column (Waters) prior to deprotection. The lyophilized product was dissolved in 0.1 mL of NaHCO₃ at pH 8.5, and added to a suspension of succinimidyl 4-(*N*-maleimidyl)butyrate (Prochem, Rockford, IL) in DMF (35 mL). After 2 h at 25 °C, the deoxyoligonucleotide containing a maleimidyl group was purified on a C₁₈ reversed-phase HPLC column, lyophilized, and characterized by MALDI-TOF-MS (Voyager DE, Perseptive Biosystems); m/z : calcd (found): **1a**: 9000.9 (9000.4), **3**: 9146.0 (9138.2), **4**: 7994.4 (7985.7), **13**: 6460.5 (6458.9).

The product bound covalently between the major coat protein (p8) of the phage (m/z 5235.7) and *N*-biotinoyl-*N'*-(6-maleimidohexanoyl)hydrazide was detected by SELDI-MS; m/z : calcd (found): 5687.2 (5689.5).

The DNA encoding the Stoffel fragment was amplified by PCR by using the template pTTQ18-TaQ and primers **7** and **8**; it was cloned as a NcoI–NotI fragment into the phagemid pHEN1^[17] to give the pHEN1–Stoffel fragment. Similarly, pHEN1–Klenow was made from the PCR amplification product of the Klenow fragment by using the template pSV–V_{NP}PolIk and primers **9** and **10**. The phagemid pHEN1–Stoffel mutant is a derivative of pHEN1–Stoffel whose NheI–XcmI fragment was replaced by the PCR amplification product by using primers **11** and **12**. Note that a free cysteine residue near the C-terminus of the Klenow fragment is lacking in the Stoffel fragment. A free cysteine was introduced at the N-terminus of the Stoffel fragment. These residues are on the far side of the polymerase away from the active site.

Phage particles were produced as described previously,^[3] except that the helper phage KM13 was used instead of VCSM13.^[18] The phage particles were purified by ultracentrifugation in a CsCl gradient followed by dialysis.^[3, 25] Size-exclusion chromatography on a column (Econo-Pac, Biorad) loaded with Sephacryl (S300, Pharmacia-Biotech) was used to purify phage polymerases (5×10^{12} tu, 10^{13} tu mL⁻¹) from free contaminating polymerase.

For ELISA, an anti-Klenow scFv isolated from a phage antibody repertoire or an anti-TaQ antibody (Taqstart, Clontech) was used for coating and an anti-M13-horseradish peroxidase (Pharmacia Biotech) used for detection in a standard protocol.^[3]

To test the catalytic activity of phage polymerases, we used a primer extension assay; 7×10^8 phage polymerases (about 7×10^{11} infective phage particles) were incubated for 10 min at 25 °C (Klenow phage) or 51 °C (Stoffel phage) in an aqueous solution (10 µL) of 15 or 150 µM of primer **5** annealed to template **6**: 250 µM dNTP, 1.5 mM MgCl₂, 33 mM α³²P-dCTP, 3.10^6 Ci mol⁻¹ in a buffer containing 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT at pH 7.5 (Klenow phage) or 10 mM Tris-HCl, 50 mM KCl at pH 9 (Stoffel phage). The reaction was stopped by addition of EDTA (0.1 M, 5 µL). Labelled oligonucleotides were detected on an polyacrylamide gel (21 %) in denaturing conditions.

In a typical selection for catalysis, phage polymerases (10^{10} infective particles, corresponding to about 10^7 infective particles after pIII cleavage with trypsin in 17 µL) in PBS were mixed with 5'-maleimidyl containing 9 µM primer **1**, 50 µM template **3**, 90 µM dATP, 0.9 µM each of dGTP and dCTP, and 0.9 µM biotinylated deoxynucleotide triphosphate **2** (Boehringer, Mannheim), 9 mM MgCl₂, and let react for one hour at 35 °C and pH 8.5. An aliquot of the phage mixture (8 µL) was then added to streptavidin-coated superparamagnetic beads (Dynabeads M-180, Dynal, 200 µL) in TEN buffer (190 µL). The mixture of phages and beads was mixed for 30 min at room temperature. The beads were then washed seven times with TEN buffer (200 µL) during 35 min and the selected phage mixture linked to the beads was finally resuspended in PBS (100 µL). The selected phage mixture (100 µL) or an aliquot of the initial phage mixture (5 µL) was incubated for 5 min at 37 °C after addition of one tenth in volume of bovine pancreas trypsin (10 g L⁻¹, Sigma). To each mixture, 1 mL of *E. coli* TG1 in 2xTY at a density of about 8×10^8 cells mL⁻¹ was added and incubated at 37 °C for 15 min. The cells were plated on ampicillin-containing plates and grown overnight at 37 °C.

The conditions used in the selection of phage polymerases by binding to DNA, were as follows: Streptavidin-coated beads (200 µL) were mixed for 30 min at room temperature with the 5'-biotinyldeoxyoligonucleotide **4** (0.3 nmol) and template **3** (0.3 nmol) in TEN buffer (100 µL). The beads were then washed three times with a PBS solution adjusted at pH 8.5 with triethylamine prior to addition of a mixture of Stoffel phage and Stoffel phage/mutant (10^{10} infective phage particles) in a PBS solution at pH 8.5. The resulting suspension was mixed for 25 min at room temperature. The beads were then washed four times with a PBS solution at pH 8.5 and trypsin was added as described previously.

The ratios between Stoffel phage and Stoffel phage mutant particles before and after selection were determined by PCR-fingerprinting as follows: Cells from single colonies were added to 19 µL of a polymerization mixture (pH 8.8) containing 0.25 mM dNTP, 0.5 µM primers **14** and **15**, 1 U Taq polymerase, 0.05 U Pfu polymerase, 20 mM Tris-HCl, 2 mM MgSO₄, 0.1 % Triton X-100, 0.1 g L⁻¹ bovine serum albumin, 10 mM KCl, and 10 mM (NH₄)₂SO₄. The amplification was made using the temperature/time sequence (°C/min): (94/10) (94/1; 61/1; 72/2)₃₀ (65/10). The restriction enzyme *AluI* (4U, New England Biolabs) was then added to the PCR

products (9 µL) mixed with a buffer (9 µL, pH 7.9) containing 10 mM Tris-HCl, 10 mM MgCl₂, 50 mM NaCl, 1 mM dithiothreitol, and the solution was incubated at 37 °C for 3 h. The resulting DNA mixtures were loaded on an agarose gel (2 %) for electrophoresis. The DNA band patterns obtained were characteristic of the polymerases: the Stoffel fragment contains a GAC GAG CTG sequence encoding amino acids 785–787 whereas the mutant Stoffel fragment contains a GCC GCA CTG sequence and lacks the AGCT *AluI* restriction site. The ratios between the number of Stoffel-phage and Klenow-phage particles were determined using the same protocol except that the amplification was done with primers **16** and **17** and the digestion using the restriction enzyme *PvuII* (9U, New England Biolabs) prior to analysis on an agarose gel (1 %).

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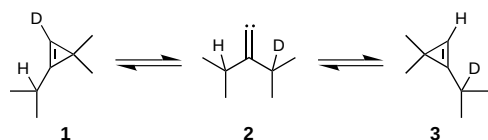
Alkyl Migration Aptitudes in the Vinylidene–Acetylene Rearrangement and Isotope Effect in the Vinylidene Formation Process from a Deuterium-Labeled Cyclopropene**

Wilhelm Graf von der Schulenburg, Henning Hopf,* and Robin Walsh*

In memory of Wolfgang Roth

Investigation of vinylidenes and their rearrangement mechanisms are difficult because good sources of these highly reactive species do not exist. They are known to be involved in the high temperature thermal rearrangements of acetylenes,^[1,2] but the very high temperature required (about 600 °C) make acetylenes an unsuitable source. We and others^[3–8] have recently shown the involvement of vinylidenes as crucial intermediates in the thermal isomerization of cyclopropene to acetylene under mild conditions. A key experimental study was that of Jones and colleagues^[5] who provided evidence for the vinylidene carbene hypothesis by means of an elegant deuterium scrambling experiment.

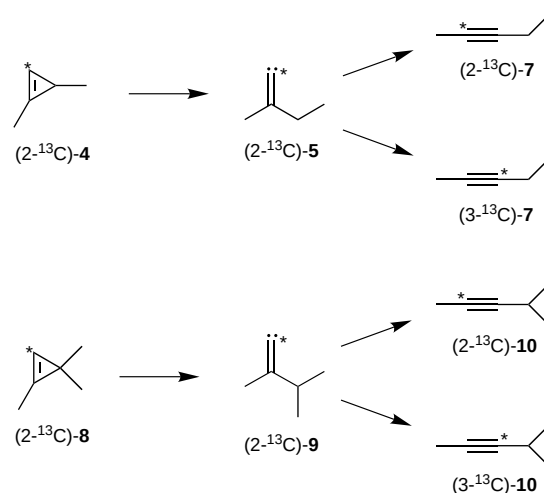
This experiment offered direct confirmation of the proposed reversibility^[4] of the cyclopropene ring-opening process, although product yields were low (0.5 % of **3** at 90 % conversion of **1**). We have also found similar evidence by



Scheme 1. Thermal generation of 1-ethyl-1-methylvinylidene **5** from cyclopropenes **4** and **6**.

generate specific vinylidenes from cyclopropenes, we have now carried out experiments to resolve the question of relative migratory aptitudes of alkyl groups in vinylidenes and we report the results here. Additionally we describe a deuterium scrambling experiment, which allows us to extract the kinetic isotope effect for the true cyclopropene ring-opening process that leads to a vinylidene.

To address the first question we have prepared specifically ¹³C-labeled cyclopropenes **4** and **8**, which are expected to yield mixtures of two differently ¹³C-labeled 2-pentyne products **7** and two differently labeled ¹³C-labeled 4-methyl-2-pentyne products **10**, respectively, on pyrolysis. Compounds (2-¹³C)-**4** and (2-¹³C)-**8** were prepared in two steps by adapting



means of a kinetic study of a nondegenerate cyclopropene–cyclopropene isomerization,^[8] namely the interconversion of 1,3-dimethylcyclopropene **4** and 1-ethylcyclopropene **6**, via 1-ethyl-1-methylvinylidene **5** (Scheme 1). Kinetic modeling of the analytical results of this study^[8] enabled us to obtain, for the first time, a quantitative measure of the relative propensities of vinylidene **5** to form stable products by C–H insertion (to form cyclopropenes **4** and **6**), and alkyl migration (to produce 2-pentyne (**7**)). However **7** can be formed from **5** by either methyl or ethyl migration and what is not known from our earlier study, or indeed any other source, is which route is preferred, and to what extent. With our proven capability to

published labeling procedures^[9–11] (use of 70 % ¹³C-enriched chloroform). Two pyrolysis runs of both pure (2-¹³C)-**4** and (2-¹³C)-**8** were used for each ¹³C NMR product sample at a pressure of 50–70 Torr and with a conversion over 95 %.^[12, 13] Their ¹³C NMR spectra were recorded using the inverse gated decoupling method to suppress NOE effects and with a pulse delay of 120 s to avoid any relaxation effects.^[14] The ¹³C shifts of the expected 2- and 3-positions of alkyne products **7** and **10** were identified by nondecoupling experiments of the pyrolyzed ¹³C samples.^[15] The distribution of the label in (¹³C)-**7** and (¹³C)-**10** was determined as the average of two to four independent runs (after correction for the only 70 % ¹³C enrichment), and the error is quoted as a single standard deviation from the mean. Pyrolysis of (2-¹³C)-**4** at 523 K gave a ratio of products [(3-¹³C)-**7**]/[(2-¹³C)-**7**] = 3.0 ± 0.1 while pyrolysis of (2-¹³C)-**8** at the same temperature gave the

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product ratio $[(3\text{-}^{13}\text{C})\text{-}\mathbf{10}]/[(2\text{-}^{13}\text{C})\text{-}\mathbf{10}] = 1.40 \pm 0.05$. Figure 1 shows the distribution of the quaternary carbon atoms of these alkynes in the ^{13}C NMR spectra. It should be noted that **7** and **10** are major products of reaction. Compound **7** represents 81 % of the products from **4**, while **10** represents 22 % of the products from **8**.

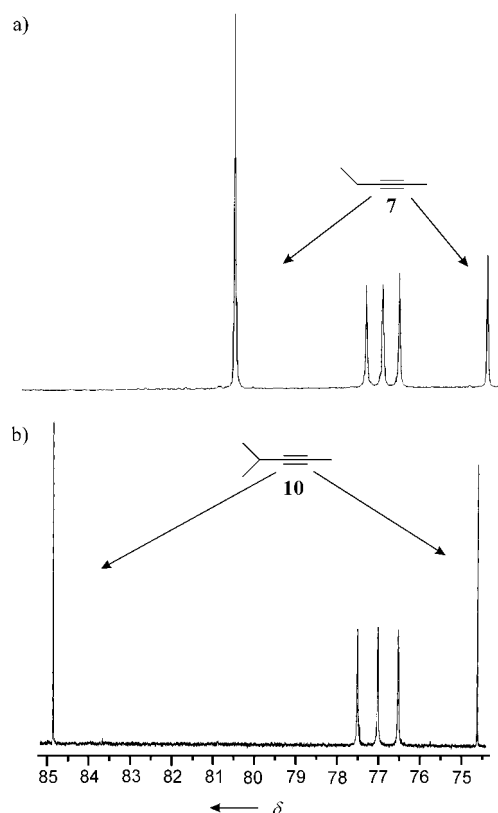


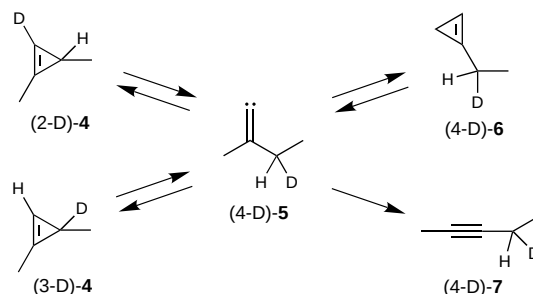
Figure 1. Distribution of the ^{13}C NMR signals (CDCl_3 , 100 MHz) for the quaternary carbon atoms in alkyne products of pyrolysis at 523 K: a) **7** from $(2\text{-}^{13}\text{C})\text{-}\mathbf{4}$; b) **10** from $(2\text{-}^{13}\text{C})\text{-}\mathbf{8}$.

Further checks showed no significant pressure or conversion dependence of the label distribution. The lack of a conversion dependence demonstrates that there is no tendency for the alkyne products **7** and **10** to undergo any degenerate scrambling processes. A small temperature dependence was detected with a label distribution ratio of 3.2 ± 0.1 for $(^{13}\text{C})\text{-}\mathbf{7}$ and 1.60 ± 0.05 for $(^{13}\text{C})\text{-}\mathbf{10}$ at $T = 482$ K, which indicates a slightly stronger selectivity for the alkyl shift (ethyl or isopropyl against methyl) at lower temperature. The preference of an ethyl or an isopropyl migration over a methyl migration in the vinylidene–alkyne reaction is unambiguously shown by these data. If we reasonably assume that the rate of methyl migration is not affected by the presence of the other alkyl substituent, this establishes a non-monotonic migration order with relative rates of Me:Et:Pr of 1:3.0:1.4. This is the first report of this migration sequence. Likhotvorik et al.^[5] attempted this measurement for vinylidene **9** with ^{12}C enrichment, but the small size of the difference signals meant that the authors could only report that both methyl and isopropyl groups migrated, but not their relative rates. The non-monotonic reactivity order is reminiscent of the sequence Me:Et:Pr of 1:1.40:0.47 obtained by

Casanova et al.^[17] in the isoelectronic isonitrile–nitrile ($\text{RN}\equiv\text{C} \rightarrow \text{RC}\equiv\text{N}$) rearrangement, except that the rate for the isopropyl group is even slower than that for the methyl group.

For the vinylidene rearrangement, as for the isonitrile isomerization, hyperconjugative effects that lead to a positively charged migrating carbon atom in the transition state will mean stabilization of the transition state by larger alkyl groups, and an expected rate acceleration. However, for the more bulky groups (isopropyl, or larger), statistical and steric effects may well play a role that lead to rate decelerations. A combination of these two effects offers a plausible explanation of the present findings. It is also consistent with the (admittedly small) temperature dependencies of these relative migratory aptitudes.

To address the second question we have prepared deuterium-labeled 1,3-dimethylcyclopropene $(2\text{-D})\text{-}\mathbf{4}$, which because of our previous kinetic analysis of the pyrolysis of unlabeled **4**^[8, 13] we expect to generate significant yields of $(3\text{-D})\text{-}\mathbf{4}$ upon pyrolysis (Scheme 2). Compound $(2\text{-D})\text{-}\mathbf{4}$ was



Scheme 2. Formation of isotopomeric cyclopropene $(3\text{-D})\text{-}\mathbf{4}$ from $(2\text{-D})\text{-}\mathbf{4}$ via vinylidene $(4\text{-D})\text{-}\mathbf{5}$.

prepared in two steps by adapting published labeling procedures.^[9–11] The gas-phase kinetics of $(2\text{-D})\text{-}\mathbf{4}$ were investigated using quantitative GC analysis, followed by ^1H NMR analysis to obtain the ratio of $(2\text{-D})\text{-}\mathbf{4}$ and $(3\text{-D})\text{-}\mathbf{4}$. The kinetic studies of $(2\text{-D})\text{-}\mathbf{4}$ were performed at two temperatures (504 and 524 K) at times covering conversions (to all products) between 20 and 80 %.^[12] Five to six runs were carried out at each temperature under the same reaction conditions with undiluted cyclopropene at a total pressure of 50 Torr for ^1H NMR analysis. The proportion of $(3\text{-D})\text{-}\mathbf{4}$ was obtained by comparing the integrated signal of the hydrogen atom at position 2 ($\delta = 6.62^{[10, 13]}$) with that of the hydrogen atom at position 3 ($\delta = 1.42^{[10, 13]}$) of **4** (total of $(2\text{-D})\text{-}\mathbf{4}$ and $(3\text{-D})\text{-}\mathbf{4}$) in the ^1H NMR spectrum. This procedure provides an estimate of the proportion of $(3\text{-D})\text{-}\mathbf{4}$ in the total mixture reliable to $\pm 5\%$.

The product–time evolution curves (Figure 2) show the formation of the isotopomeric cyclopropene $(3\text{-D})\text{-}\mathbf{4}$ as a transient product with a maximal yield of about 8 %. This demonstrates clearly the thermal interconversion of the two isotopomers $(2\text{-D})\text{-}\mathbf{4}$ and $(3\text{-D})\text{-}\mathbf{4}$. It is worth noting that a small amount of the deuterium-labeled 1-ethylcyclopropene $(4\text{-D})\text{-}\mathbf{6}$ is also present. The data of Figure 2 were fitted to a kinetic model^[18] (based on an extended version of the mechanism of Scheme 2 that included all products) and optimum values of the rate constants were obtained. These were

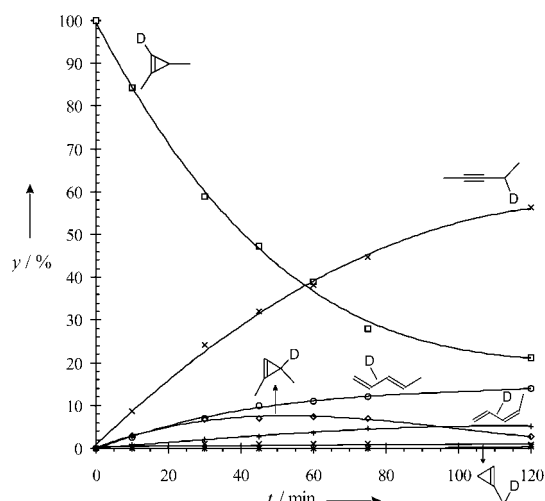
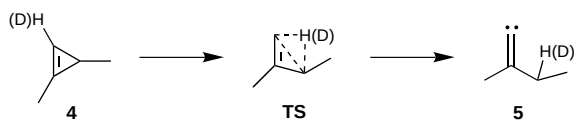


Figure 2. Product-time evolution curves for the decomposition of (2-D)-4 at 504 K (y: percentage composition of the reactants).

compared with values for the unlabeled system (extended version of Scheme 1^[8]) calculated from the appropriate Arrhenius equations at 504 K. From a steady-state analysis of the kinetic scheme^[18] these values yielded, inter alia, the isotope effect for the specific rate constant for formation of the vinylidene involved, namely for (2-D)-4 → (4-D)-5. The value of $k_H/k_D = 1.32 \pm 0.07$ obtained corresponds to the isotope effect for the ring-opening process of cyclopropene. A similar value of 1.30 ± 0.06 was obtained at 524 K.

The values of intermolecular kinetic isotope effects for unimolecular gas phase reactions are dependent not only on the reaction involved but also the conditions (temperature and pressure).^[19] For example, for the [D₆]cyclopropane case, the overall intermolecular isotope effect at 783 K and high pressures is 2.0.^[20, 21] Since this value is for the process that leads to [D₆]propene, and involves a rate-determining 1,2 H(D)-shift, this is clearly a primary isotope effect, although augmented by secondary effects. For the more specific ring-opening process of 1,2-dideuteriocyclopropane, not involving H(D)-transfer, calculations give values in the region of 1.13–1.18 at 696 K^[22, 23] for the specific secondary isotope effect per deuterium atom. Thus the value for the isotope effect found here, based on these analogues, looks to be too large to be secondary. This suggests strongly that the 1-H(D) atom is involved in the transition state (TS) in the ring-opening process of 4.



This result then is entirely consistent with the cyclopropene-vinylidene proposition. A theoretical calculation of the isotope effect has not yet been carried out but is planned. While a contribution of 1,3-biradicals to the formation of acetylenes from cyclopropenes cannot be entirely ruled out, the findings of this paper make this possibility rather unlikely.

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The First Total Synthesis of 6-Sulfo-de-*N*-acetylsialyl Lewis^x Ganglioside: A Superior Ligand for Human L-Selectin**

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Ten Feizi,* Reiji Kannagi,* and Makoto Kiso*

Selectins (E-, P-, and L-)^[1, 2] are a family of carbohydrate-binding proteins found on leukocytes, vascular endothelium, and platelets, which mediate cell adhesion through recognition and binding of cell-specific oligosaccharide ligands on a variety of glycoconjugates such as glycoproteins, glycolipids, and glycosaminoglycans. There is now a general agreement that all three selectins can bind sialyl Lewis^x (sLe^x), sialyl Lewis^a (sLe^a), and structurally related Lewis blood group oligosaccharides found on many cancer cells,^[3] and it has been suggested that the selectin–carbohydrate interactions might be involved in many physiological events such as leukocyte trafficking, inflammation, thrombosis, and cancer metastasis. This has stimulated research activities aimed at the development of novel synthetic carbohydrate compounds and their mimetics as therapeutic agents against reperfusion injury, chronic inflammatory diseases, allergy, autoimmunity, and cancer.^[4] It is also known that L- and P-selectin can efficiently bind to sulfated carbohydrates such as fucoidan, sulfatide, sulfated glucuronyl oligosaccharides, heparin, sulfo Le^x, and sulfo Le^a structures.^[1, 2, 5–7]

L-selectin (leukocyte endothelial cell adhesion molecule 1) is a lymphocyte homing receptor involved in the binding of lymphocytes to the high endothelial venules (HEV) in peripheral lymph nodes. One of the endothelium-derived counter-receptors for L-selectin is GlyCAM-1, a mucin-like glycoprotein with sulfated, sialylated, and fucosylated oligosaccharide sequences.^[8, 9] We have demonstrated with chemically synthesized gangliosides^[10a] that the sLe^x sequence with 6-O-sulfation at *N*-acetylglucosamine (6-sulfo sLe^x) is the preferred ligand for human L-selectin.^[11] In addition, anti-sialyl Lewis^x antibodies that bind to 6-sulfo sLe^x reacted to HEV in lymph nodes and inhibited the binding of L-selectin to HEV.^[10b] The 6-sulfo sLe^x sequence has also been identified

as a major carbohydrate capping group of the L-selectin ligand on HEV in human lymph nodes by using newly established monoclonal antibodies directed against the chemically synthesized sulfo sLe^x gangliosides and their derivatives.^[12] In the course of work with the chemically synthesized gangliosides, a novel de-*N*-acetylated form of 6-sulfo sLe^x has been identified as a superior L-selectin ligand to the *N*-acetyl form of 6-sulfo sLe^x. This novel molecule was originally discovered^[11] as a minor by-product of the parent 6-sulfo sLe^x hexasaccharide ganglioside. To corroborate the superior reactivity of this novel molecule we undertook, selectively, for the first time, the total synthesis of the 6-sulfo de-*N*-acetylsialyl Le^x ganglioside **1** (see Table 1 for spectroscopic and physical data), and examined its binding by human L-selectin.

The most important problems in the total synthesis of the title compound **1** are a) selective protection of the amino group in neuraminic acid, b) regio- and α -stereoselective glycosylation of sialic acid, c) selective protections of the 3- and 6-hydroxyl groups of *N*-acetylglucosamine (GlcNAc) that undergo fucosylation and sulfation, respectively, and d) efficient construction of the glycolipid structure. The first problem was solved by protecting the amino group of neuraminic acid with the trifluoroacetyl (TFAC) group, which

Table 1. Selected physical data of **4**, **7**, **10**, **12**, **14**, and **1**.

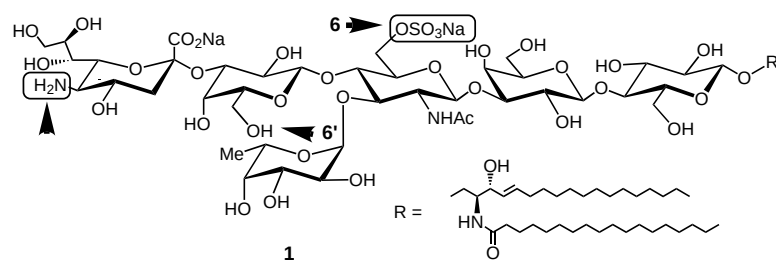
4 : ¹ H NMR (500 MHz, CDCl ₃): δ = 1.04 (m, 2H, CH ₂ SiMe ₃), 1.99, 2.01, 2.12, 2.17 (4 × s, 12H, 4AcO), 2.74 (dd, 1H, $J_{\text{gem}} = 13$, $J_{3\text{eq},4} = 4.6$ Hz, H-3b _{eq}), 3.67 (s, 3H, COOMe), 4.44 (dd, 1H, $J_{1,2} = 7.8$ Hz, H-1a), 4.97 (m, 1H, $J_{3\text{ax},4} = J_{4,5} = 10.5$ Hz, H-4b), 5.28 (dd, 1H, $J_{6,7} = 2.1$, $J_{7,8} = 8.9$ Hz, H-7b), 5.35 (s, 1H, CHPh), 5.42 (m, 1H, H-8b), 7.25–7.48 (m, 5H, Ph); ¹⁹ F NMR (476.5 MHz, CDCl ₃) δ (relative to fluorobenzene) = 37.03 (s, CF ₃ CO); $[\alpha]_{\text{D}}^{25} = -3.9^\circ$ ($c = 1.3$ in CHCl ₃).
7 : ¹ H NMR (400 MHz, CDCl ₃): δ = 1.00 (m, 2H, CH ₂ SiMe ₃), 1.41, 1.48, 1.94, 2.00, 2.04, 2.06, 2.10, 2.18 (8 × s, 24H, 6AcO, AcN, and AcCH ₂ CH ₂), 2.51 (m, 4H, AcCH ₂ CH ₂), 2.53 (dd, 1H, $J_{\text{gem}} = 13$, $J_{3\text{eq},4} = 4.8$ Hz, H-3e _{eq}), 3.75 (s, 3H, MeOPh), 3.84 (s, 3H, COOMe), 5.30 (dd, 1H, $J_{1,2} = 8.1$, $J_{2,3} = 10$ Hz, H-2d), 6.76–8.21 (m, 40H, 8Ph); $[\alpha]_{\text{D}}^{25} = +11.3^\circ$ ($c = 1.1$ in CHCl ₃).
10 : ¹ H NMR (500 MHz, CDCl ₃): δ = 0.99 (m, 2H, CH ₂ SiMe ₃), 1.21 (3H, $J_{5,6} = 6.4$ Hz, H-6f), 1.48, 1.90, 1.93, 2.02, 2.03, 2.04, 2.05, 2.08, 2.15, 2.18 (10 × s, 30H, 8AcO, AcN, and AcCH ₂ CH ₂), 2.46–2.54 (m, 4H, AcCH ₂ CH ₂), 2.57 (dd, 1H, $J_{\text{gem}} = 12.4$, $J_{3\text{eq},4} = 4.1$ Hz, H-3e _{eq}), 3.85 (s, 3H, COOMe), 5.07 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1f), 7.06–8.15 (m, 40H, 8Ph); $[\alpha]_{\text{D}}^{25} = -9.4^\circ$ ($c = 1.7$ in CHCl ₃).
12 : ¹ H NMR (500 MHz, CDCl ₃): δ = 1.24 (d, 3H, $J_{5,6} = 6.4$ Hz, H-6f), 1.48 (s, 3H, AcN), 1.94–2.20 (16 × s, 48H, 15AcO and AcCH ₂ CH ₂), 2.56 (dd, 1H, $J_{\text{gem}} = 12.6$, $J_{3\text{eq},4} = 4.4$ Hz, H-3e _{eq}), 2.61–2.80 (m, 4H, AcCH ₂ CH ₂), 3.87 (s, 3H, COOMe), 6.47 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1a), 7.52–8.17 (m, 5H, Ph), 8.66 (s, 1H, C=NH); $[\alpha]_{\text{D}}^{25} = +5.8^\circ$ ($c = 1.3$ in CHCl ₃).
14 : ¹ H NMR (400 MHz, CDCl ₃): δ = 0.88 (t, 6H, $J_{\text{vic}} = 6.6$ Hz, 2MeCH ₂), 1.46 (s, 3H, AcN), 1.63–2.22 (15 × s, 45H, 15AcO), 2.57 (dd, 1H, $J_{\text{gem}} = 12$, $J_{3\text{eq},4} = 4.4$ Hz, H-3e _{eq}), 3.89 (s, 3H, COOMe), 5.86 (dt, 1H, $J_{4,5} = 13.5$, $J_{5,6} = 7.0$ Hz, H-5 of sphingosine), 7.42–8.18 (m, 10H, 2Ph); ¹⁹ F NMR (476.5 MHz, CDCl ₃) δ (relative to fluorobenzene) = 36.82 (s, CF ₃ CO); $[\alpha]_{\text{D}}^{25} = -17.6^\circ$ ($c = 1.2$ in CHCl ₃).
1 : FAB-MS (negative ion mode, triethanolamine matrix): m/z : 1795.6 [$M - H + Na$] [–] , 1773.0 [$M - H$] [–] , 1750.6 [$M - Na$] [–] , 1500.8 [$M - H - \text{neuraminic acid sodium salt}$] [–] , 1479.8 [$M - \text{neuraminic acid sodium salt} - Na$] [–] , 1339 [$1500.8 - \text{Gal}$] [–] , 1317.8 [$1479.8 - \text{Gal}$] [–] , 888.7 [lactosylceramide] [–] , 726.7 [glucosylceramide] [–] , 564.5 [ceramide] [–] ; calcd for C ₇₇ H ₁₃₆ N ₃ Na ₂ O ₃₇ S ($M - H$): 1772.8369; found: 1773.05.
FAB-MS (negative ion mode, [18]crown-6 in 3-nitrobenzyl alcohol matrix): m/z : 1750.9 [$M - Na$] [–] , 1728.90 [$M - 2Na + H$] [–] , 1479.2 [$1728.9 - \text{neuraminic acid}$] [–] ; calcd for C ₇₇ H ₁₃₇ N ₃ NaO ₃₇ S ($M - Na$): 1750.8549; found: 1750.64.

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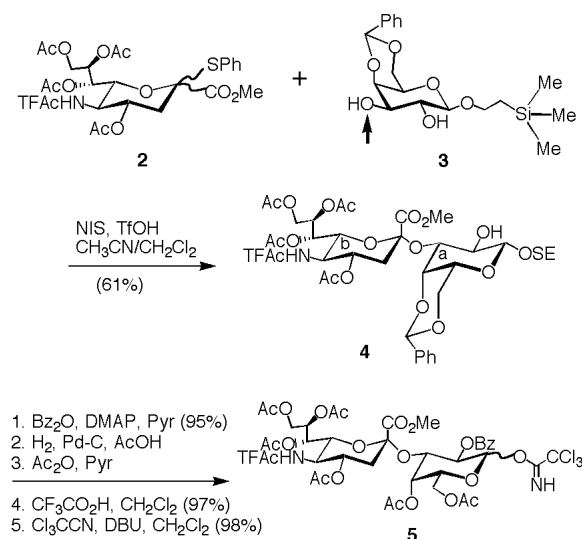
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is stable in acidic conditions but can be readily removed by alkaline treatment. For the second problem, we have already developed^[13] a highly efficient, regio- and α -stereoselective glycosylation of sialic acid by using the 2-thioglycoside derivatives of sialic acid as glycosyl donors. The third problem could be solved by constructing the trisaccharide building block **6**^[10a] in which the 3- and 6-hydroxyl groups of GlcNAc are suitably protected by the *p*-methoxybenzyl (MPM) and levulinoyl (Lev) groups, respectively.

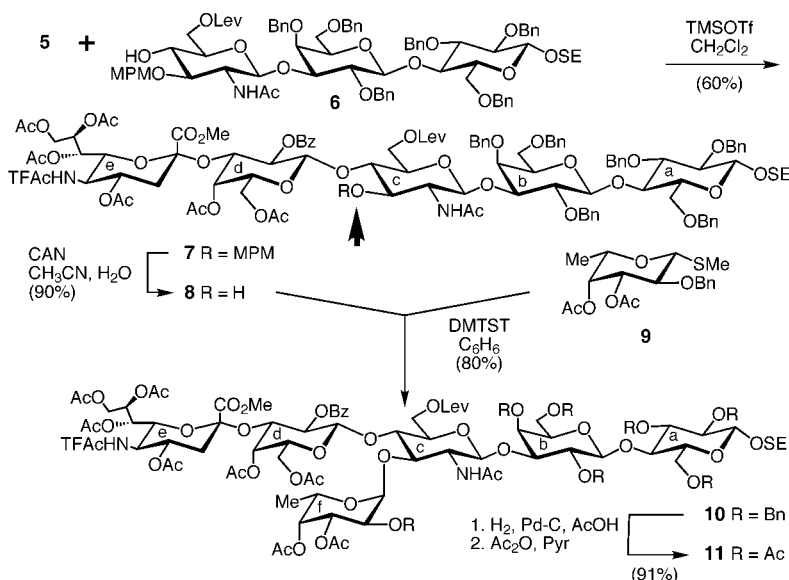
Iodonium-ion promoted,^[14] regio- and α -stereoselective glycosylation of **2**, which was readily prepared from the phenyl 2-thioglycoside of *N*-acetylneuraminic acid,^[10a] with **3**^[10a] was performed at -30°C in a solution of acetonitrile/dichloromethane (5/1) to give the desired sialyl $\alpha(2\rightarrow3)\text{Gal}$ derivative **4** in 61% yield. Compound **4** was converted into the trichloroacetimidate derivative **5** in high yield by 2-*O*-benzoylation, successive debenzylideneation and acetylation, selective cleavage^[15] of the 2-(trimethylsilyl)ethyl (SE) group, and treatment^[16] with trichloroacetonitrile and DBU, (Scheme 1).

Coupling of **5** with the suitably protected trisaccharide acceptor **6** gave the sialyl $\alpha(2\rightarrow3)\text{neolactotetraose}$ derivative



Scheme 1. Synthesis of **5**. Ac = acetyl, TFAc = trifluoroacetyl, NIS = *N*-iodosuccinimide, Tf = F_3CSO_2 , DMAP = 4-dimethylaminopyridine, SE = 2-(trimethylsilyl)ethyl, Bz = benzoyl, Pyr = pyridine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

7, which was treated with ceric ammonium nitrate (CAN) for 1 h at room temperature in a solution of acetonitrile/water (9/1) to give **8** in 90% yield. The 3-hydroxyl group of the GlcNAc residue in **8** was then fucosylated with **9** in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)^[17] in benzene (Scheme 2). The resulting sialyl Le^x hexasaccharide derivative **10** (80%) was hydrogenolized, followed by treatment with

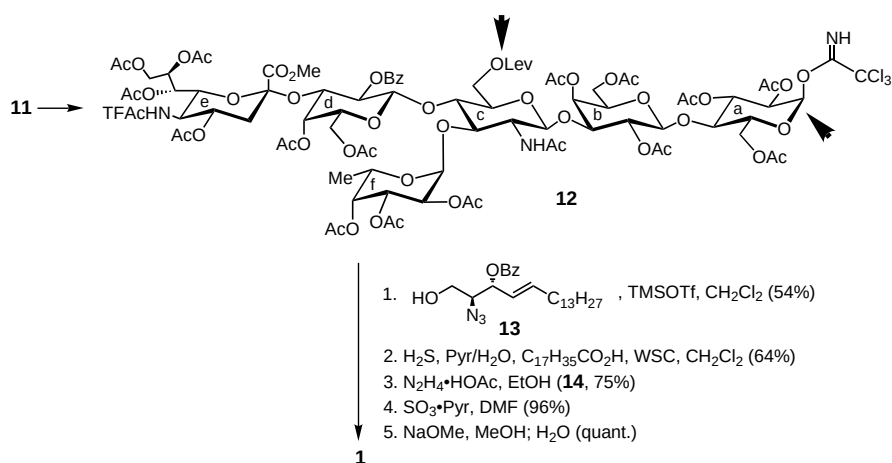


Scheme 2. Synthesis of **10** and **11** by coupling of the building blocks **5**, **6**, and **9**. Bn = benzyl, MPM = *p*-methoxybenzyl, TMS = trimethylsilyl, Lev = levulinoyl, CAN = ceric ammonium nitrate, DMTST = dimethyl(methylthio)sulfonium triflate.

Ac_2O in pyridine, to afford **11** (91%), which was converted into the trichloroacetimidate derivative **12** in nearly quantitative yield as described for **5**.

Glycosylation of the azidosphingosine^[18, 19] derivative **13** with **12**, and successive reduction of the azido group and *N*-acylation were carried out by the established method^[20–22] (Scheme 3). The levulinoyl group was selectively removed by treatment with hydrazine monoacetate in ethanol for 2 h at room temperature to give **14** without cleavage of the TFAc group. The deprotected 6-hydroxyl group of the GlcNAc residue was then sulfated in an almost quantitative yield by treatment with a sulfur trioxide-pyridine complex in DMF. Finally, removal of all protective groups under the basic conditions furnished the target molecule **1**.

In binding experiments with the multivalent human L-selectin^[11] for the immobilized synthetic ganglioside **1** and the 6-sulfo sLe^x we corroborated the superior binding to the former (Figure 1). The amounts of **1** and of 6-sulfo sLe^x required for 50% of maximal binding were 18 and 40 pmol per well, respectively. The structure of the synthetic by-product (negative control) has been identified as a 6-sulfo sLe^x derivative containing “lactamized” neuraminic acid.^[11] We observed previously^[11] that 6-*O*-sulfation at the GlcNAc residue of the sLe^x (6-sulfo sLe^x) sequence results in substantial enhancement of the binding activity, while the 6'-sulfo sLe^x sequence (6-*O*-sulfation at the Gal residue) gives



Scheme 3. Synthesis of 6-sulfo-de-*N*-acetylsialyl Lewis^x ganglioside **1**. WSC = *N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide hydrochloride.

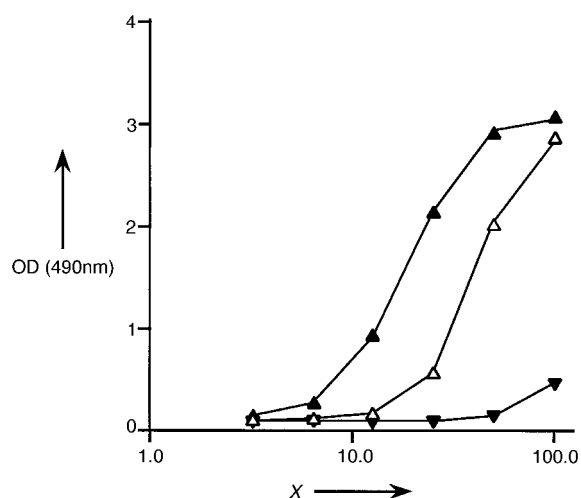


Figure 1. Binding of multivalent L-selectin (50 ng per well) to the chemically synthesized 6-sulfo-de-*N*-acetyl-sialyl Lewis^x (**1**) and related sLe^x structures. *X*: glycolipid added (pmol per well). ▲: **1**, △: 6-sulfo sLe^x, ▼: synthetic by-product (negative control) with "lactamized" neuraminic acid. For further information see the text.

little or no detectable binding. In addition, the presence of the additional sulfate as in 6,6'-bis-sulfo sLe^x results in impairment of the binding.^[11] In conclusion, the order of binding ability of the sLe^x series with L-selectin was **1** > 6-sulfo sLe^x > (nonsulfated) sLe^x[20b] = 6,6'-bis-sulfo sLe^x ≫ 6'-sulfo sLe^x. We have raised the possibility that the de-*N*-acetylsialyl(neuraminyl) forms of 6-sulfo sLe^x and related structures may be among the high affinity endogenous ligands for L-selectin on HEV that are involved in the interaction of leukocytes with the vascular endothelium. This question will be solved by generating specific antibodies to 6-sulfo-de-*N*-acetyl sLe^x and related oligosaccharide structures by using the chemically synthesized gangliosides.

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Metabolites with a Novel C₃₀ Backbone from Marine Ciliates**

Graziano Guella, Fernando Dini, and Francesco Pietra*

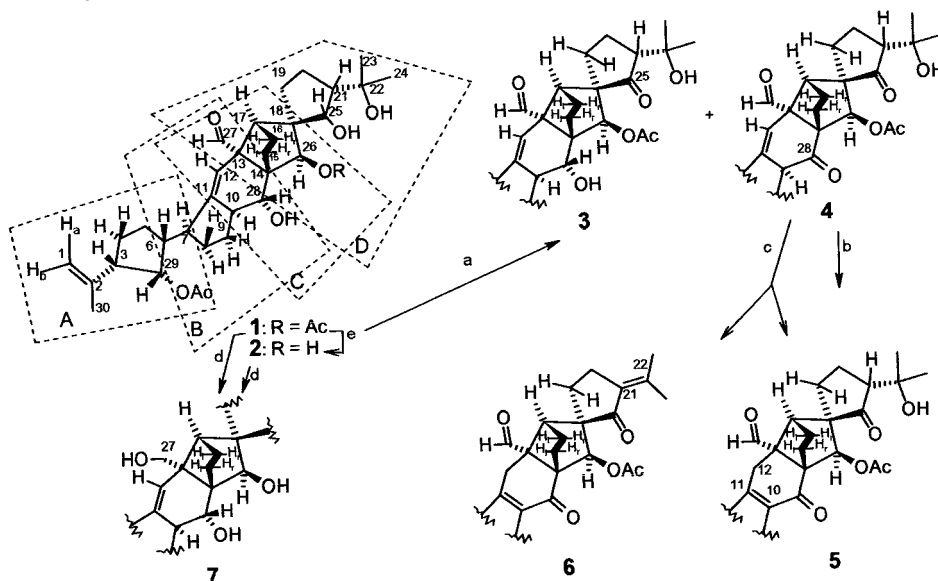
In memory of Antonino Fava

Unicellular organisms in the phylum Ciliophora (the ciliates) are a recent addition to marine microbes that are of growing interest as sources of secondary metabolites, particularly unusual sesqui- and diterpenoids.^[1] We describe here two compounds with an unusual C₃₀ backbone isolated from tropical strains of *Euplotes vannus*, an interstitial ciliate which plays a fundamental role in matter recycling within the marine ecosystem, which demonstrate the diverse nature of compounds produced by these organisms.

The structures of the new compounds, vannusal A (**1**) and vannusal B (**2**), isolated in this order of abundance from strain Sil21, and in the reverse order of abundances from strain BUN3 of *E. vannus*, and those of their derivatives **3–7** (Scheme 1), were fully assigned, with the exception of the absolute configuration, by a combination of mass spectrometry (MS) and NMR spectroscopy (Table 1).

With **1**, fast-atom bombardment (FAB) MS showed a protonated molecular ion ($[M+H]^+$ m/z 585) and a fragment ion m/z 507 ($[M+H-H_2O-CH_3COOH]^+$), whereas high resolution electron-impact MS measurements on the highest mass peak m/z 506 ($[M-H_2O-CH_3COOH]^+$) established the composition C₃₄H₄₈O₈ and thus the degree of unsaturation and the number of rings, which were in agreement with the NMR spectra. These allowed us to identify four structural blocks, A–D, which are partly superimposed and which define the entire molecule. Block A is characterized by

the observations that the pivotal H29 is ³*J* heterocoupled with C29–O–C=O, C4, and C5, and homocoupled and NOE-related with H3 and H6. Block B is defined by ³*J* couplings of H7 with C10 and C12, H12 with C7 and C14, H27 with C14, and H28 with C9, C11 and C13. Moreover, a large coupling of H6 with H7 infers the dominance of a *trans*-diaxial conformation, which is in agreement with the lowest energy conformation calculated by molecular-mechanics (Figure 1). The allylic positions of H7 and H10 are confirmed both by their deshielding and ⁴*J* coupling with H12. The pseudoaxial position for C28–OH is supported by a small coupling *J*_{10,28}. Further support for the linkage between blocks A and B is given by NOESY maps for H29 with H_β8, H7 with H_α8 and H10, H12 with H₂5, and H28 with H10 and H_β9. Block C,



Scheme 1. Structure of vannusal A (**1**) and vannusal B (**2**) and their transformation products. a) PCC in CH₂Cl₂; b) slow on standing in CDCl₃ at RT; c) fast in CH₂Cl₂ containing catalytic *p*-toluenesulfonic acid; d) NaBH₄ (3 equiv) at RT, 20 min; e) KOH/MeOH, RT, fast.

which is critical because of the high density of quaternary stereocenters^[2] at C13, C14 and C18 (which poses as many problems for assignment as it will in any attempt at the total synthesis of vannusal A) and problems arising from the spin isolation of C11, is defined by the coupling pattern of H26 (³*J* heterocoupling with C26–O–C=O, C15, and C28, and ⁴*J* homocoupling with H₁15), the coupling of H₁15 with both protons at C16, and, for H17, ³*J* homocoupling with H₁16 and ³*J* heterocoupling with C14, C15, and C26. The pseudoequatorial position of C26–OAc and the bridgehead configuration are supported by NOE enhancements for H27 with H17 and

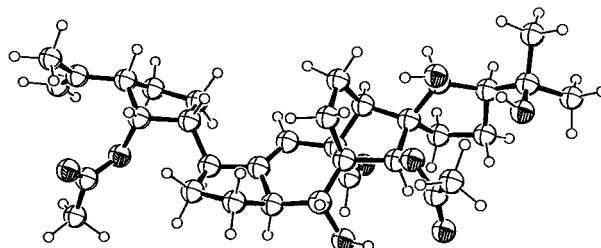


Figure 1. Lowest energy conformation of vannusal A (**1**) from molecular mechanics calculations.

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Table 1. NMR spectral data for vannusal A (**1**) in CD₃OD and C₆D₆ (¹H at 600 MHz, ¹³C at 150 MHz).

Atom	In CD ₃ OD	¹³ C	In C ₆ D ₆	¹³ C
1	$\begin{cases} \alpha \text{ 4.757 dq (1.4, 0.9)} \\ \beta \text{ 4.825 q (1.4)} \end{cases}$	111.45 t	$\begin{cases} \alpha \text{ 4.881 dq (1.4, 0.9)} \\ \beta \text{ 4.951 q (1.4)} \end{cases}$	111.60 t
2	—	144.78 s	—	144.32 s
3	2.547 br. ddd (3.3, 7.9, 11.4)	52.11 d	2.197 br. ddd (3.5, 7.4, 11.8)	51.74 d
4	$\begin{cases} \alpha \text{ 1.99 m} \\ \beta \text{ 1.78 m} \end{cases}$	26.61 t	$\begin{cases} \alpha \text{ 1.98 m} \\ \beta \text{ 1.62 m} \end{cases}$	26.30 t
5	$\begin{cases} \alpha \text{ 1.93} \\ \beta \text{ 1.74} \end{cases}$	28.97 t	$\begin{cases} \alpha \text{ 1.45 m} \\ \beta \text{ 1.65 m} \end{cases}$	28.76 t
6	2.009 dq (3.3, 9.7)	50.76 d	1.384 dq (3.5, 10.0)	50.27 d
7	2.477 dddd (1.8, 6.6, 7.8, 9.7)	45.20 d	2.385 dddd (1.3, 6.6, 8.4, 10.0)	44.53 d
8	$\begin{cases} \alpha \text{ 1.475 tdd (6.6, 11.8, 13.0)} \\ \beta \text{ 1.859 dddd (1.8, 6.6, 7.8, 13.0)} \end{cases}$	31.05 t	$\begin{cases} \alpha \text{ 1.209 tdd (6.6, 11.9, 12.6)} \\ \beta \text{ 1.817 dddd (1.8, 6.6, 8.4, 12.6)} \end{cases}$	30.63 t
9	$\begin{cases} \alpha \text{ 2.248 dddd (1.8, 6.6, 8.1, 13.0)} \\ \beta \text{ 1.309 dddd (1.8, 7.8, 11.8, 13.0)} \end{cases}$	33.65 t	$\begin{cases} \alpha \text{ 2.131 dtd (1.8, 8.4, 12.7)} \\ \beta \text{ 1.142 dtd (7.2, 11.9, 12.7)} \end{cases}$	33.62 t
10	2.278 dddd (1.8, 2.4, 7.8, 8.1)	51.82 d	2.448 dddd (1.8, 3.2, 7.2, 8.4)	51.23 d
11	—	157.08 s	—	156.54 s
12	5.555 dd (1.8, 2.4)	114.72 d	5.302 dd (1.3, 3.2)	114.32 d
13	—	69.21 s	—	68.78 s
14	—	57.59 s	—	57.34 s
15	$\begin{cases} \text{left 1.041 ddt (2.4, 2.8, 12.7)} \\ \text{right 1.975 ddd (5.7, 9.0, 12.7)} \end{cases}$	25.41 t	$\begin{cases} \text{left 1.026 ddt (1.3, 2.5, 12.7)} \\ \text{right 1.943 ddd (5.9, 9.0, 12.7)} \end{cases}$	25.30 t
16	$\begin{cases} \text{left 1.529 ddt (3.8, 5.7, 12.7)} \\ \text{right 2.493 ddd (2.8, 9.0, 12.7)} \end{cases}$	22.59 t	$\begin{cases} \text{left 1.441 ddt (3.8, 5.9, 12.7)} \\ \text{right 2.753 ddd (2.5, 9.0, 12.7)} \end{cases}$	22.45 t
17	2.635 d (3.8)	51.67 d	2.417 d (3.8)	50.74 d
18	—	56.78 s	—	55.38 s
19	$\begin{cases} \alpha \text{ 1.463 m} \\ \beta \text{ 1.545 ddd (6.3, 7.8, 12.6)} \end{cases}$	39.87 t	$\begin{cases} \alpha \text{ 1.373 m} \\ \beta \text{ 1.635 ddd (6.3, 7.8, 12.6)} \end{cases}$	38.47 t
20	$\begin{cases} \alpha \text{ 1.634 dddd (3.1, 6.3, 7.8, 12.6)} \\ \beta \text{ 1.345 dddd (6.6, 10.6, 11.4, 12.6)} \end{cases}$	24.12 t	$\begin{cases} \alpha \text{ 1.082 dddd (3.1, 6.3, 7.8, 12.6)} \\ \beta \text{ 1.405 m} \end{cases}$	23.61 t
21	1.808 ddd (6.6, 7.8, 11.4)	60.55 d	1.680 ddd (7.8, 8.3, 10.7)	57.43 d
22	—	71.90 s	—	72.30 s
23	1.238 s	29.24 q	1.118 s	30.35 q
24	1.160 s	28.18 q	1.004 s	26.33 q
25	4.162 d (6.6)	76.54 d	4.189 d (8.3)	77.56 d
26	5.160 d (2.4)	79.40 d	5.552 d (2.4)	79.62 d
27	9.690 s	200.76 d	9.690 s	198.07 d
28	3.537 d (1.8)	71.06 d	3.784 dd (1.8, 8.9)	70.40 d
29	5.433 t (3.3)	78.06 d	5.433 t (3.5)	76.69 d
30	1.762 br. t (0.9)	24.02 q	1.876 br. t (0.9)	24.45 q
C26–OC(Me)=O	—	172.10 s	—	169.75 s
C26–OC(Me)=O	1.972 s	20.94 q	1.689 s	21.12 q
C29–OC(Me)=O	—	173.00 s	—	171.63 s
C29–OC(Me)=O	2.084 s	21.53 q	1.781 s	21.34 q
HO-22	—	—	1.72 br. s	—
HO-25	—	—	2.03 br. s	—
HO-28	—	—	4.608 d(8.9)	—

H₂₆, H₁₅ with H₁₆ and H₂₈, and H₂₈ with C₂₈–OH. The W relationship between H₁₅ and H₂₈ rests on their mutual weak coupling, while the C₂₅–OH...O=C(Me)O–C₂₆ hydrogen bond, unusually strong for an eight-membered ring, is reflected in the independence of the C₂₅–OH resonance from the sample concentration in C₆D₆. Finally, block D is characterized by ³J heterocorrelations of H₂₅ with C₁₉, C₂₂, and C₂₆, H₁₇ with C₁₉, both H₂₃ and H₂₄ with C₂₁, and by ²J heterocorrelations of C₁₈ with H₂₅, H₂₆, and H₂₉. The relative configurations at both C₁₈ and C₂₅ are established by NOE enhancements for H₂₅ with both H₁₆ and H₁₇, H₂₆ with H₁₉, and H₁₇ with H₁₉.

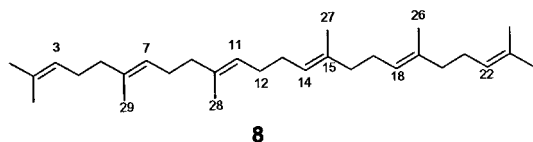
The structure of vannusal B (**2**) rests on similar spectral evidence, and further structural support for the structures of both **1** and **2** was obtained from their interconversion and

from the MS and NMR spectra of their chemical transformation products (Scheme 1). Thus, the secondary alcoholic groups of vannusal A (**1**) were oxidized by pyridinium chlorochromate (PCC) to give **4** as the major product together with minor amounts of **3** (route a). The former underwent isomerization to the α,β -conjugated enone **5** (route b), slowly on standing in CDCl₃, but more rapidly on addition of *p*-toluenesulfonic acid, which also induced dehydration to give a mixture of **5** and **6** (route c). Reduction of the aldehyde group of **1** to a primary alcohol with NaBH₄/MeOH (route d) was crucial as it allowed us to locate the cryptic environment of the aldehyde group from the differential chemical shifts in **7** (largest $\Delta\delta(^1\text{H})$ at H₁₂ (+0.25), H₁₇ (–0.34), H₇ (–0.10), H₁₀ (+0.07) and $\Delta\delta(^{13}\text{C})$ at C₁₃ (–14), C₁₂ (–11), and C₁₁ (–11)). Notably, reduction of the aldehyde group of **1** was

accompanied by regiospecific hydrolysis of the C26 ester group. This found parallels in the facile hydrolysis of **1** to **2** in basic methanol (route e) and in the clean reduction of **2** to **7** in NaBH₄/MeOH (route d).

No evidence could be obtained for microbial symbionts of *E. vannus* that could explain the formation of **1** or **2**, which are not produced by the organism food, *Dunaliella salina*. Therefore, both **1** and **2** must be products of the ciliate.

Disconnections at C3–C29, C7–C11, and C21–C25 of both vannusal A (**1**) and vannusal B (**2**) reveal the C1–C9 and C20–C25 portions of the squalene (**8**) backbone, which, however, cannot be obtained in its entirety by further bond disconnections. A route to **1** and **2** may be envisaged to occur



from a hypothetical squalene-type precursor^[3] which involves unprecedented C15–C28 and C12–14 bond formations followed by C13 extrusion. More appealing, particularly in the light that abzymes may contravene chemical rules,^[4] is an unorthodox abstraction of the disfavored primary allylic proton in the squalene pathway^[5] to close the central ring of **1** or **2** through bond C28–C14. Whether these are real possibilities is left to challenging biosynthetic experiments.

In summary, the molecular diversity described here suggests that prolific ciliates such as strains of *E. vannus* are border-zone species^[6,7] that deserve particular care in the planning of biodiversity.^[6]

Experimental Section

Stocks Sil21 and BUN3 of *E. vannus* (Müller, 1786) were collected in August 1996 along the coasts of the Siladen and Bunaken Islands, respectively, northwest of Manado, Indonesia, in the Celebes Sea. Their membership to *E. vannus*, comprising the cluster of the marine single dargyrome cirrotype 10 forms, *E. vannus-crassus-minuta*, was defined according to the classical (morphological) taxonomic practice.^[8] To obtain mass cultures, stocks Sil21 and BUN3 were grown separately in large, shallow 30 liter tanks and fed the green microalga *Dunaliella salina*. Cultures were maintained at two fissions per day by feeding excess food and were kept at 23 ± 1 °C while exposed to alternate light and darkness with a rhythm of 12 h. Centrifuged, closely-packed cells of the Sil21 strain (14 mL; about 2 × 10⁸ individuals) were suspended in ethanol which was then evaporated. The residue was subjected to high-performance liquid chromatography (HPLC) on a Merck Lichrosphere RP18 25 × 1 cm column, eluant CH₃CN/H₂O (7/3, 5 mL min⁻¹) to yield vannusal A (**1**) and vannusal B (**2**). **1**: *t*_R = 8.7 min, 9.3 mg; m.p. (water): 95 °C; [α]_D²⁰ = –17, [α]_D³⁰ = –287 (*c* = 0.3, MeOH); CD (MeOH): λ_[nm] (Δε [deg L mol⁻¹ cm]) = 229 (+6.2), 304 (–5.4); UV (MeOH): λ_{max} [nm] (ε [M⁻¹ cm⁻¹]) = 229 sh (6600), 304 (1300); **2**: *t*_R = 6.2 min, 1.4 mg; amorphous powder; [α]_D²⁰ ≈ 0, [α]_D³⁰ = –85 (*c* = 0.05, MeOH); CD (MeOH): λ_[nm] (Δε [deg L mol⁻¹ cm]) = 228 (+5.4), 308 (–3.1); UV/Vis (MeOH): λ_{max} [nm] (ε [M⁻¹ cm⁻¹]) = 229 (5100), 301 (1100). NMR spectra were recorded with Bruker AMX-600 and Varian XL-300 spectrometers equipped for inverse detection; chemical shifts are reported relative to residual solvent signal (for CD₃OD δ(¹H) = 3.310 and δ(¹³C) = 49.00; for C₆D₆ δ(¹H) = 7.150 and δ(¹³C) = 128.50) and *J* in Hz at probe temperature 20 °C. A conformational space search was carried out with the program GMMX (steric energy minimization program based on the MMX force field by Serena Software, Bloomington, IN) and minima were refined with the MM3(96) program (by N.L. Allinger et al., distributed by QCPE, Indiana University, 17/1,

February 1997) by the block diagonal Newton-Raphson energy minimization method with default parameters.

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Molecular Recognition within a Self-Assembled Cylindrical Host**

Thomas Heinz, Dmitry M. Rudkevich, and Julius Rebek, Jr.*

We recently reported the synthesis and characterization of the capsular assembly **1** (Figure 1).^[1] It is a cylinder-shaped dimer held together by eight bifurcated hydrogen bonds in apolar organic solvents. The dimer has the capacity to select one large molecule or two small molecules from the bulk solution and encapsulate them reversibly. Further expressions of the capsule's unique behavior have emerged from the use of rigid, flexible and complex guests, and we relate them here.

Rigid structures such as stilbenes **2–5** offer a set of “rulers” for measuring the interior dimensions of the capsule (Figure 2). The (*E*)-4,4'-dimethylstilbene **2** is indeed readily-

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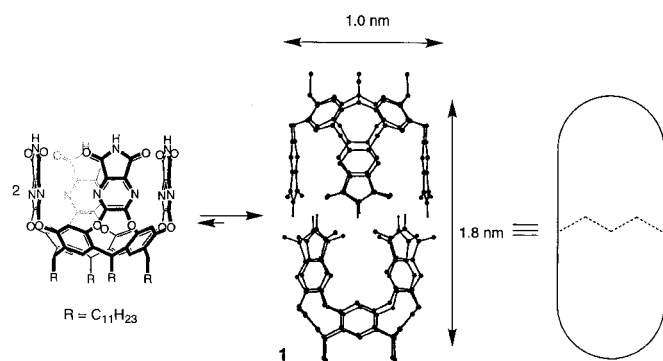


Figure 1. Self-assembly and structure of capsule **1**. In the center is the energy-minimized^[4] (MacroModel 5.5, Amber* force field) structure; the long alkyl chains and CH hydrogen atoms are omitted for clarity. Right: The cartoon representation^[1] used in Figures 2–6.

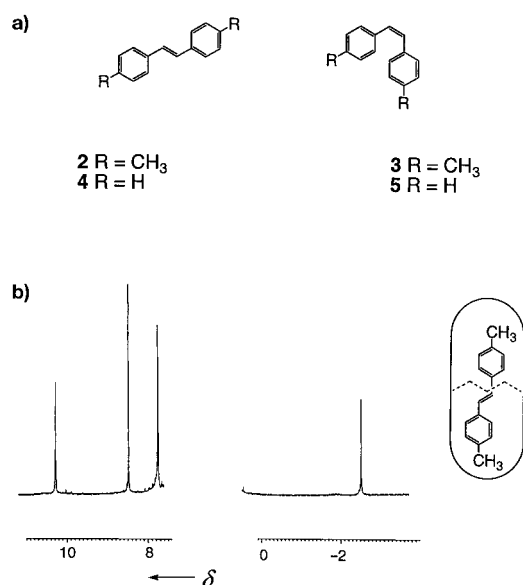


Figure 2. a) Stilbenes **2**–**5**. b) Downfield and upfield regions of the ¹H NMR spectrum^[2] upon encapsulation of (*E*)-stilbene **2**.

encapsulated by **1** in [D₁₂]mesitylene, and relevant regions of the NMR spectrum are shown in Figure 2b.^[2] The large upfield shifts of the guest methyl groups ($\delta \approx -2.8$, $\Delta\delta \approx 5$) place them near the ends of the capsule. Initial experiments with an > 50-fold excess *Z* isomer **3** gave the same spectra as were seen with **2**, but this was traced to contamination of the sample with small amounts (some 1 to 2 %) of **2**. Direct competition experiments using **2** and **3** gave no evidence for the encapsulation of **3**; only the spectra for **2** emerged. When a smaller excess (ca 16-fold) of **3** was employed, no encapsulation was observed. Instead, the characteristic (and complex) pattern of signals observed for mesitylene occupancy persisted.^[1] The shorter unsubstituted (*E*)-stilbene **4** was also readily encapsulated, but again the *Z* isomer **5** was not. Accordingly, the selectivity **2/3** is at least 50:1 and is beyond the ¹H NMR detection limits.

Typical secondary amides such as anilide **6** and benzylamide **7** (Figures 3 and 4) are nearly as rigid as the stilbenes in that

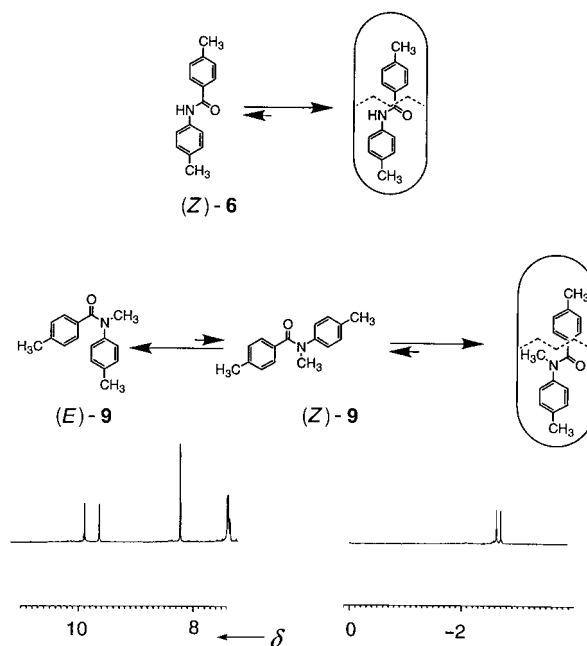


Figure 3. Anilides **6** and **9**, and portions of the ¹H NMR spectra^[2] of encapsulated (*Z*)-**9**. Downfield region: imide-NH and arene-CH signals of **1**; upfield region: methyl signals of the guest molecule.

they exist primarily in the *Z* conformation shown. Either amide is readily encapsulated by **1** in [D₁₂]mesitylene and the NMR spectra (not shown) indicate that the two ends of the complex are now different: these guests can spin rapidly about the long axis of the capsule, but can not “tumble” within, at least not on the NMR timescale. In contrast, tertiary amides are flexible structures insofar as they exist as interconverting *Z* and *E* conformations. For example, amide **8** exists as a roughly equal mixture of *E* and *Z* isomers in CDCl₃ or [D₁₂]mesitylene. Upon encapsulation, a set of upfield shifted signals emerge and these appear at nearly the same chemical shifts seen for the *Z* amide **7** inside (Figure 4).

The effect of encapsulating the tertiary anilide **9** is profound. Shudo and co-workers^[3] have shown that such tertiary anilides greatly favor the *E* conformation in CD₂Cl₂ and CDCl₃. Yet inside the capsule the signals are shifted in the just the manner observed for the *Z* secondary anilide **6** (Figure 3). Unquestionably, tertiary amides **8** and **9** are fixed in the *Z* conformation within the complex. Molecular modeling^[4] indicated that the corresponding *E* conformers cannot fit inside the capsule without severe steric problems with the walls. Whatever forces favor the *E* conformation in solution are overcome by these steric clashes and the CH- π , van der Waals and dipolar interactions offered by the interior surface of the capsule to the *Z* conformation.^[5] Since the ¹H NMR spectrum (Figure 4) did not change even when only 1.5 equivalents of **8** was present, encapsulation of this flexible molecule imposes a conformation unfavored in the bulk solution.

Hydrogen bonding stabilizes the capsule and causes dimerization of small self-complementary molecules as well. The dimers of 2-pyridone/2-hydroxypyridine and carboxylic acid are particularly well-studied cases.^[6] These molecules, specifically pyridone **10** and benzoic acid **11** were encapsulated as dimers within **1** (Figure 5 a); the ¹H NMR spectra showed the

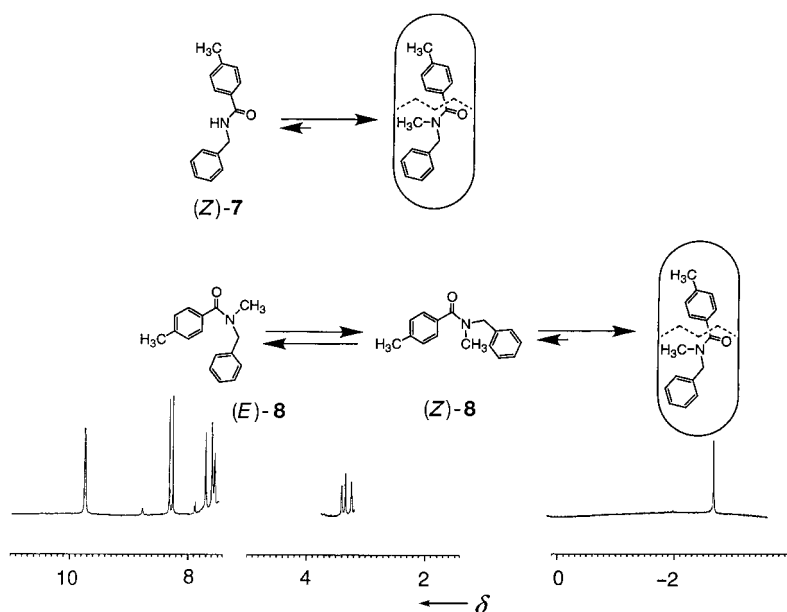


Figure 4. Amides **7** and **8**, and portions of the ^1H NMR spectra^[2] of encapsulated (*Z*)-**8**. Downfield region: imide-NH and arene-CH signals of **1**; upfield region: methyl signals of the guest molecule. The arene-CH signals of the encapsulated guest ($\delta = 4\text{--}2$) are also shown.

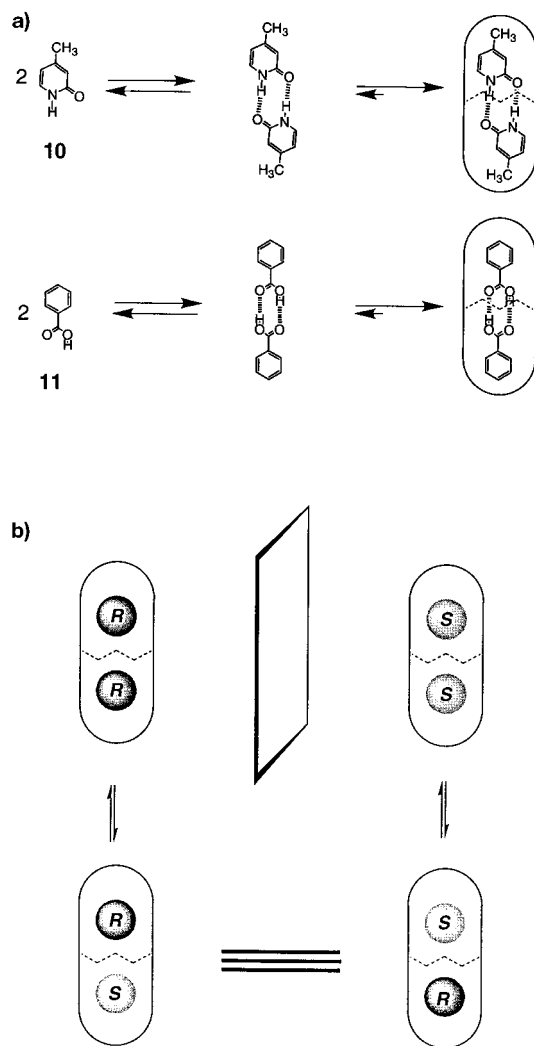


Figure 5. a) Hydrogen-bonded dimers of 4-methylpyridine-2(1*H*)-one (**10**) and benzoic acid (**11**) inside **1**. b) Cartoon representation of diastereomers formed upon encapsulation of racemic molecules.

characteristic features for the encapsulation and the integration confirmed the stoichiometry. Under the same conditions, the *p*-toluic acid dimer was not encapsulated; its length exceeds the limits imposed by the hydrogen bonds that hold the assembly together.

Encapsulation of chiral guests gives rise to diastereomeric complexes. Many cases of selective intermolecular interactions in racemic systems exist;^[7] and these attractions appear to be intensified within the confines of the capsule (Figure 5b). Two different species are observed in the presence of the racemic *trans*-1,2-cyclohexanediol **12**,^[8] while only one appears if only the single enantiomer **13** is available (Figure 6). In either case, integration indicates there are two guests inside each capsule, but the intensity of the signals for the enantiopure versus the racemic guests indicates more of the latter—the capsule prefers to be filled with a guest and its mirror image rather than two identical

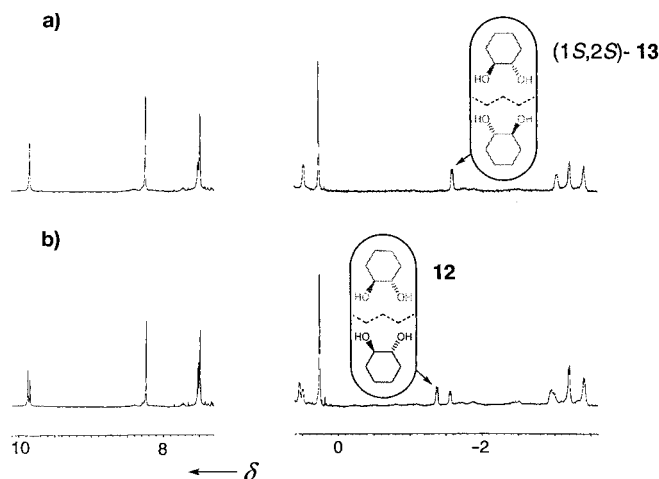


Figure 6. Portions of the ^1H NMR spectra^[2] a) encapsulated (1*S*,2*S*)-*trans*-(1,2)-cyclohexanediol (**13**) and b) encapsulated racemic *trans*-1,2-cyclohexanediol (**12**). Downfield region: imide-NH and arene-CH signals of **1**; upfield region: cyclohexane-CH signals of the guest compounds **12/13**.

molecules. The systems shown in Figure 5 and Figure 6 represent complexes within complexes, or second-order supramolecules.

Finally, the encapsulation process shows a range of rates. The uptake, release, and exchange of small guests is rapid: benzene, toluene, and xylene equilibrate with the capsule in $[\text{D}_{12}]$ mesitylene in less than one minute at ambient temperatures and NMR (millimolar) concentrations. The appropriate complexes containing two guests in each host are obtained. Likewise, a large guest (e.g. anilide **6** or stilbene **2**) replaces the poorly accommodated solvent mesitylene, on the same timescale. In contrast, the exchange rates between large guests are slow: displacement of encapsulated **2** by **6** (or vice versa) takes days to reach equilibrium. The sequence of events—hydrogen bond rupture, conformational changes, and

guest substitution—must occur for either large or small guests. It seems likely that flaps^[9] can open in the capsule to permit the exchange of small guests. The exchange of large guests may require the complete dissociation of the superstructure.

In summary, the encapsulation behavior of self-assembled capsule **1** derives from its considerable size and elongated shape. These features guarantee a selectivity for congruent molecules as guests. Even hydrogen-bonded systems—assemblies within assemblies—are temporarily frozen in space and time. The formation and dissipation of the systems ranges from seconds to days, and encapsulated species enjoy an environment insulated from the intrusions of the bulk solution where weakly bound complexes are forced to change of partners frequently. It should be possible to observe reactive intermediates whose lifetimes are on the appropriate time-scales within these chambers.

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Is the Bis(μ-oxo)dicopper Core Capable of Hydroxylating an Arene?*

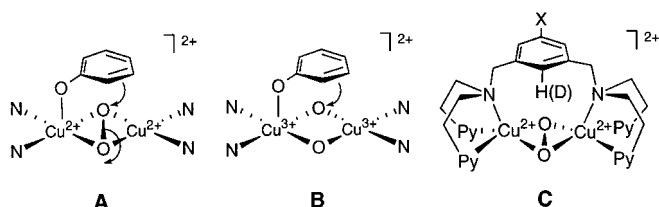
Patrick L. Holland, Kenton R. Rodgers, and William B. Tolman*

A critical mechanistic issue in C–H bond activations by metal–dioxygen species in catalytic and biological systems concerns the sequence of the O–O and C–H bond-breaking events.^[1] In the context of tyrosinase, a metalloenzyme that performs aromatic hydroxylations^[2] with O₂ via a spectroscopically characterized (μ-η²:η²-peroxo)dicopper(II) intermediate,^[3] a key question is whether this intermediate attacks the arene substrate directly (**A**), or whether the O–O bond first breaks to yield a bis(μ-oxo)dicopper unit that then performs the hydroxylation (**B**, Scheme 1). Studies of synthetic systems that model the protein active site often have used dinucleating ligands with *meta*-xylyl spacers that undergo hydroxylation upon oxygenation of their dicopper(II)

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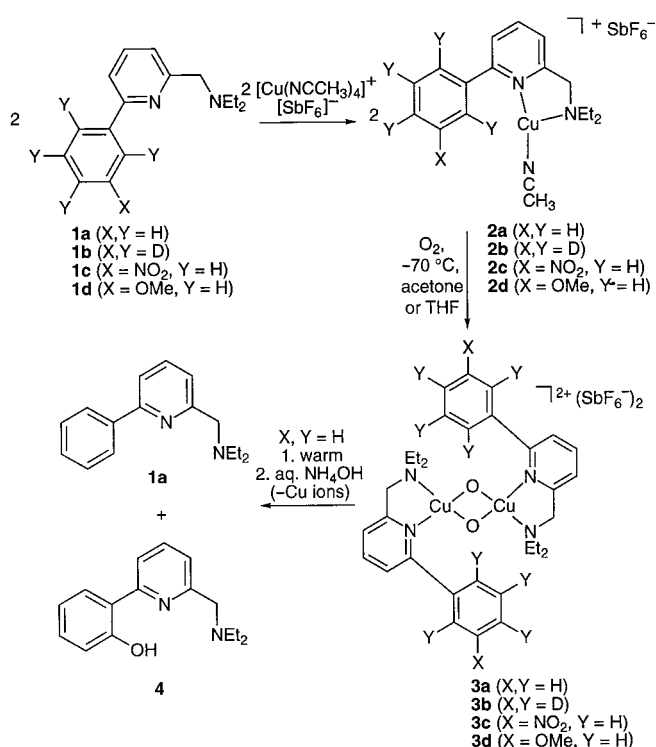


Scheme 1. Proposed intermediates in the hydroxylation of arenes. In **A** and **B** N represents nitrogen-containing ligands. Py = pyridyl.

complexes.^[4, 5] In one such system well-studied by Karlin and co-workers, there is good evidence for a (μ - η^2 : η^2 -peroxo)dicopper(II) intermediate (**C**). Electron-withdrawing groups X on the *meta*-xylyl ring slow its hydroxylation rate, and there is a negligible H/D kinetic isotope effect.^[5] Together, these results, as well as others on related systems,^[4] imply that the hydroxylation involves attack of an electrophilic peroxide oxygen on the π system of the aromatic ring, which due to its bridging position is predisposed toward this intramolecular reaction.

The discovery that $[\text{Cu}_2(\mu\text{-}\eta^2\text{:}\eta^2\text{-O}_2)]^{2+}$ and $[\text{Cu}_2(\mu\text{-O})_2]^{2+}$ units can interconvert through a low-energy pathway in a model system has raised the possibility that the above synthetic and enzymatic arene hydroxylations could proceed through the intermediacy of a bis(μ -oxo)dicopper isomer like **B**.^[6, 7] This possibility appears unlikely in the arene-bridged models, however, as indicated by the absence of spectroscopic features due to even small amounts of the *meta*-xylyl-bridged $[\text{Cu}_2(\mu\text{-O})_2]^{2+}$ unit in oxygenated solutions.^[4g, 8] Also, the dominant reaction pathway observed to date for bis(μ -oxo)dicopper complexes (heretofore capped solely by tertiary amine ligands that stabilize the formal Cu^{III} oxidation state) is intramolecular monooxygenation/N-dealkylation at aliphatic ligand C–H bonds α to the amine N-donor, even in the presence of arene appendages.^[4g, 8, 9] Herein, on the other hand, we report the facile hydroxylation of aryl rings from spectroscopically observable bis(μ -oxo)dicopper complexes supported by bidentate ligands with mixed imine/amine ligation and a pendant phenyl group. In addition to enlarging the class of bis(μ -oxo)dicopper(III) complexes to include those supported by ligands with imine donors,^[10] these results suggest that a $[\text{Cu}_2(\mu\text{-O})_2]^{2+}$ unit could attack arene substrates in tyrosinase and model compounds.

Addition of 2-(diethylaminomethyl)-6-phenylpyridine (**1a**) to one equivalent of $[\text{Cu}(\text{NCCH}_3)_4][\text{SbF}_6]$ gave the 1:1 complex **2a** (Scheme 2), which was confirmed to have a three-coordinate T-shaped geometry by X-ray crystallography [Cu–N_{amine} 2.186(3); Cu–N_{imine} 1.974(3); Cu–N_{nitrile} 1.867(4) Å].^[11] Analogous copper(I) complexes were synthesized with [D₅]phenyl (**2b**), *m*-nitrophenyl (**2c**), and *m*-methoxyphenyl (**2d**) appendages. In acetone or THF at -70°C , oxygenation of **2a–c** yielded EPR-silent yellow solutions with an optical feature ($\lambda_{\text{max}} = 404 \pm 2$ nm for **3a–c**; $\epsilon = 13 \text{ mm}^{-1} \text{ cm}^{-1}$ for **3c** in THF), which we attribute to a charge-transfer transition for transient bis(μ -oxo)dicopper species **3a–c** by analogy to previously reported data (Figure 1, Table 1).^[6, 7, 12, 13] The absorption data are not consistent with a (μ - η^2 : η^2 -peroxo)dicopper complex ($\lambda_{\text{max}} = 340\text{--}380$ nm, $\epsilon = 11\text{--}23 \text{ mm}^{-1} \text{ cm}^{-1}$; $\lambda_{\text{max}} = 510\text{--}580$ nm, $\epsilon \approx 1 \text{ mm}^{-1}$



Scheme 2. Synthesis and decomposition of complexes **3a–d**. Complex **3d** was not observed.

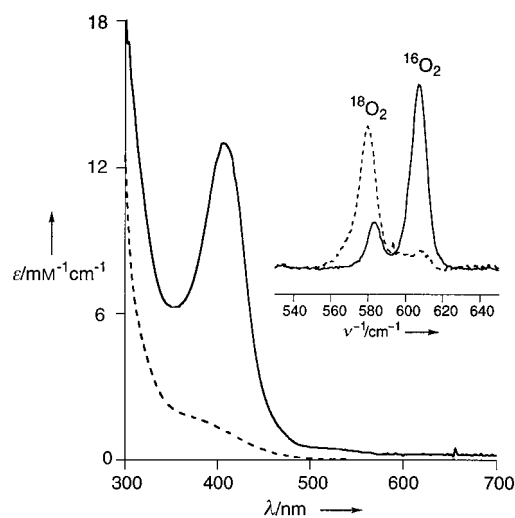


Figure 1. Absorption spectra of copper(I) complex **2c** (dashed line) and of the product of its oxygenation, **3c** (solid line), as acetone solutions at -70°C . Inset: Resonance Raman spectra of **3c** (solid line) and its ^{18}O isotopomer (dashed line), as solutions in acetone at -85°C ($\lambda_{\text{ex}} = 406.7$ nm).

cm^{-1}).^[14] Likewise, resonance Raman (RR) spectra ($\lambda_{\text{ex}} = 406.7$ nm, $T = -85^\circ\text{C}$) of solutions of **3c** in acetone (Figure 1, inset) show an intense polarized ($I_{\perp}/I_{\parallel} = 0.35$) feature at 607 cm^{-1} which shifted to 580 cm^{-1} when $^{18}\text{O}_2$ was used.^[15] Complexes with a bis(μ -oxo)dicopper core have such characteristic vibrations near 600 cm^{-1} ($\Delta(^{18}\text{O}) = 19\text{--}27 \text{ cm}^{-1}$) due to the symmetric breathing mode of the tetraatomic Cu_2O_2 unit,^[16] while (μ - η^2 : η^2 -peroxo)dicopper complexes typically

Table 1. Characterization and decomposition data for bis(μ -oxo)dicopper complexes in acetone.

Complex	$\lambda_{\text{max}}^{\text{[a]}}$ [nm]	$\tilde{\nu}(\text{Cu}_2\text{O}_2)^{\text{[b]}}$ [cm ⁻¹]	$k^{\text{[c]}}$ [10 ⁻⁴ s ⁻¹]	1,4 ^[d] [%]	Yield ^[d] [%]
3a	406	606 (578)	6 ± 2	70, 30	60
3b	406	— ^[e]	6 ± 2	70, 30	60
3c	404	606 (579)	0.4 ± 0.1	80, 0	< 10
3d	—	—	—	60, 40	80
[(L _{ME}) ₂ Cu ₂ O ₂] ²⁺ ^[f]	401	610 (587)	—	—	—

[a] Acetone solution, -70 °C for **3**; CH₂Cl₂ solution for [(L_{ME})₂Cu₂O₂]²⁺.^[7]
 [b] Values of the ¹⁸O₂ isotopomer in parentheses, λ_{ex} = 406.7 nm. [c] Acetone solution, -70 °C. [d] Yield of **4** [%] × 50 % (theoretical yield of monooxygenase reaction). [e] Not measured. [f] L_{ME} = (1*R*,2*R*)-*N,N'*-diethyl-*N,N'*-dimethyldiaminocyclohexane.^[7] [g] Recovered.

show O—O vibrations at 710–760 cm⁻¹.^[14] No O—O vibrations were observed in RR spectra (λ_{ex} = 514.5 nm) of **3c**, showing that the amount of a potential peroxo complex must be small (less than about 10 %).

When solutions of **3a–c** were allowed to stand at -70 °C or to warm, the absorption near 400 nm in the visible spectrum gradually disappeared. Although it has not yet been possible to isolate clean copper(II) decomposition products in quantity, changes in the ligand were examined by removing copper with NH₄OH and examining the organic products by GC/MS and NMR spectroscopy. Treatment of **3a** in this way gave **1a** and **4** in an approximate 2:1 ratio (Scheme 2, Table 1). In a monooxygenation reaction, one ligand per bis(μ -oxo)dicopper complex would undergo hydroxylation; thus, the observed 2:1 product ratio corresponds to about 70 % monooxygenase yield. This ratio was the same when **2a** was oxygenated at room temperature, and did not vary between acetone and THF solvents. We conclusively identified **4** on the basis of GC/MS and ¹H NMR spectroscopy; moreover, ¹⁸O labeling of **3a** and deuterium labeling of the phenyl group confirmed that the oxygen atom in **4** derives from O₂ and is incorporated into the phenyl ring. Analogous organic products were obtained from the oxygenation of **2b** and **2d**, while the oxygenation of **2c** gave no phenol-containing products.

The decay of **3a–c** (monitored by the disappearance of the absorption at ca. 400 nm) was first-order in [**3**] in each case, yielding the first-order rate constants shown in Table 1. The decomposition clearly involves reaction with the aryl group because the aryl substituents significantly influence the decay rate. There is little, if any, kinetic isotope effect on replacing the aryl C—H bonds with C—D bonds, indicating that attack on the C—H bond is not rate-determining. A substantial electronic effect on the rate of decomposition is evident: an electron-withdrawing group causes a decrease in the decay rate constant and an electron-donating substituent makes the decomposition so fast that no intermediate could be observed. These observations are reminiscent of electrophilic aromatic substitution reactions^[17] and of the electrophilic hydroxylation observed in **C**.^[5] Complicating matters, however, are the negligible yield of hydroxylation from the slow decomposition of **3c** and the high yield from the rapid decay of the presumed intermediate **3d**. These data are consistent with the operation of a competitive pathway for degradation of the bis(μ -oxo)dicopper compounds that does not yield phenolic prod-

ucts and is less dependent on the substituents on the aryl group than the hydroxylation reaction. The nature of this second pathway is currently unclear, but appears not to involve attack on solvent on the basis of identical decomposition rates in acetone and [D₆]acetone (observed with **3a** and **3c**).

We have shown that the formation of bis(μ -oxo)dicopper species is not limited to complexes capped solely by amine donor ligands; thus, the identification of compounds **3** with softer imine donors makes the biological histidine imidazolyl ligand set a plausible scaffold for the [Cu₂(μ -O)₂]²⁺ core.^[10] Complexes **3** decompose through electrophilic attack on aromatic rings, suggesting that the bis(μ -oxo)dicopper core is capable of hydroxylating an arene directly and making the possibility of stepwise O—O bond breaking followed by C—H bond activation in tyrosinase appear more feasible. Thus, aromatic hydroxylation has now been observed to arise from both isomeric [L₂Cu₂O₂]²⁺ cores: those like **C** in which the O—O bond is intact and those like **3** in which the O—O bond has already been cleaved. In each case, however, the experimental data do not allow one to rule out unambiguously rapid preequilibration of the observed (major) complex with a small amount of the other (unseen) isomer that may be more reactive. As a result, it is possible that either core is solely responsible for the observed reactivity, or that each is capable of direct attack on a properly positioned arene ring. Further experiments are necessary to resolve this dilemma.

Experimental Section

The methods used here were described previously.^[16a] Resonance Raman spectra were collected using CCD detectors interfaced with Winspec software; those with λ_{ex} = 457.9 and 514.5 nm were recorded for frozen samples at 77 K, and samples with λ_{ex} = 406.7 nm for liquid acetone samples at -85 °C. Raman shifts were referenced externally through a quadratic fit to the known spectrum of indene at room temperature.^[18] 2-(Chloromethyl)-6-phenylpyridine^[19] was isolated as the free base and purified by Kugelrohr distillation (90 °C, 0.05 Torr), and phenyl-substituted analogues were synthesized by analogous procedures.

1a: Powdered KOH (1.0 g, 56 mmol), 2-(chloromethyl)-6-phenylpyridine (0.41 g, 2.0 mmol), and HNEt₃ (3 mL) were refluxed under N₂ for 14 h, and then all volatile materials were removed in vacuo. Water (5 mL) and brine (5 mL) were added to the brown residue, and the solution was extracted with diethyl ether (2 × 20 mL). The organic fractions were dried over Na₂SO₄, dried in vacuo, and purified by Kugelrohr distillation (100 °C, 0.05 Torr), giving **1a** (469 mg, 98 %) as a pale yellow liquid. ¹H NMR (CDCl₃): δ = 7.98 (dd, 2H, *J* = 2, 8 Hz), 7.69 (t, 1H, *J* = 8 Hz), 7.56 (d, 1H, *J* = 8 Hz), 7.44 (m, 3H), 7.38 (tt, 1H, *J* = 8, 1 Hz), 3.82 (s, 2H), 2.62 (q, 4H, *J* = 7 Hz), 1.08 (t, 6H, *J* = 7 Hz); ¹³C NMR (CDCl₃): δ = 160.6, 156.3, 139.6, 136.8, 128.6, 128.5, 126.8, 120.9, 118.2, 59.4, 47.3, 12.0; correct C,H,N analysis. Ligands **1b–d** were synthesized and characterized in a similar fashion.

2a: Under N₂ atmosphere, a solution of **1a** (91.1 mg, 0.379 mmol) in anhydrous THF (1 mL) was added to [Cu(NCMe)₄][SbF₆] (175.6 mg, 0.379 mmol), causing a color change to yellow. Anhydrous diethyl ether (10 mL) was used to precipitate the product as a yellow oil, which turned into a pale yellow solid upon exposure to vacuum (198 mg, 90 %). ¹H NMR ([D₆]acetone): δ = 8.15 (t, 1H, *J* = 6 Hz), 7.92 (ddd, 2H, *J* = 1, 2, 7 Hz), 7.85 (ddd, 1H, *J* = 1, 2, 7 Hz), 7.6 (m, 4H), 4.15 (s, 2H), 2.91 (q, 4H, *J* = 7 Hz), 2.36 (s, 3H), 1.27 (t, 6H, *J* = 7 Hz); ¹³C NMR ([D₆]acetone): δ = 159.9, 159.5, 141.1, 140.7, 130.7, 129.5, 129.3, 124.4, 123.3, 118.9, 60.8, 50.3, 12.3, 2.2; Correct C,H,N analysis. Complexes **2b–d** were synthesized and characterized in a similar fashion.

Ligand recovery: In a typical experiment, **2a** (14.4 mg, 24.8 μmol) was dissolved in acetone (2 mL) in a vial under nitrogen, oxygen was bubbled through the solution at room temperature for 0.5 h, and then volatile materials were removed from the brown solution in vacuo. The residue was dissolved in concentrated NH_4OH (2 mL), and the blue mixture was extracted with CH_2Cl_2 (3×2 mL). The combined organic fractions were dried over MgSO_4 and filtered, and volatile materials were removed in vacuo to leave a yellow oil (5.0 mg). ^1H NMR spectroscopy with an internal standard (1,1,2,2-tetrachloroethane) was used to verify the yield as $>90\%$ and to determine the ratio of **1a** and **4** (7:3). GC/MS (30 m $\times$ 0.25 mm HP-5 5% crosslinked PhMe-silicone column, 1 mL min^{-1} He flow, initial solvent delay 2 min at 50°C , ramp rate $20^\circ\text{C min}^{-1}$ to 250°C): $t_{\text{R}} = 11.78$ (m/z 239, **1a**), 13.12 min (m/z 255, **4**); ^1H NMR of **4** (CDCl_3): $\delta = 14.7$ (br s, OH, exchangeable with D_2O), 7.02 (dd, $J = 8.1, 1.3$ Hz, *ortho* to OH), 6.90 (ddd, $J = 8.1, 6.9, 1.3$ Hz, *para* to OH), 3.77 (s, CH_2) (other resonances were hidden below those of **1a**). These shifts and coupling constants compare favorably to the known spectrum of 2-(2-hydroxyphenyl)pyridine.^[20]

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- [12] The extinction coefficient (in THF solution) was determined with **3c**, because its stability was greater than that of **3a**. Intense pyridine-to-metal charge-transfer bands obscured the presumed bis(μ -oxo)dicopper band at about 300 nm.
- [13] *Syn* and *anti* isomers of **3** that differ with respect to the relative position of the imine and amine donors of the two ligands are possible, but we favor the *anti* form drawn in Scheme 1 because molecular models suggest that steric clashes between arene rings prohibit adoption of a *syn* geometry. Consistent with this idea, a preliminary crystal structure of a bis(μ -hydroxo)dicopper(II) complex ligated to **2a** has the *anti* conformation (P. L. Holland, W. B. Tolman, unpublished results).
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Cyclopropanation Enantioselectivity Is Pressure Dependent in Supercritical Fluoroform**

Dolores C. Wynne and Philip G. Jessop*

In liquid solvents the enantioselectivity of asymmetric catalysis is dependent on the solvent pressure, but only over extremely large pressure ranges of several kbar.^[1–3] Supercritical fluids (SCFs), with their tunable dielectric constants and high compressibilities, offer the possibility of pressure-dependent selectivity at much lower pressures. This possibility has been realized for uncatalyzed diastereoselective reactions,^[4, 5] enzymatic catalysis,^[6, 7] and conformational equilibria,^[8] but not yet for homogeneous catalysis. Herein we describe the first example of enantioselective homogeneous catalysis that is dependent on solvent pressures below 100 bar.

A number of factors can affect the rates or selectivities of reactions in SCFs,^[9–12] but the dielectric constant (ϵ) is the factor we have chosen to exploit in our preliminary studies. At 30 °C the dielectric constant of fluoroform (scCHF₃, T_c = 25.9 °C, P_c = 48.2 bar) varies greatly with pressure (Figure 1,

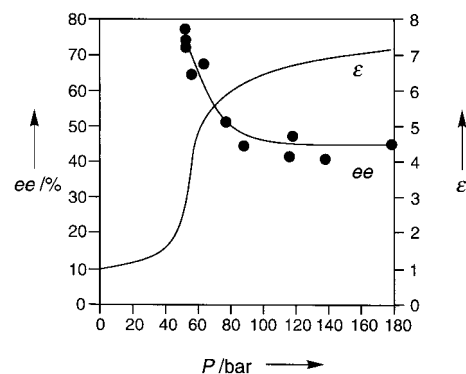


Figure 1. The dependence of the *ee* of product **2** and the dielectric constant^[13] of scCHF₃ on the pressure. Conditions: 1 h reaction, 30 °C, 0.69 μ mol catalyst, 56 μ mol diazo reagent, 440 μ mol styrene, 160 mL vessel.

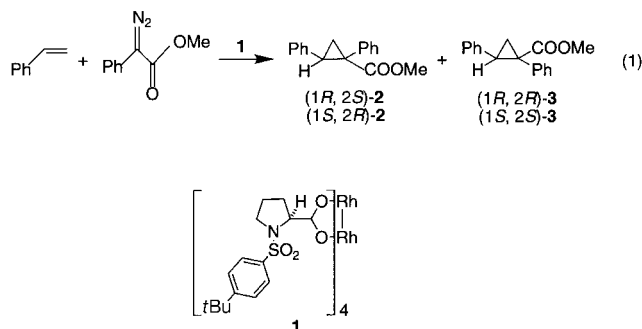
right axis).^[13] At low pressures the dielectric constant of scCHF₃ is 1–3, meaning that it is a very nonpolar solvent analogous to liquid pentane. At high pressures, however, the dielectric constant is 6–8, and the solvent is as polar as ethyl acetate or THF. Thus it is possible, by changing the pressure, to test the effect of changing the solvent's dielectric constant on the enantioselectivity of a reaction *without changing solvents*.

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We chose to test a cyclopropanation reaction between styrene and methyl phenyldiazoacetate catalyzed by the *tert*-butylbenzenesulfonyl-L-proline dirhodium catalyst **1**^[14, 15] in scCHF₃ [Eq. (1)]. Liquid-phase studies of this reaction^[16] and



a related reaction^[15, 17] have found a strong solvent dependence. The enantiomeric excess (*ee*) of the major product **2** was 61 % for the reaction in methylene chloride (ϵ = 9.08) and 85 % in pentane (ϵ = 1.84), the major enantiomer being (1*R*,2*S*).^[16] The explanations offered by Davies et al.^[17] and Doyle et al.^[16] invoke only the polarity of the solvent rather than its coordinating ability or any other solvent property. Hence we anticipated that the enantioselectivity of this reaction should be strongly pressure dependent in scCHF₃.

Our results (see Figure 1) show that this is indeed the case, especially near the critical point of the solvent. The reaction was performed repeatedly in scCHF₃ at 30 °C, each reaction at identical molar concentrations of reagents and catalyst but with different pressures of the SCF. Although the critical pressure of CHF₃ is 48.2 bar, no experiments were attempted at pressures below 52 bar because of insufficient solubility of the reagents under such conditions (see the supporting information). Very low concentrations of reagents were used so that the critical point of the mixture would be very close to that of pure CHF₃. The enantiomeric excess of product **2** varied from 40 % *ee* for reactions performed at pressures above 100 bar (P_r/P_c = 2.07) to as high as 77 % *ee* for those at 52 bar (P_r = 1.08).

In contrast, the enantioselectivity is pressure independent in scCO₂, within experimental error. At 34 °C the selectivity was 83 % *ee* at 79 bar (P_r = 1.07), 84 % *ee* at 96 bar (P_r = 1.30), and 80 % *ee* at 110 bar (P_r = 1.49). The lack of variation in enantioselectivity is presumably due to the fact that the dielectric constant of scCO₂ increases only slightly with pressure, ranging between 1.3 and 1.5 under these conditions.^[18] Thus, varying the pressure and density has little effect on enantiomeric excess in the absence of a dielectric constant change. We can also conclude that cyclopropanation enantioselectivities in scCO₂ are as good as the best results in liquid solvents.

If the selectivity trends observed in scCHF₃ are due to the variation in dielectric constant, then the enantioselectivity should change in scCHF₃ as the dielectric constant changes near the critical point. It is evident from Figure 1 that this is the case. An alternative presentation of the data, a plot of enantiomeric ratio versus the dielectric constant of the reaction medium (Figure 2), shows that the scCHF₃ data

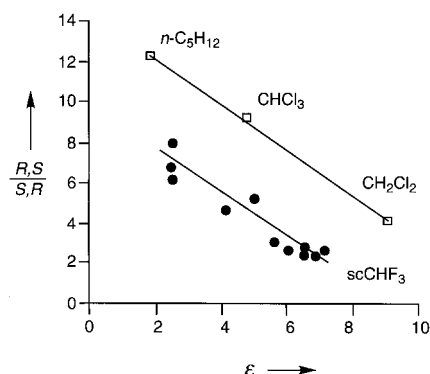


Figure 2. The dependence of the ratio of enantiomers of **2** on the dielectric constant of scCHF_3 at the conditions of Figure 1. The result in liquid chloroform is new data, while the results in CH_2Cl_2 and pentane are those reported by Doyle et al.^[16]

exactly parallels the trend observed in liquids. There are two other explanations of the results, but they are both considered less plausible than a dielectric constant effect: a) the catalyst could be less soluble in scCHF_3 at the lower pressures, but that would most likely cause a decrease in the *ee*, not an increase (see the supporting information for more evidence against this explanation), and b) the delivery of the diazoacetate into the vessel could be slower or less complete at lower pressures of scCHF_3 than at higher pressures, but tests showed that halving the amount of diazoacetate at 150 bar has no effect on the enantiomeric excess within experimental error. We are currently investigating the cause of the lower selectivity in scCHF_3 relative to liquids. Whatever the cause, it does not seem to decrease the enantioselectivity in scCO_2 . The development of a method for the gradual addition of the diazoacetate may increase the enantioselectivity.

The solvent dependence of the enantioselectivity has been proposed to be due to the stabilizing effect of polar solvents on charge-separated transition states,^[17] although the stability of different rotamers of the catalyst may also be affected by solvent polarity.^[16, 17] Our data is entirely consistent with a polarity effect, and there is no need to invoke either the coordinating ability of the solvent or “clustering” near the critical point to explain the observations.

SCFs are unique tools for probing solvent effects because the properties of a single medium can be varied continuously. This example demonstrates that pressure-controlled variation of the dielectric constant allows the researcher to tune the selectivity of a reaction by adjusting the pressure. Thus, SCFs are the tunable analogues of liquid solvents, just as homogeneous catalysts are the tunable analogues of heterogeneous catalysts.

Experimental Section

Reactions were performed by placing **1** (0.69 μmol), styrene (440 μmol), and a stir bar in a 160-mL high-pressure vessel, which was then sealed and warmed to 303 K in a water bath. The vessel was purged and pressurized with CHF_3 to at least P_c but below the desired reaction pressure. After a 10 min delay to allow for dissolution of the styrene, methyl phenyldiazoacetate (56 μmol) was injected with an HPLC-style injector. This diazoacetate was then flushed into the vessel by the remaining CHF_3 required to bring the pressure up to the desired level. After 1 h the

reaction was stopped by submerging the vessel in a dry ice/acetone bath until the pressure fell to approximately 1 bar. The vessel was then vented and allowed to warm to room temperature. The product was dissolved in acetone and the *ee* determined by GC (Chirasil-DEX CB column, 170 °C). Tests to ensure repeatability and solubility are described in the supporting information.

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A Diazoalkane Derivative of a Polyoxometalate: Preparation and Structure of $[\text{Mo}_6\text{O}_{18}(\text{NNC}(\text{C}_6\text{H}_4\text{OCH}_3)\text{CH}_3)]^{2-}$ **

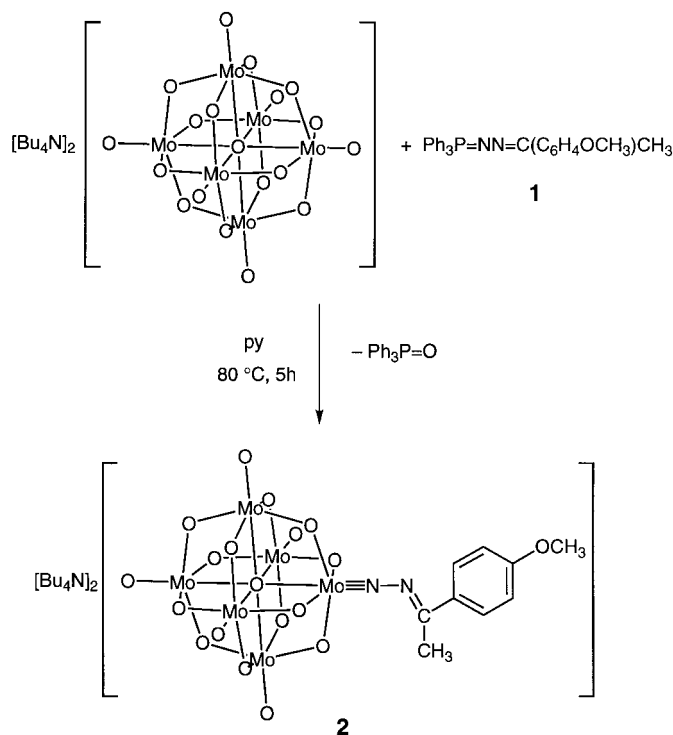
Haidoo Kwen, Victor G. Young, Jr., and Eric A. Maatta*

Dedicated to Professor Rupert A. D. Wentworth

Diazoalkane complexes $[\text{L}_n\text{M}(\text{N}_2\text{CR}_2)]^{[1]}$ are distinguished by their utility in important organic transformations^[2] and by the variety of their coordination modes.^[3] Such systems are generally prepared by the direct interaction of R_2CN_2 with low-valent ($\leq d^2$) metal complexes; in unusual and noteworthy cases, diazoalkane complexes have been prepared by elaboration of ligated N_2 ^[4] and $[\text{NNH}_2]$ groups.^[5] When compared with the chemistry of related organoimido complexes $[\text{L}_n\text{M}(\text{NR})]$,^[6] that of diazoalkane complexes is less systematic, in part because of the restrictions imposed by available preparative routes. We illustrate here a high-yield metathetical synthesis, involving the exchange of oxo and diazoalkane ligands, which promises to allow the introduction of diazoalkane ligands into previously inaccessible coordination environments.

Reaction of $[\text{Bu}_4\text{N}]_2[\text{Mo}_6\text{O}_{19}]$ with phosphazene **1** in pyridine at 80 °C for 5 h produces $\text{Ph}_3\text{P}=\text{O}$ and the diazoalkane–hexamolybdate complex **2** (Scheme 1). After solvent evaporation and washing the residue with toluene (to remove the phosphane oxide byproduct), **2** is obtained as an analytically pure dark orange powder in 84 % yield. To our knowledge, **2** is the first diazoalkane–polyoxometalate complex.^[7] Other singly functionalized derivatives of $[\text{Mo}_6\text{O}_{19}]^{2-}$ bearing multiply bonded nitrogen ligands include $[\text{Mo}_6\text{O}_{18}(\text{NO})]^{3-}$,^[8] $[\text{Mo}_6\text{O}_{18}(\text{NNAr})]^{3-}$,^[9] $[\text{Mo}_6\text{O}_{18}(\text{NNMePh})]^{2-}$,^[10] and a variety of organoimido complexes $[\text{Mo}_6\text{O}_{18}(\text{NR})]^{2-}$.^[11]

In the IR spectrum of **2**, the $\text{C}=\text{N}$ stretching vibration occurs at 1610 cm^{-1} . In the $\text{Mo}=\text{O}$ stretching region, a sharp and distinct band at 986 cm^{-1} is observed as a shoulder on the main feature at 956 cm^{-1} ; this pattern is characteristic of many monosubstituted hexamolybdate species.^[11] The lowest energy band in the electronic spectrum of a solution of **2** in CH_3CN occurs at $\lambda_{\text{max}} = 397\text{ nm}$ ($\epsilon = 7.7 \times 10^4$) which we assign as arising from nitrogen-to-molybdenum charge transfer. A second prominent band at $\lambda_{\text{max}} = 311\text{ nm}$ ($\epsilon = 7.3 \times 10^4$) is assigned as a $\pi \rightarrow \pi^*$ transition within the $[\text{N}_2\text{CMeAr}]$ ligand; in the spectrum of the corresponding hydrazone $\text{H}_2\text{NNCMeAr}$, this feature is observed at $\lambda_{\text{max}} = 271\text{ nm}$ ($\epsilon = 3.2 \times 10^4$).



Scheme 1. Metathetical synthesis of the diazoalkane–hexamolybdate **2**.

Cyclic voltammetry studies of **2** (CH_3CN ; 298 K; Pt electrode; $[\text{Bu}_4\text{N}][\text{PF}_6]$ supporting electrolyte; scan rate 100 mV s^{-1}) in the range from +1.0 V to –1.0 V (vs. Ag/Ag^+) reveal a one-electron reduction wave at –0.894 V ($\Delta E_p = 0.147\text{ V}$); under these conditions, the electrochemical couple of deliberately added $[\text{Mo}_6\text{O}_{19}]^{2-}$ was observed at $E_{1/2} = -0.706\text{ V}$ ($\Delta E_p = 0.085\text{ V}$). The cathodic shift in reduction potential of **2** versus that of $[\text{Mo}_6\text{O}_{19}]^{2-}$ is comparable to those observed for various $[\text{Mo}_6\text{O}_{18}(\text{NR})]^{2-}$ complexes,^[11] indicating that the donor ability of the $[\text{N}_2\text{CMeAr}]$ group is superior to that of the oxo ligand and similar to that of an $[\text{NR}]$ group.

The structure^[12] of the anionic cluster within **2** is shown in Figure 1. The diazoalkane ligand is bound at a terminal position in a monodentate fashion. Its metrical parameters ($\text{Mo1}-\text{N1}\ 1.738(11)$, $\text{N1}-\text{N2}\ 1.337(14)$, $\text{N2}-\text{C1}\ 1.31(2)\text{ Å}$; $\text{Mo1}-\text{N1}-\text{N2}\ 172.0(10)$, $\text{N1}-\text{N2}-\text{C1}\ 117.3(11)^\circ$) are consistent

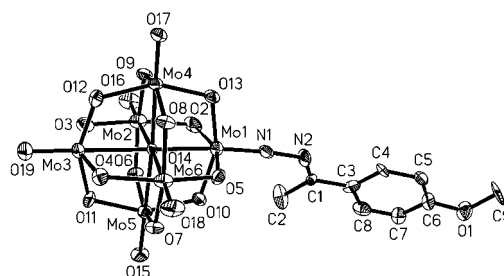


Figure 1. Thermal ellipsoid drawing of the $[\text{Mo}_6\text{O}_{18}(\text{N}_2\text{CMeC}_6\text{H}_4\text{OMe})]^{2-}$ anion within **2**. Selected bond lengths [Å] and angles [°]: $\text{Mo1}-\text{N1}\ 1.738(11)$, $\text{N1}-\text{N2}\ 1.337(14)$, $\text{N2}-\text{C1}\ 1.31(2)$, $\text{Mo1}-\text{O5}\ 1.954(8)$, $\text{Mo1}-\text{O2}\ 1.963(9)$, $\text{Mo1}-\text{O13}\ 1.949(8)$, $\text{Mo1}-\text{O10}\ 1.990(8)$, $\text{Mo1}-\text{O14}\ 2.164(7)$, $\text{Mo3}-\text{O3}\ 1.923(8)$, $\text{Mo3}-\text{O4}\ 1.913(9)$, $\text{Mo3}-\text{O11}\ 1.892(9)$, $\text{Mo3}-\text{O12}\ 1.926(9)$, $\text{Mo3}-\text{O14}\ 2.399(7)$; $\text{Mo1}-\text{N1}-\text{N2}\ 172.0(10)$, $\text{N1}-\text{N2}-\text{C1}\ 117.3(11)^\circ$.

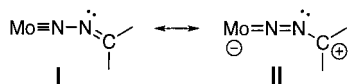
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with a description as a dianionic “hydrazone-type” species, involving contributions from the resonance structures **I** and **II**, with form **I** dominating.



No discernable variations are observed among the Mo–O_t bond lengths which span the narrow range from 1.679 to 1.698 Å; these values are quite close to those in [Mo₆O₁₉]^{2–}.^[13] Within the equatorial belt of the cluster, a regular pattern of bond length alternation^[14] is evident: each O_b atom forms one long (2.00 Å) and one short (1.88 Å) bond to its pair of Mo atoms. Bond lengths from the equatorial set of Mo atoms to the central O14 atom range from 2.335 to 2.346 Å, again similar to the corresponding distances in [Mo₆O₁₉]^{2–} (2.32 Å). At the diazoalkane binding site, Mo1–O_b distances are distinctly longer than the corresponding Mo3–O_b distances at the *trans* terminal oxo site: Mo1–O_b distances range from 1.949 to 1.990 Å (av 1.964 Å), while Mo3–O_b distances range from 1.892 to 1.926 Å (av 1.914 Å). Since the [N₂CMeAr] ligand is superior to an oxo ligand as an electron donor, this longitudinal shift of O_b electron density away from Mo1 provides a mechanism to equalize the valence at Mo1 and Mo3. Along the Mo1–Mo3 axis, the central O14 atom is substantially nearer to Mo1 (2.164 Å) than to Mo3 (2.399 Å). The large discrepancy in these relatively weak interactions is expected given the much larger *trans* influence of terminal oxo ligands as compared to that of multiply bonded nitrogen ligands.^[15]

In summary, we have demonstrated a new route to metal complexes of diazoalkane ligands. As illustrated by the preparation of **2**, this metathetical approach promises to provide diazoalkane complexes in previously inaccessible environments. Studies to discern both the range of diazo functionality which may be transferred and the generality of this process with other oxo–metal complexes are underway.

Experimental Section

p-Methoxyacetophenone hydrazone^[16] was converted to the corresponding triphenylphosphazene **1** by reaction with Ph₃PBr₂ in benzene in the presence of two equivalents of Et₃N.^[17] Compound **1** (0.46 g, 1.1 mmol) and [Bu₄N]₂[Mo₆O₁₉]^[18] (1.00 g, 0.73 mmol) were combined in pyridine (10 mL) and stirred at 80 °C for 5 h under N₂. The reaction mixture was filtered, volatiles were removed under vacuum, and the red residue was washed successively with Et₂O and toluene to yield 0.93 g (84 %) of **2** as an analytically pure dark orange solid. Crystals were grown by diffusion of Et₂O vapor into a CH₃CN solution at 25 °C. Elemental analysis for C₄₁H₈₂N₄O₁₉Mo₆ (%): calcd: C 32.60, H 5.47, N 3.71; found: C 32.84, H 5.54, N 3.82; ¹H NMR (400.1 MHz, CD₃CN, 25 °C): δ = 7.85, 7.83, 6.98, 6.96 (AA'BB', 4H, C₆H₄), 3.85 (s, 3H, OCH₃), 2.73 (s, 3H, CH₃), 3.09 (m, 16H, N-CH₂), 1.60 (m, 16H, CH₂), 1.34 (m, 16H, CH₂), 0.96 (t, 24H, CH₃); IR (Nujol, cm^{–1}): ν̄ = 1610 (C=N), 986, 956 (Mo=O); UV/Vis (CH₃CN): λ_{max} (ε) = 397 (7.7 × 10⁴), 311 nm (7.3 × 10⁴).

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Heterosupramolecular Chemistry: Programmed Pseudorotaxane Assembly at the Surface of a Nanocrystal**

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The size-dependence of the electronic and optical properties of metal and semiconductor nanocrystals has been studied in detail.^[1] These investigations have been facilitated by the preparation of dispersions of essentially defect-free nanocrystals that possess narrow size distributions and are stabilized by chemisorbed long-chain alkanes.^[2–8] Fundamental insights into the evolution of bulk electronic and optical properties in metals and semiconductors have resulted.^[3, 9]

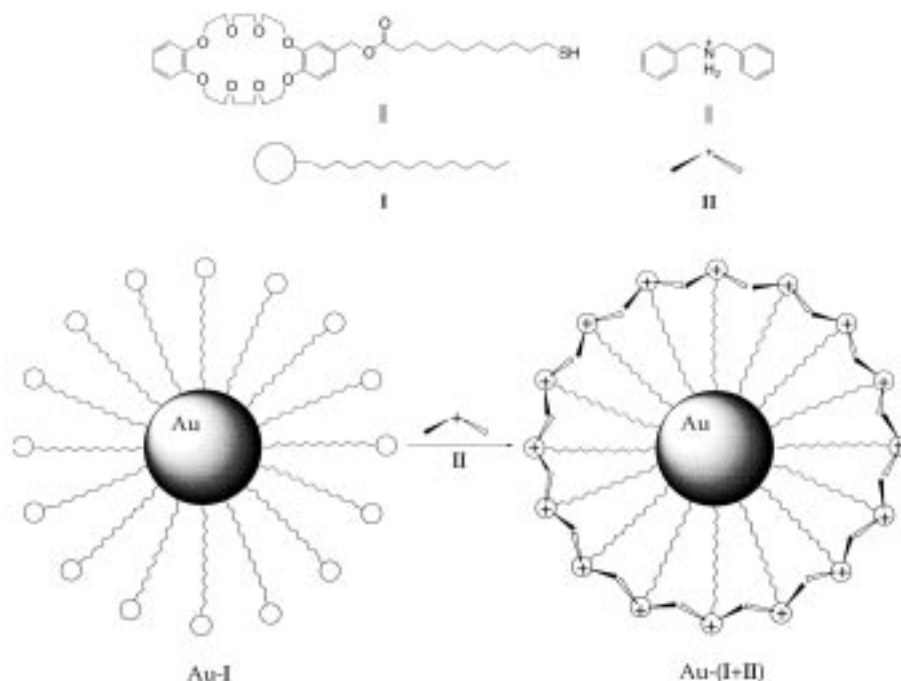
Increasingly, it is the collective electronic and optical properties of metal and semiconductor nanocrystals that are being investigated.^[3, 10, 11] These studies are being facilitated by the preparation of organized nanocrystal assemblies.^[1, 5, 10–14] Of particular interest is how the size-dependence of the electronic and optical properties of the constituent nanocrystals may be exploited to tune their collective properties.

Organized nanocrystal assemblies are generally prepared at an air–water interface using Langmuir–Blodgett techniques (two-dimensional),^[12] or on a suitable substrate by controlled solvent evaporation (three-dimensional).^[10] Both approaches, however, are limited by the fact that only relatively simple nanocrystal architectures may be realized. For this reason, strategies that may permit the assembly of complex nanocrystal architectures are of particular interest.

One strategy is to prepare a dispersion of nanocrystals that is stabilized by molecules that incorporate one or more binding sites. These binding sites serve to define uniquely the position of a nanocrystal from a dispersion in the nano-

crystal architecture being assembled. Upon mixing a number of such dispersions each nanocrystal recognizes and binds selectively a nanocrystal from another dispersion or a well-defined region on the surface of a suitably patterned substrate. By this means it should be possible to program the parallel assembly of identical, multiple copies of the desired nanocrystal architecture in solution or at a substrate. Underpinning the above strategy are efforts directed toward the development of a systematic chemistry of both condensed phase and molecular components, that is, a systematic heterosupramolecular chemistry.^[15–17]

Despite a growing number of examples of programmed nanocrystal assembly in solution, much remains to be done.^[15–21] Specifically, there is a pressing need for a robust model system, the study of which could provide detailed insights into the factors that control nanocrystal assembly in solution or at a suitable substrate. Toward this end, we have prepared a dispersion of gold nanocrystals that possess a narrow size distribution and are stabilized by a chemisorbed monolayer of **I**. It was expected that these nanocrystals, denoted **Au-I**, would recognize and bind selectively the dibenzylammonium cation **II** in solution to form the pseudo-



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rotaxane-containing assembly **Au-(I + II)**.^[22–24] Presented here are the findings of our initial studies that show these expectations are largely justified and, interestingly, which point to similarities between the binding of a molecule in solution by the receptor sites on the surface of a nanocrystal and the binding of a drug molecule by the receptor sites on the surface of a cell.^[25]

Transmission electron microscopy (TEM) establishes that the average diameter of a gold nanocrystal in **Au-I** is 42 ± 8 Å (Figure 1).^[26] Elemental analysis establishes that the average number of **I** adsorbed at the surface of each nanocrystal is

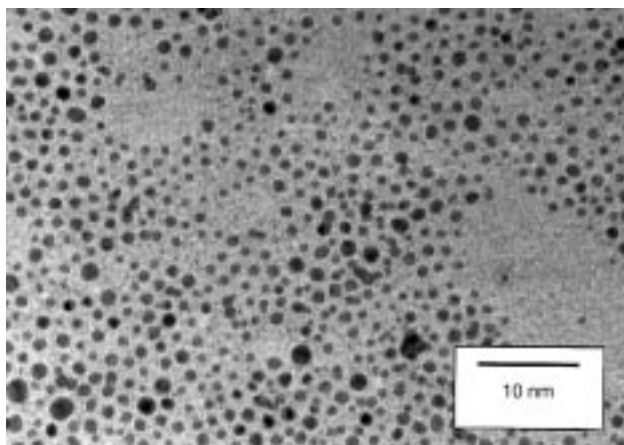


Figure 1. TEM picture of gold nanocrystals stabilized by a chemisorbed monolayer of **I** (Au-I).

300.^[27] On this basis^[28] it has been calculated that the average surface area occupied by each molecule **I** is 18 Å². This value is smaller than that for a monolayer of **I** self-assembled at a planar gold substrate (21 Å²),^[29] but, as expected, somewhat larger than those reported for an alkanethiol adsorbed at the surface of a gold nanocrystal (16 Å²).^[30] These findings can be explained by the extreme curvature of the gold nanocrystal and the steric hindrance associated with the terminal crown moiety of **I**, respectively.

The ¹H NMR spectra of **I** and Au-I in CDCl₃ are shown in Figure 2. In solution the resonances assigned to the methylene protons α, β, and γ to the sulfur atom in **I** arise at δ 2.52 (2H,

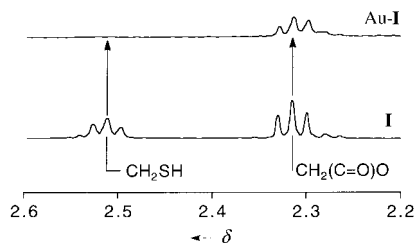


Figure 2. Section of the ¹H NMR spectrum of free **I** and Au-I.

q), 1.61 (2H, m), and 1.36 (2H, m), respectively. When, however, **I** is adsorbed at the surface of a gold nanocrystal, these same resonances are broadened to such an extent that the α-methylene proton resonance is no longer observed.^[30] This observation contrasts with the fact that the resonances assigned to the methylene protons α, β, and γ to the ester moiety in **I** are not significantly broadened. Thus, it is concluded that all molecules of **I** in Au-I are adsorbed at the surface of a gold nanocrystal. It is also concluded, because of the curvature of the gold nanocrystal, that the motions of the methylene groups close to the sulfur atoms and the surface of the gold nanocrystal are constrained. However, this situation does not pertain to the methylene groups near the oxycarbonyl group in that region of **I** that extends into the solvent.

Dibenzo[24]crown-8, the precursor of **I**, recognizes and binds **II** selectively in CDCl₃.^[22–24] It has also been established that the crown is threaded by **II** to form a [2]pseudorotaxane.

It was expected, therefore, that **I** would also recognize and bind **II** selectively in CDCl₃ and that a similar complex would be formed. The ¹H NMR spectra (not shown) of **I**, **II**, and an equimolar mixture of **I** and **II**—(**I** + **II**)—were measured in CDCl₃ to establish whether or not this is the case. The observed changes in the spectra are similar to those reported for an equimolar mixture of dibenzo[24]crown-8 and **II**.^[22–24] Furthermore, an analysis of these changes also yields values for *K*_a (2.7 × 10⁴ L mol^{−1}) and Δ*G*⁰ (−25 kJ mol^{−1}) that are similar.^[24] On this basis, it is concluded that an equimolar mixture of **I** and **II** self-assemble to form a [2]pseudorotaxane.^[22–24] Having established that an equimolar mixture of **I** and **II** forms a [2]pseudorotaxane, it was expected that Au-I would recognize and bind **II** selectively to form a similar complex under similar conditions. To establish whether or not this is the case, the ¹H NMR spectra of Au-I, **II**, and an equimolar mixture of Au-I and **II**—Au-(**I** + **II**)—were measured in CDCl₃ (Figure 3).

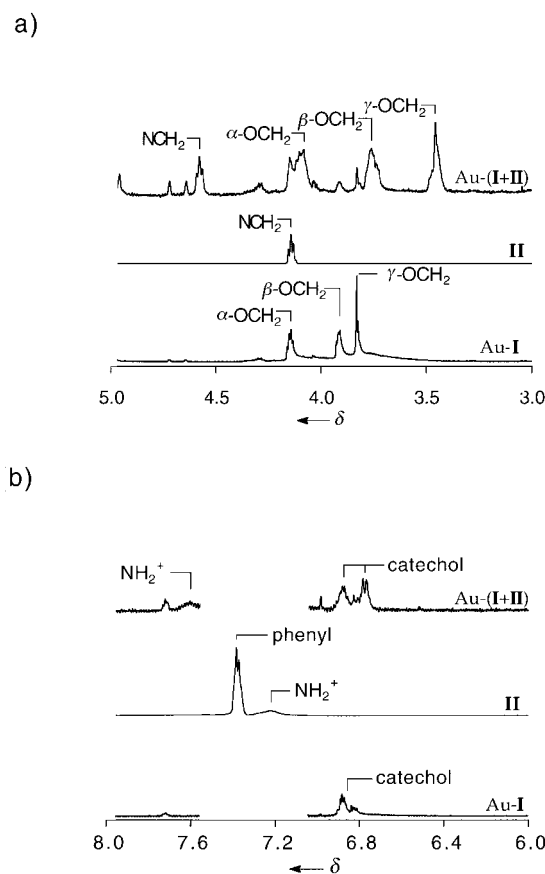


Figure 3. Two sections of the ¹H NMR spectra of Au-I, **II**, and an equimolar mixture of Au-I and **II**.

In the aliphatic region of the spectrum of Au-I (Figure 3 a), the resonances assigned to the α-, β-, and γ-OCH₂ protons of the dibenzo[24]crown-8 ring moiety appear at δ = 4.14 (8H, t), 3.92 (8H, t), and 3.83 (8H, s), respectively. In the aliphatic region of the spectrum of **II** the resonance assigned to the benzylic methylene protons of the dibenzylammonium cation appear at δ = 4.15 (4H, t). In Au-(**I** + **II**), the resonances assigned to the α-, β-, and γ-OCH₂ protons of the diben-

zo[24]crown-8 moiety are shifted upfield by $\Delta\delta = 0.05$, 0.15, and 0.37, respectively, while the resonance assigned to the benzylic methylene protons of the dibenzylammonium cation are shifted downfield by $\Delta\delta = 0.43$. In the aromatic region (Figure 3b), the resonance assigned to the catechol protons of the dibenzo[24]crown-8 moiety of Au-I appear at $\delta = 6.80$ –6.92 (7 H, m), while the resonances assigned to the phenyl and ammonium cation protons of II appear at $\delta = 7.37$ –7.40 (10 H, m) and 7.22 (2 H, brs), respectively. The resonance assigned to the catechol protons of Au-I in Au-(I + II) are shifted upfield by $\Delta\delta = 0.1$, while the resonances assigned to the phenyl and ammonium cation protons of II are shifted upfield and downfield by $\Delta\delta = 0.2$ and 0.4, respectively. The above spectral changes are similar to those previously reported for an equimolar mixture of dibenzo[24]crown-8 and II,^[22–24] and observed for an equimolar mixture of I and II. On this basis it can be concluded that an equimolar mixture of I, adsorbed at the surface of a gold nanocrystal, and II form a [2]pseudo-rotaxane.

A quantitative analysis of the spectra in Figure 3 indicates that 86% of the dibenzo[24]crown-8 binding sites at the surface of each gold nanocrystal in Au-I are complexed with a dibenzylammonium cation II. As a consequence Au-(I + II) precipitates from CDCl_3 over a period of three hours. It should be noted, that addition of 10% by volume of CD_3CN results in the precipitated nanocrystals being re-dispersed. It should also be noted, however, that the fraction of complexed dibenzo[24]crown-8 binding sites at the surface of each gold nanocrystal in Au-I is reduced to 45%. As the crown ether binding sites incorporated in I are not in solution but are adsorbed at the surface of a gold nanocrystal, a single point determination of K_a based on the nominal concentrations of Au-I, II, and Au-(I + II) is not appropriate.^[25] The preparation of a Scatchard plot with a slope equal to $-K_a$ and an intercept equal to NK_a [Eq. (1)] is more appropriate.^[25, 31]

$$\frac{P}{C} = NK_a - PK_a \quad (1)$$

P is the fraction of crown binding sites complexed with a cation, C is the concentration of I added to solution, and N is the number of crown binding sites on each gold nanocrystal. The Scatchard Plot obtained, however, is highly nonlinear (Figure 4a). The positive slope observed at low concentration C indicates that the fraction of crown receptor sites that have bound a dibenzylammonium cation is less than expected. This, in turn, indicates that binding is co-operative. Under such conditions an analysis based on the preparation of a Hill Plot (Figure 4b) with a slope equal to the Hill coefficient n_H , and an intercept equal to $-n_H \lg(1/K_a)$ [Eq. (2)] is necessary.^[25, 32] The slope of the Hill Plot in Figure 4b yields a value for n_H equal to 2.21 (correlation coefficient $R = 0.991$), which is consistent with the nonlinearity of the Scatchard Plot, and confirms a positive co-operation in the binding process exists.

$$\lg\left(\frac{P}{1-P}\right) = n_H \lg C - n_H \lg\left(\frac{1}{K_a}\right) \quad (2)$$

The origin of the positive co-operativity is likely to result from the following: An increased affinity of II for the surface of Au-I as the surface of the nanocrystal become progressively

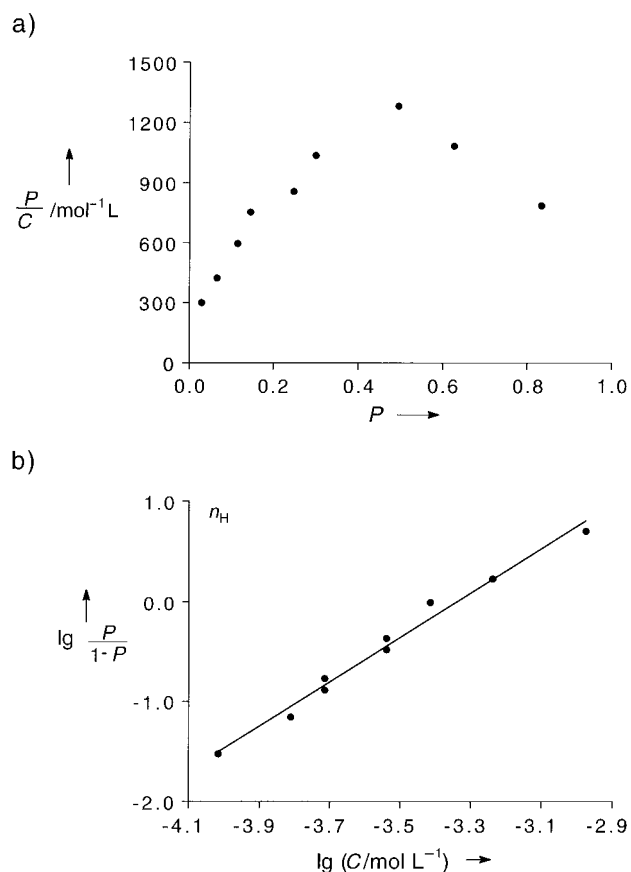


Figure 4. a) Scatchard Plot and b) Hill Plot for Au-I (0.25–1.00 equivalents of II added).

more polar/hydrophilic. It should be noted that the above suggestion is consistent with the observation that Au-(I + II) precipitated from CDCl_3 . Our results raise the intriguing possibility of using such systems as models for cell–drug binding studies and, possibly, as in vivo sensors for such interactions.

Experimental Section

(2-Formyl)dibenzo[24]crown-8, prepared as described in detail elsewhere,^[33] was converted into (2-hydroxymethyl)dibenzo[24]crown-8,^[34] which was subsequently coupled to 11-sulfanylundecanoic acid to form I.^[35] Compound II was prepared as described elsewhere.^[22]

Gold nanocrystals, stabilized by chemisorbed I, were prepared following the method reported by Brust et al.^[36] HAuCl_4 (0.155 g) in deionized H_2O (15 mL) was added to a phase transfer catalyst $n\text{Oct}_4\text{NBr}$ (1.101 g) in CDCl_3 (12 mL), and the resulting mixture stirred vigorously for approximately 10 min. The organic layer was recovered, the stabilizer (I, 0.070 g) in CDCl_3 (4 mL) and the reducing agent (NaBH_4 , 0.190 g) in H_2O (13 mL) added, and the resulting mixture stirred overnight at room temperature. Gold nanocrystals were precipitated by concentrating the reaction mixture (to 4 mL), adding it EtOH (300 mL), and stirring vigorously. The precipitated nanocrystals were isolated by centrifugation, re-dissolved in a minimum of CDCl_3 , and re-precipitated by addition of EtOH (30 mL). Gold nanocrystals stabilized by chemisorbed I, Au-I, were recovered by centrifugation and dried in air.

^1H NMR studies were carried out on Au-I and employed $1 \times 10^{-6} \text{ M}$ (particle concentration) dispersions in CDCl_3 , corresponding to a $4 \times 10^{-4} \text{ M}$ solution of adsorbed I. Studies on equimolar mixtures of Au-I and II employed 1.0 mL of the above dispersion to which had been added a 30 μL aliquot of $1.35 \times 10^{-2} \text{ M}$ stock solution of II in CD_3CN , which

corresponded to a final concentration of the added compound of 4×10^{-4} M. Binding studies on Au-I employed a 3×10^{-6} M (particle concentration) dispersion in CDCl_3 and CD_3CN (10 vol %), corresponding to a 1×10^{-3} M solution of adsorbed I. The desired volume of a 1.35×10^{-2} M stock solution of II, which corresponds to between 0.25 and 1.00 equivalents, was added to 0.7 mL of the above dispersion.

All ^1H NMR spectra were recorded at 25°C using a Varian 500 FT spectrometer. Transmission electron micrographs were recorded using a JEOL 2000 FX Temscan (150 KeV) for samples deposited on carbon coated copper grids.

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